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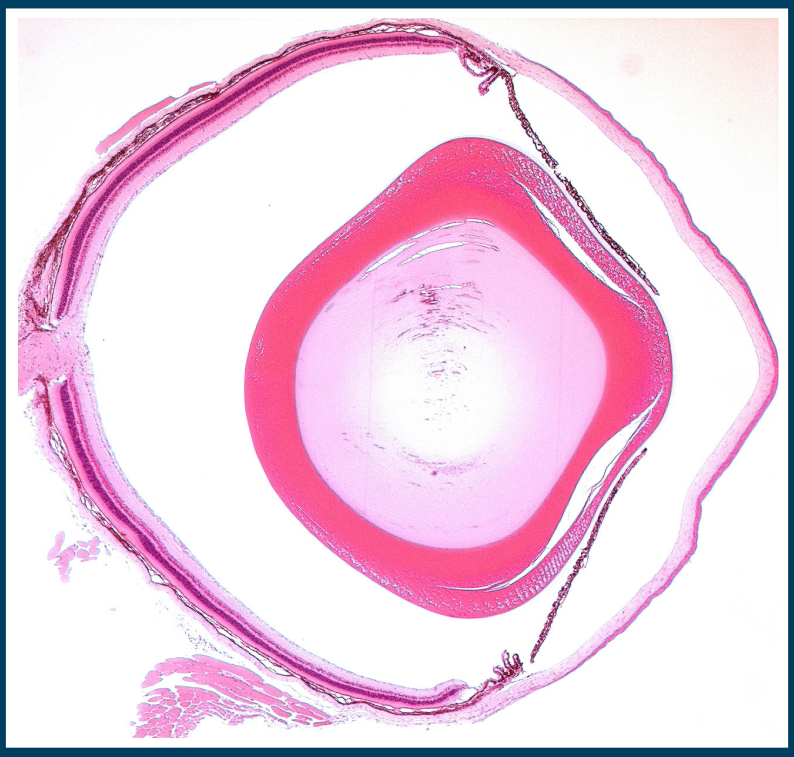
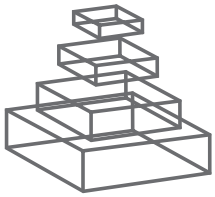


GOOD NEWS - BAD NEWS: THE TWO FACES OF IMMUNE PRIVILEGE

Topic Editors
Rachel R. Caspi and Joan Stein-Streilein

frontiers in IMMUNOLOGY



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GOOD NEWS - BAD NEWS: THE TWO FACES OF IMMUNE PRIVILEGE

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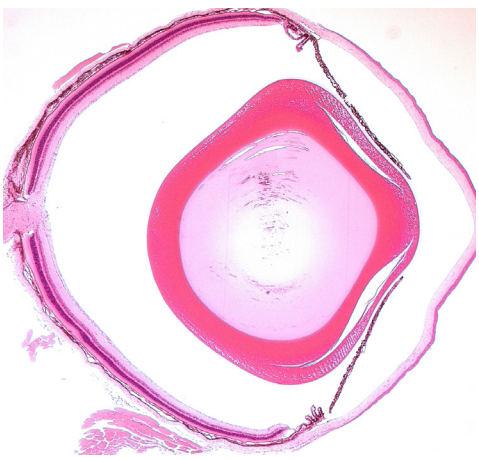

A hematoxylin and eosin-stained section through the pupillary-optic nerve plane of a healthy mouse eye. It represents the typical mammalian eye, with an anterior chamber residing between the cornea and the lens, and a vitreous chamber filled with the vitreous gel which extends all the way from the back of the lens to the retina. Both of these chambers are immunologically privileged compartments in the eye.
Image by Rachel R. Caspi

Immune privilege was once thought to be the property of a few select sites that include the eye, brain, testis, pregnant uterus and (of all things) the hamster cheek pouch, and was believed to be mainly based on sequestration behind blood-tissue barriers. This view has changed. Immune privilege is now considered to constitute a more general phenomenon through which tissues are able to actively direct and control immune responses taking place in their "territory" to preserve their structural and functional integrity in the face of inflammatory processes. These positive aspects of immune privilege can be hijacked by tumors to their survival advantage and to the detriment of the host. This Research Topic dissects the beneficial and deleterious consequences of immune privilege in terms of the cellular and molecular mechanisms that various tissues and tumors use, each in its own fashion, to regulate immune processes that affect them, at the local and the systemic level.

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EDITORIAL
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Immune privilege and the philosophy of immunology

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Keywords: immune privilege, tolerance, immune suppression, eye, regulatory cells

Like other scientists, immunologists use two types of approaches to research: one reduces the problem to its parts; the other studies the emergent phenomenon produced by the parts. Scientists that reduce the problem to its parts are sometimes called reductionists. The conclusions of reductionist experiments are often applied to the greater whole, when in actuality they may only apply to that particular experimental set. We, reductionists, are the ones who think our immune behavior exists solely because of genes, the presence of TGFβ, the presence of inflammatory cytokines, and appearance of a receptor. We tend to interpret the outcome in the colors of our interests (*ergo* "to a hammer, everything is a nail"). We measure parts and from the parts, we draw parallels to far-removed outcomes in terms of health and disease. More often than not, however, the results from the study of the parts do not predict the whole, and often the whole becomes greater than the sum of its parts. For example, a toll-like receptor does one thing when there is a bacterial invasion but the same toll-like receptor may lead to a different outcome when activated by danger signals during injury.

The approach that is perhaps more applicable to biology, and more specifically, to immunology, is the chaos theory. The chaos theory deals with the multiple layers of conditions and unexpected turns and restarts that can effect the outcome. It is applicable to studies of dynamic systems like the weather, and in our case, dynamic biological/immunological systems. The chaos theory points out that small differences in initial conditions make it impossible to predict the outcome. Thus, the behavior of the parts does not make the outcome predictable.

Our point? Immune privilege is broadly understood as the ability of the tissue to actively regulate and direct immune responses that take place in its "territory." The articles in this Research Topic in Frontiers in Immunology, "Good news–bad news", support the idea that to understand how immune privilege works, one has to understand the dynamics of the different tissues in terms of initiation, expression, regulation, and behavior, before one can begin to predict an outcome.

This Research Topic contains a number of papers that deal with immune privilege in the eye: a review that explores the relationship of immune privilege to ocular disease (1); a contribution on local regulation of immune $CD8^+$ T cells in the eye (2); a review discussing the intriguing parallels between the mechanisms of ocular immune privilege and uveal melanoma (3); a thoughtful contribution on how $CD8^+$ Treg cells might enable ocular tumor growth (4); a review proposing that (paradoxically) immune privilege emerges as an enabler of immune cells that heal, as well as of regulator immune cells that promote tissue damage (5).

While the eye is considered the prototypic immune privileged tissue, it is not the only one. We therefore shift to reviews of immune privilege and its mechanisms in other tissues and organs, underscoring the universality of the phenomenon: the reproductive tract (6), the testis (7), tumor environment (8), and finally chronic inflammatory diseases (9). While the eye, testes, reproductive tract, tumors, and chronic immune diseases all seem to share some "immune privileged" mechanisms, each has developed unique features of its own. Recent reports have repeatedly shown that immune regulation is "tailored" to the individual tissue in which it is taking place.

Each immune privileged tissue has a unique function. The eye must protect the light path and signals that stimulate the retina, and photoreceptors to preserve vision. The testis has to protect the sperm as they proceed to the epididymis where they mature. The maternal reproductive tract has to protect its eggs both before and after fertilization and thus has developed specialized mechanisms to modify the body's response to foreign antigens. These unique challenges require different solutions and lead to unique immune privilege mechanisms. However, although microenvironment and the stromal cells that carry out the particular function may vary between tissues, and consequently the mechanisms that promote regulation may be unique to that cell or tissue, the goal is the same: limit collateral damage to preserve tissue integrity and maintain homeostasis to the extent possible, without compromising host defense.

Many of the studies in the tissues other than the eye reveal areas of investigations that have not been well studied in the eye. IDO exerts profound effects on immune and tissue cells that suppress pro-inflammatory and immune stimulatory responses to a variety of insults. While the eye does not have frequent exposure to foreign antigens, its many layers of immune regulation appears to allow unmatched cornea grafts long-term survival without systemic immunosuppression. It is clear that we do not know all the immune privilege paradigms, but by understanding the emergent phenomena that are produced by the parts of immune privilege that is used by other tissues, may help to understand the concept of immune privilege as a whole. The needs of the tissue, its environment, and consequently the mechanisms used by each tissue to immunoregulate may vary. But yet, many of the basic concepts may be shared. Thus, out of the parts, emerges a whole that is greater than the sum of the parts.

We hope this Research Topic has successfully outlined the many layers involved in immune privilege, established that they vary with each tissue and clarified that the outcome cannot always be predicted. Because clinical expectations of medical research often hurry scientific discoveries prematurely to therapeutic applications, the scientists who break new ground should also use caution. Caution, that premature application of the basic science *good news* without sufficient understanding and application of the chaos theory, may lead to *bad news*.

REFERENCES

1. Forrester JV, Xu H. Good news-bad news: the Yin and Yang of immune privilege in the eye. *Front Immunol* (2012) **3**:338. doi:10.3389/fimmu.2012.00338
2. McPherson SW, Heuss ND, Gregerson DS. Regulation of CD8(+) T cell responses to retinal antigen by local FoxP3(+) regulatory T cells. *Front Immunol* (2012) **3**:166. doi:10.3389/fimmu.2012.00166
3. Niederkorn JY. Ocular immune privilege and ocular melanoma: parallel universes or immunological plagiarism? *Front Immunol* (2012) **3**:148. doi:10.3389/fimmu.2012.00148
4. McKenna KC, Previte DM. Influence of CD8+ T regulatory cells on intraocular tumor development. *Front Immunol* (2012) **3**:303. doi:10.3389/fimmu.2012.00303
5. Benhar I, London A, Schwartz M. The privileged immunity of immune privileged organs: the case of the eye. *Front Immunol* (2012) **3**:296. doi:10.3389/fimmu.2012.00296
6. Clark GF, Schust DJ. Manifestations of immune tolerance in the human female reproductive tract. *Front Immunol* (2013) **4**:26. doi:10.3389/fimmu.2013.00026
7. Li N, Wang T, Han D. Structural, cellular and molecular aspects of immune privilege in the testis. *Front Immunol* (2012) **3**:152. doi:10.3389/fimmu.2012.00152
8. Biragyn A, Lee-Chang C. A new paradigm for an old story: the role of regulatory B cells in cancer. *Front Immunol* (2012) **3**:206. doi:10.3389/fimmu.2012.00206
9. Li L, Huang L, Lemos HP, Mautino M, Mellor AL. Altered tryptophan metabolism as a paradigm for good and bad aspects of immune privilege in chronic inflammatory diseases. *Front Immunol* (2012) **3**:109. doi:10.3389/fimmu.2012.00109

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Good news–bad news: the Yin and Yang of immune privilege in the eye

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The eye and the brain are prototypical tissues manifesting immune privilege (IP) in which immune responses to foreign antigens, particularly alloantigens are suppressed, and even completely inhibited. Explanations for this phenomenon are numerous and mostly reflect our evolving understanding of the molecular and cellular processes underpinning immunological responses generally. IP is now viewed as a property of many tissues and the level of expression of IP varies not only with the tissue but with the nature of the foreign antigen and changes in the limited conditions under which privilege can operate as a mechanism of immunological tolerance. As a result, IP functions normally as a homeostatic mechanism preserving normal function in tissues, particularly those with highly specialized function and limited capacity for renewal such as the eye and brain. However, IP is relatively easily bypassed in the face of a sufficiently strong immunological response, and the privileged tissues may be at greater risk of collateral damage because its natural defenses are more easily breached than in a fully immunocompetent tissue which rapidly rejects foreign antigen and restores integrity. This two-edged sword cuts its swathe through the eye: under most circumstances, IP mechanisms such as blood–ocular barriers, intraocular immune modulators, induction of T regulatory cells, lack of lymphatics, and other properties maintain tissue integrity; however, when these are breached, various degrees of tissue damage occur from severe tissue destruction in retinal viral infections and other forms of uveoretinal inflammation, to less severe inflammatory responses in conditions such as macular degeneration. Conversely, ocular IP and tumor-related IP can combine to permit extensive tumor growth and increased risk of metastasis thus threatening the survival of the host.

Keywords: immune privilege, para-inflammation, eye

INTRODUCTION

The first Ophthalmology textbook in English, written in the first half of the nineteenth century, contained a description of sympathetic ophthalmia (SO), an intraocular inflammatory disease which develops in the fellow eye several months after penetrating injury to the first eye (Mackenzie, 1830). SO was a pre-eminent example of "horror autotoxicus" (reviewed in Mackay, 2010) and the search was on to define the autoantigen(s) (Faure, 1980), many of which are located in the retina (Wacker, 1991). At the same time as immunity and autoimmunity were being recognized, the remarkable acceptance of corneal allografts compared to skin allografts (ZIrm, 1906), which had been reported in the early part of the twentieth century, allowed Medawar to formulate the concept of immune privilege (IP). IP was a property of certain tissues (specifically the eye and the brain; Medawar, 1961) in which foreign antigens placed in those tissues failed to evoke a conventional immune response. Such tissues were seen to be afforded a level of protection from immunological damage (termed IP).

The concept of IP has since been extended and is now regarded as a relative term, not unique to the eye or brain; it is a property of many tissues, develops *de novo* in accepted vascularized grafts (Cobbold, 2009; Huang et al., 2010) and constitutes part of the immune response to tumors (Mellor and Munn, 2008). Ocular IP is inducible and transferable (through adoptive transfer of $CD8^+$ T regulatory cells (Tregs) — infectious tolerance; Griffith et al., 2011) and thus has informed immunology generally on regulatory mechanisms.

Despite ocular IP, autoimmune and immune-mediated diseases of the eye occur with demoralizing frequency; for instance, 5 year survival rates of corneal allografts in humans are lower than those of solid organ grafts (Williams and Coster, 1997), although this statistic can be somewhat misleading since most corneal allografts in humans are performed without tissue matching (see below); also, both innate and adaptive immune mechanisms underlie several blinding ocular diseases, the scourge of populations wordwide, such as age-related macular degeneration (AMD), infectious corneal blindness, glaucoma and the "Cinderella" disease, uveitis (see **Box 1**).

Ocular IP has been reviewed several times recently (Caspi, 2006; Niederkorn, 2006; Ferguson and Griffith, 2007; Forrester et al., 2008b). This review therefore will focus on the place of IP as an immunoregulatory, tolerance-inducing mechanism, and discuss its limitations in the context of sight-threatening diseases.

Box 1 | Uveitis.

Terminology for Uveitis is confusing and as a result the condition has been somewhat neglected as a global cause of blindness (thus it is a "Cinderella" syndrome) mainly because clinicians have had difficulty reaching agreement as to what constitutes uveitis. However, a recent initiative is aimed at developing international criteria for the various entities that come under the umbrella of uveitis (Standardization of Uveitis Nomenclature, SUN; Jabs et al., 2005).

The term "Uveitis" is a misnomer since it suggests that the focus of inflammation is the uvea. Discrete parts of the uvea can be affected separately: the iris (iritis), ciliary body (cyclitis, iridocyclitis), choroid (choroiditis), or entire uvea (panuveitis; see **Figures 1A,B**). However, the triggering antigens (either foreign or self-antigens from retina, lens, cornea) can be located in any of the tissues, including the uveal tract itself. The most potent autoantigens have been identified in the retinal photoreceptors. Accordingly, uveitis (uveoretinitis) is also classified under the term "Intraocular Inflammation," and sub-classified as to whether it affects the anterior segment of the eye ("anterior segment intraocular inflammation," ASII) in which it is restricted to the cornea, anterior chamber, iris, ciliary body, and lens, or it selectively affects the posterior segment, which includes the pars plana region of the ciliary body (pars planitis), the vitreous gel (vitritis), the retina (retinitis), the retinal vessels (retinal vasculitis), the choroid (choroiditis), or the optic nerve (papillitis, optic neuritis; posterior segment intraocular inflammation, PSII).

Uveitis according to the SUN criteria is classified by its underlying cause and then according to its anatomic location (**Table 1**).

Table 1 | Classification of uveitis (SUN criteria) with some examples.

Classification	Type of uveitis/uveoretinitis/intraocular inflammation	
	Infectious	**Non-infectious**
Anterior	Viruses	HLA B27-associated uveitis
	e.g., HSV, CMV, VZV	
Intermediate	Toxocara	Pars planitis
	Toxoplasmosis	Idiopathic vitritis
		Intermediate uveitis
Posterior	Toxoplasmosis	Retinal vasculitis
	Tuberculosis	Multifocal choroiditis
	Syphilis	PIC*
	Lyme disease	Behcet's disease
	Candida	Vogt–Koyanagi–Harada disease
Panuveitis	All of above	All of above

**PIC, punctate inner choroidopathy, a chronic low grade inflammation with subretinal neovascularization (see text).*

IMMUNOLOGICAL PROPERTIES OF THE EYE

CELLS AND TISSUES

Several ocular tissues such as the uvea (middle layer of the eye comprising the iris, ciliary body, and choroid), the cornea, the conjunctiva, and periocular fascia (Forrester et al., 2008a; see **Figure 1**), contain rich networks of innate immune cells (bone marrow-derived resident macrophages and dendritic cells, DCs) which, together with the parenchymal cells, secrete a wide range of mediators which underpin IP (Forrester et al., 2008b, 2010). The retina contains specialized myeloid cells (microglia), similar to brain microglia, recently reported to originate from yolk sac precursors (Ginhoux et al., 2010). In addition, the central (around the optic nerve) and peripheral (at pars plana, **Figure 1**) rims of the retina contain a small population of DCIR^{+} MHC Class II^{hi} DCs, as does the corneal periphery (Xu et al., 2007b).

Recently, a small population of retinal DCs has been described in mice expressing CD11c-GFP (Heuss et al., 2012) although the specificity of CD11c for DCs is open to question. The central cornea has few DCs but contains MHC Class II^{+} macrophages (Brissette-Storkus et al., 2002; Sosnova et al., 2005; Kuffová et al., 2008) while peripheral corneal epithelial Langerhans cells and stromal Langerin^{+} cells also reside in the cornea (Hattori et al., 2012). The lens contains no myeloid cells while the normal extravascular tissue of the eye is devoid T or B cells.

BLOOD–OCULAR BARRIERS

The intraocular compartments (**Figure 1B**) are separated from the blood and lymphatic circulations by the blood–aqueous barrier and the blood–retinal barrier (BRB; Forrester et al., 2008a).

Blood–aqueous barrier

Blood–aqueous barrier has two components – tight junctions between the endothelial cells of the ciliary blood vessels and similar junctions between the lining epithelial cells (**Figure 1**). The epithelial cells of the ciliary body maintain the intraocular pressure by pumping fluid which drains through the porous trabecular meshwork of the anterior chamber into the blood and lymphatic vessels of the episclera (**Figure 1B**). The sclera itself, like the central cornea, is avascular.

Blood–retinal barrier

Blood–retinal barrier also comprises two components — tight junctions of the retinal vessels and of the retinal pigment epithelium (RPE; **Figure 2**). The RPE is a terminally differentiated layer of neuroectoderm-derived cells formed embryologically by cells of the developing outer layer of the optic cup (Kim and Kim, 2012) whose function is to maintain the physiology of the photoreceptors and remove waste products (see below).

OCULAR CONNECTIONS TO SECONDARY LYMPHOID TISSUES

Although the periocular tissues such as the conjunctiva and the episclera (see **Figure 1**) contain lymphatics, the intraocular compartment of the eye lacks traditional lymphatics. Aqueous fluid from the anterior chamber, presumably containing soluble antigen shed physiologically, drains *via* episcleral blood vessels (aqueous veins) through the venous circulation to thymus, liver, and spleen (**Figure 3**). There is also a site-specific eye-draining lymph node (DLN) which receives soluble and cell-associated antigenic material from the eye (Plskova et al., 2002; Kuffová et al., 2008). Fluid also tracks by transscleral flow from the vitreous cavity across the retina driven by a RPE $Na^{+}K^{+}$ ATPase pump which, when damaged, causes subretinal fluid accumulation (Forrester et al., 1990), but the likelihood of shed ocular antigens

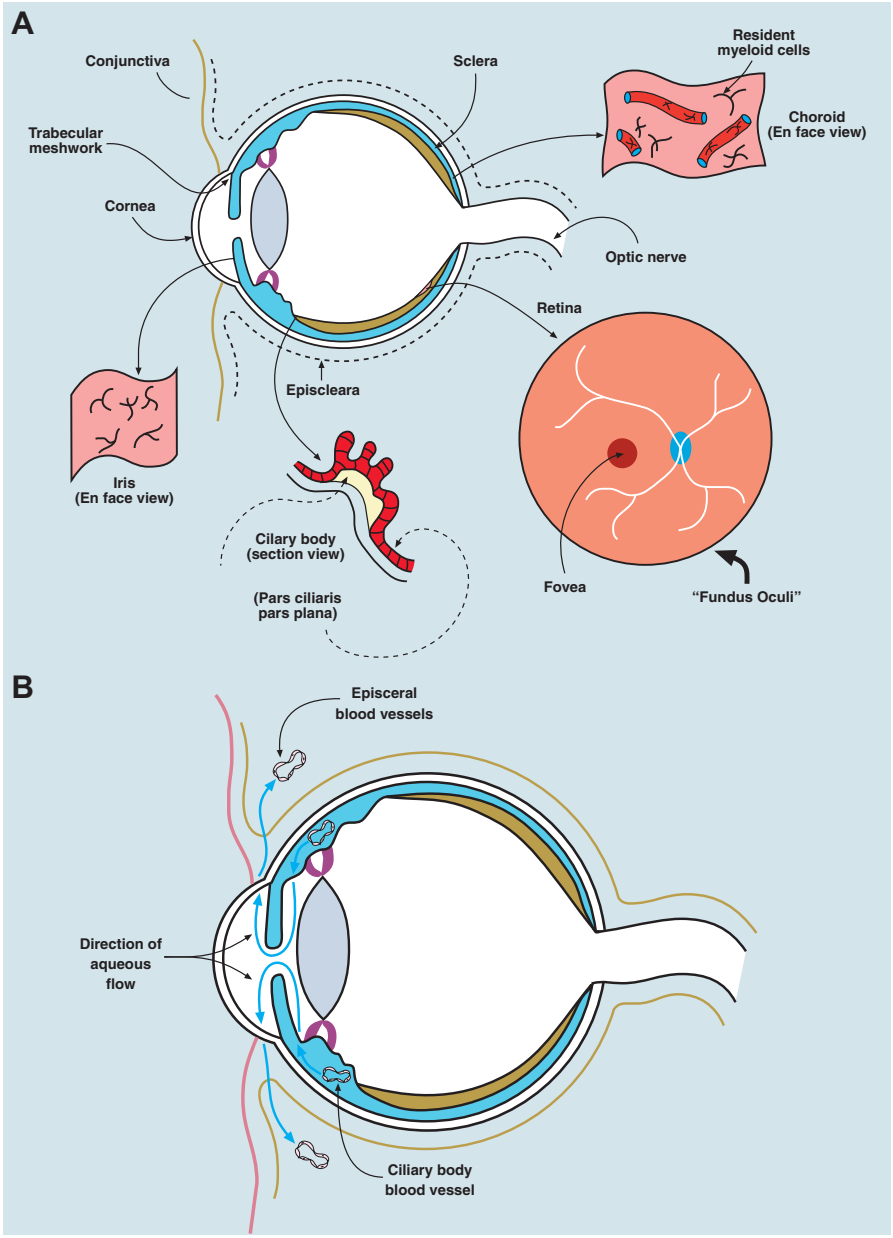

FIGURE 1 | Anatomy/physiology of the eye to include ocular immune cells. (A) The eye is composed of three layers: an outer layer (cornea/sclera), an inner layer (retina), and a middle layer (uvea, a continuous structure comprising iris, ciliary body, and choroid). The anterior chamber lies between the iris and the cornea, the posterior chamber between the lens and the iris, and the vitreous cavity (containing the vitreous gel, a type II/XI collagenous avascular extracellular matrix) describes the main chamber of the eye behind the lens. The human eye maintains a pressure between 10 and 20 mm Hg, which is generated by the unidirectional flow of fluid (aqueous humor secreted by the ciliary body) from the posterior chamber into the anterior chamber, and leaving the eye via the trabecular meshwork, to drain into the episcleral veins. The "fundus oculi" is the view of the retina/choroid seen through the ophthalmoscope; the central fovea/macula is a cone photoreceptor-rich area, 500 microns in diameter, subserving central visual acuity. The remaining retina provides peripheral vision (visual field) and all visual information is transmitted through retinal neuronal cells, via the optic nerve, which synapse in the lateral geniculate body intracranially. The uvea contains a network of resident innate immune cells (DCs and macrophages) and is highly vascular (seen in section and *en face* views in the figure). The retina contains few conventional resident myeloid cells, but has a population of microglial cells (see text). Normal ocular tissues are devoid of lymphocytes. The cornea contains a population of passenger leukocytes mostly in its peripheral rim as well as some lymphatics in this region connecting with lymphatics in the conjunctiva. **(B)** Eye health is dependent on having a normal intraocular pressure, which is maintained between 12 and 20 mm Hg by the flow of aqueous fluid from the posterior chamber of the eye (the space between the posterior surface of the iris and the anterior surface of the lens) and the anterior chamber (the space between the posterior surface of the cornea and the anterior surface of the iris). The vitreous cavity is the intraocular compartment behind the lens and in front of the retina. Aqueous fluid is secreted by the epithelial cells of the ciliary body into the posterior chamber and flows through the pupil of the iris into the anterior chamber to drain through the trabecular meshwork at the angle of the eye between the iris and the cornea, into the subconjunctival space, to be finally removed by interstitial fluid flow into the episcleral veins and the subconjunctival lymphatics.

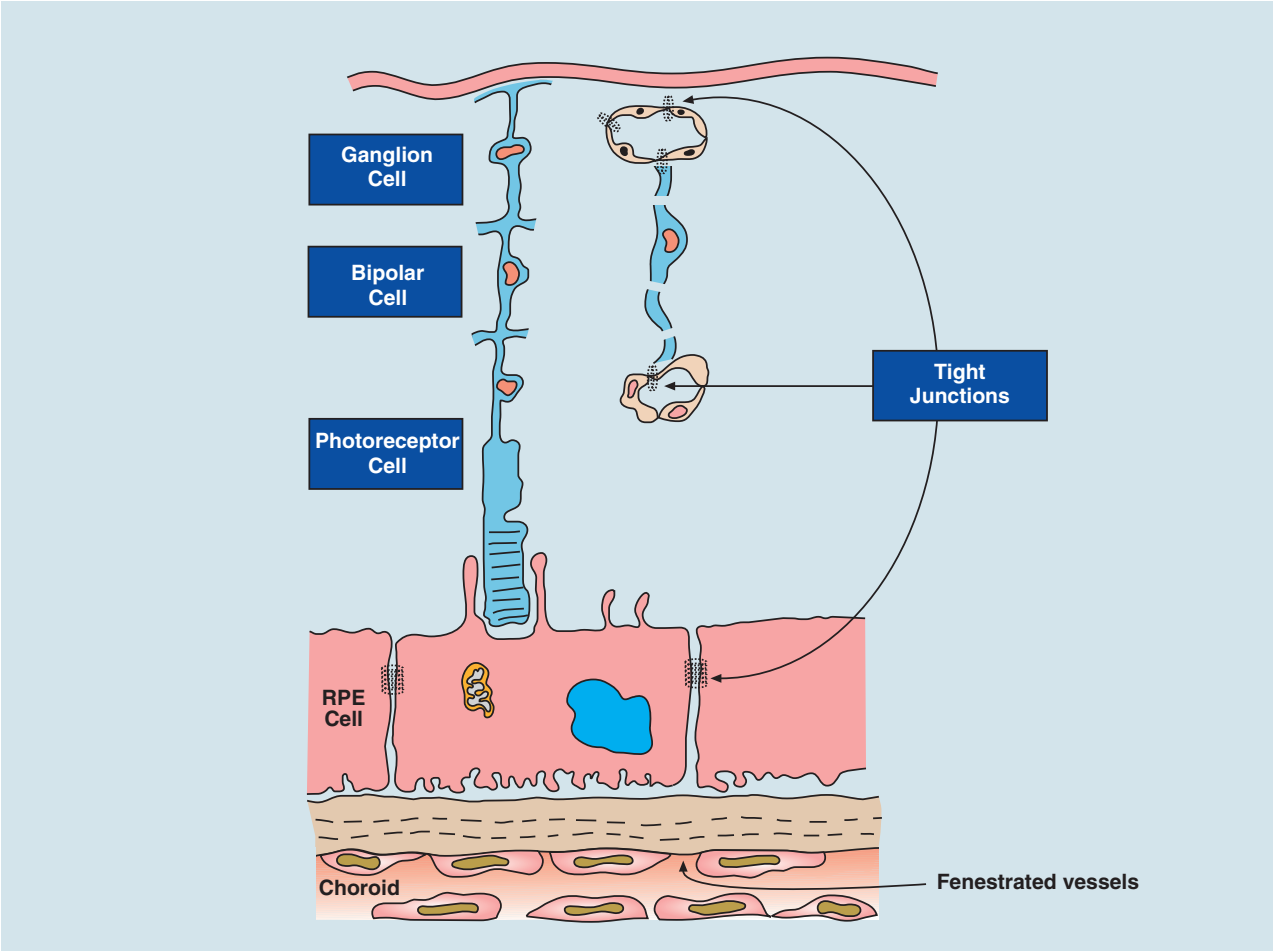

FIGURE 2 | Blood–retinal barrier (BRB). The BRB is created by tight junctions at two sites: between endothelial cells of the retinal vessels that supply the inner retina (ganglion cells and bipolar cells) and the retinal pigment epithelium (RPE cell; which filters blood from the fenestrated, leaky choroidal vessels). The RPE regulates two-way transport of fluid, nutrients, and waste between the outer retina (photoreceptors) and the fast flowing, high volume choroidal bloodstream. The choroid stroma contains resident innate immune cells to maintain homeostasis in the outer retina (see Forrester et al., 2010) as well as fibroblasts and melanocytes. Breakdown of the BRB can thus occur either at the retina vessels or at the RPE layer.

reaching lymphatics through this route is low, given the extremely high flow rates of blood through the choroidal vessels (Forrester et al., 2008a).

NEURAL CONNECTIONS

Aside from the neural connections to the brain *via* the visual pathways, for which there are several types of photoreceptors, as well as light-sensitive melanopsin-containing neurons generating circadian rhythms (Do and Yau, 2010), the eye has a full complement of motor, sensory, and autonomic nerves. Some provide, or respond to, immunoregulatory moieties with IP-promoting properties, including the neuropeptides, αMSH, CGRP and VIP, PACAP, melanocortin and retinoids (reviewed in Forrester et al., 2008b). Others provide routes for immune attack and evasion as in herpes virus infection of the cornea (*via* the Vth cranial nerve). CD8 T cells have a unique role in maintaining HSV latency at the trigeminal ganglion (Sheridan et al., 2007; Frank et al., 2012).

THE HAZARDOUS NATURE OF OCULAR IMMUNE PRIVILEGE

The term IP was coined to indicate that certain tissues or cells have an advantage over others, allowing them to modulate immune responses to foreign antigens or to be regarded themselves as non-immunogenic when transplanted (e.g., stem cells; Robertson et al., 2007; Zhou and Caspi, 2010). However, IP as such carries risks to the host since it permits foreign antigens/organisms to "hide" in IP sites (Wekerle, 2006).

WHAT IS IMMUNE PRIVILEGE?

Different levels of IP apply to many tissues, including some which are normally expected to mount full blown immune responses to foreign antigens (skin, lung, and gut). Conceptual shifts in the self/non-self paradigm underpinning adaptive immunity have emerged from evidence that innate immune recognition of foreign antigens has some degree of specificity [*via* pathogen-associated molecular patterns (PAMPs) and pathogen recognition receptors (PRRs; Kumar et al., 2011)]. This idea has evolved beyond selective

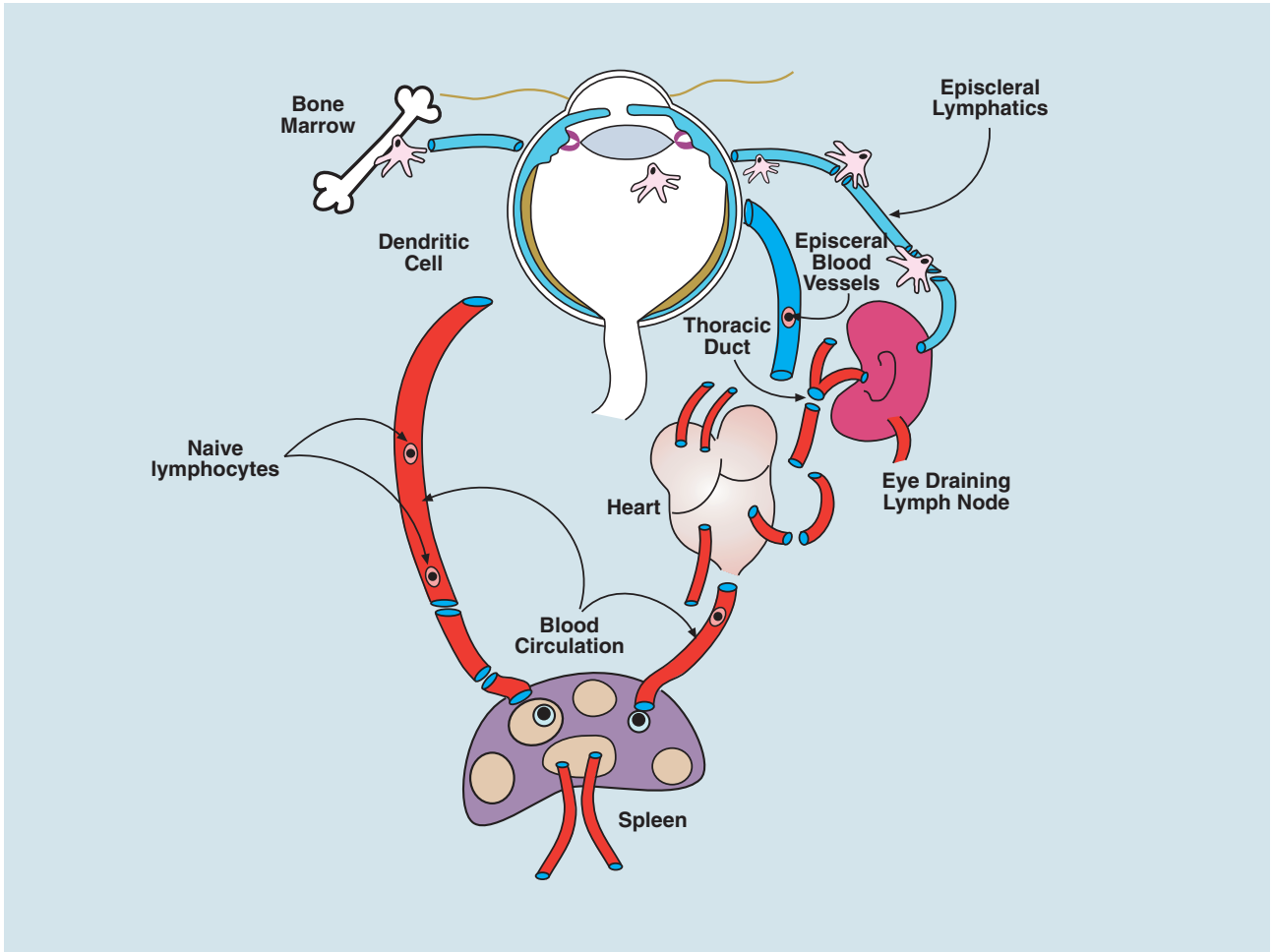

FIGURE 3 | Circulation of immune cells to and from the eye. Myeloid cells (macrophages and DCs) traffic from the bone marrow to the eye via the blood and populate some ocular tissues, predominantly the uvea (iris, ciliary body, and choroid); a few cells enter the peripheral retina and cornea. When ocular tissues are perturbed, bone marrow-derived cells, carrying antigen from the eye, can be found in the eye-draining lymph node (DLN; see text). However whether there is transport of steady-state antigen to the DLN is not known. Both cell-associated and soluble antigen injected into the eye, or applied to the abraded cornea, can be detected in the spleen after several hours. Resting T and B cells circulate normally through the uveal blood vessels of eye but do not cross the blood–ocular barriers; it is presumed they communicate with eye-derived antigen presenting cells in the secondary lymphoid tissues (spleen and lymph node) and respond appropriately to promote tolerance or immunity as in other tissues (see text).

recognition of pathogens, to recognition of "Danger" by the host (Matzinger, 2001; Miller et al., 2011), and to the notion that different tissues contribute variably to immune regulation particularly of the effector response, depending on which specific structures or functions are threatened (Matzinger, 2007; Matzinger and Kamala, 2011). While the Danger hypothesis has greatly broadened our understanding of how immunological responses are initiated, a certain caution might be exercised since it is difficult to define "Danger" in molecular or cellular terms. Accordingly, since IP itself as a concept is contingent upon the self/non-self paradigm of adaptive immunity, it is timely to review exactly what IP means.

At its core, IP is a form of tolerance expressed through tissue-specific properties (see above), is shown to be inducible toward foreign and alloantigens, and is assumed to underpin tolerance to ocular self-antigens. As a general hypothesis therefore, we can consider tissue-centered immunological tolerance as additional to the well-established central and peripheral tolerance mechanisms (Matzinger, 2007). Tissue-centered tolerance (TCT; Matzinger and Kamala, 2011) varies with the nature, properties, and vascularity of the tissue and is optimal in tissues such as eye and brain. In other tissues, TCT or IP can be induced, for instance as in accepted allografts and even in the stem cell niche (Robertson et al., 2007; Cobbold, 2009; Waldmann, 2010; Fujisaki et al., 2011). Interestingly, in these environments Treg induction seems to be the main mechanism of immune tolerance. As such, tissues which depend on their intrinsic properties for immunological protection or privilege (IP) are at greatest risk when tolerance is breached.

Several mechanisms are proposed to explain ocular IP including sequestration of antigen from the developing immune system behind blood–ocular barriers (immunological ignorance), lack of

lymphatics, absence of MHC Class II$^+$ professional antigen presenting cells (APCs; Hamrah and Dana, 2007; Forrester et al., 2010; negates CD4 T cells), lack of expression of MHC Class I on tissue cells (negates CD8 T cells), expression of Qa-1 and HLA-G/E (negates NK cells), presence of immune modulators such as TGFβ, CD200, CD55, and CD46, decay-acceleration factor (DAF; modifies APCs), and lack of thymic expression of tissue antigens (no autoreactive T cells to escape to the periphery; Forrester et al., 2008b; Zhou and Caspi, 2010).

In addition, parenchymal cells of the eye have the capacity, at least *in vitro*, to exert T cell suppressive activity (Caspi et al., 1987; Ferguson and Griffith, 2007) as well as generate Tregs (Taylor et al., 1997; Yoshida et al., 2000; Zamiri et al., 2007; Sugita et al., 2010). Expression of molecules such as FasL (Ferguson and Griffith, 2007), PDL1 (Usui et al., 2008), CTLA4 (Sugita et al., 2006), and the recently described CTLA2 (Sugita et al., 2008) by ocular cells particularly the iris, ciliary body, and RPE cells generate an immunosuppressive microenvironment (Stein-Streilein, 2008). The PD-1 pathway is interesting since it appears to have the capacity to promote T effector cell death similar to the Fas/FasL pathway, but also has the potential to promote Tregs (Francisco et al., 2010). Recently, retinoids (specifically retinoic acid, RA) have been implicated in the induction of Tregs by the RPE *in vitro* (Kawazoe et al., 2012). RA produced by CD103$^+$ lamina propria DCs has been identified as a major inducer of intestinal Tregs and this property of DCs has been extended to non-intestinal DCs (for review, see Nagy et al., 2012). In these conditions, the Treg-inducing effects of RA required TGFβ (Cong et al., 2009). Recent data showed that vitamin A-deficient mice were unable to convert naïve T cells to Tregs in the uninflamed eye *in vivo* which was interpreted as being due to an absence of locally produced RA, presumably by intraocular DCs (Zhou et al., 2012). However, in conditions of active experimental autoimmune uveoretinitis (EAU), committed T effector cells could not be converted to Tregs suggesting that this component of IP was lost during inflammation. Previous studies had, in fact, shown that RA promoted the immunogenicity of DCs in a pro-inflammatory environment (Geissmann et al., 2003) and this may explain the results from the above experiment in uveitis.

A potential role of RA in IP in the eye is a logical conceptual extension given the role and high concentrations of retinoids in the visual cycle. The RPE specifically expresses one of the enzymes (RALDh10) required for metabolizing retinal (vitamin A) to RA, and all-trans retinal itself has recently been shown to induce Tregs *in vitro* (Jeon et al., 2012). However, most of the retinoids in the RPE, if not used in the visual cycle, are stored as inactive retinyl esters, and are converted to retinal by RALDh10 if vitamin A blood levels decline. *In vivo* evidence that visual cycle retinoids themselves participate in IP is lacking, probably because their escape from the visual cycle is tightly regulated. Indeed it has been shown that accumulation of all-trans-retinal in mice lacking the ABCA4 transporter (ATP-binding cassette transporter 4) and RDH8 (retinol dehydrogenase 8) are liable to retinal degeneration, particularly that induced by light (Maeda et al., 2009). However, there are large networks of ocular tissue-resident DC which are likely to be a source of retinoids for Treg induction (Forrester et al., 2010). Indeed this may be a universal property of all tissues related to tissue-resident, homeostasis-promoting, self-tolerizing DCs, which is dependent on the density and phenotype of the DC, and is at a high level in the eye. Interestingly, Tregs are important for maintaining IP in the brain and they maintain this function even in the face of acute viral encephalitis, thus minimizing bystander damage and presumably preventing autoimmunity associated with the viral infection (Cervantes-Barragan et al., 2012). Induction of Tregs by DC in allografts and other sites has been described as a form of acquired IP and has been attributed in part to the expression of indoleamine oxidase (IDO; for review, see Huang et al., 2010; Kushwah and Hu, 2011) probably underscoring the fact that there are multiple potential routes for induction of Tregs, but more importantly highlighting the link between IP and Treg induction. Interestingly, RA- and TGFβ-mediated Treg induction does not carry over to IL-10 Tr1 regulatory cell induction, in which these molecules do not have any effect and may in fact have a negative role (Maynard et al., 2009).

How Tregs might mediate privilege is unclear. Mice with defects in central tolerance (such as those deficient in the autoimmune regulator gene *Aire* required for negative selection of certain tissue-specific antigens presented by mTECs) develop mild to moderate ocular inflammation as part of a multiple autoimmune diathesis (Anderson et al., 2002; DeVoss et al., 2006). However, *Aire* is also involved in induction of natural Tregs in the thymus (Aschenbrenner et al., 2007) and *Aire* has also been detected in the periphery, both factors indicating that this gene probably does not have a restricted role in central tolerance (Metzger and Anderson, 2011). Mice with defined immunodeficiencies involving Tregs are not known to have ocular pathology which would suggest that either Tregs do not contribute to ocular IP or that tissue-centered "IP-like" mechanisms (TCT) are sufficient to sustain tolerance in the eye. This has however, to our knowledge, not been directly tested in Treg-deficient mice, using conventional models of ocular autoimmunity, such as EAU. Despite this, patients with uveitis are reported to have decreased levels of circulating Tregs which suggests that peripheral tolerance, if not IP, is necessary to maintain immunological homeostasis. Tregs occur as part of the infiltrating inflammatory cell population in the retina in EAU, indicating that they almost certainly play a role. Since TCT is one aspect of tolerance generally, it may be somewhat semantic to attribute exclusivity in these mechanisms.

It can be seen that there are many possible mechanisms which explain the immunosuppressive properties of the eye associated with IP, all of which have been shown to be only partially validated, if functional at all *in vivo*; moreover, one single mechanism is unlikely to account fully for ocular IP; instead, each probably contributes to overall immune homeostasis. In this context, the eye's IP properties offer strong supportive evidence for Matzinger's TCT hypothesis but as discussed below, in many circumstances IP/TCT is insufficient to fully protect the eye from Danger.

DOES IP HAVE MEMORY?

Adaptive immune responses to foreign antigens are characterized by memory. Tolerance to self-antigen involves antigen-specific responses leading to deletion, peripheral anergy, and/or regulation with memory. Tolerance operates when there is risk of self-antigen

exposure to the immune system, for instance during apoptotic turnover of cells, by processes which suppress immune-mediated inflammation and encourage "silent" clearance of cellular debris. Much of this activity is conducted in secondary lymphoid tissues like the liver and spleen, which drain the interstitial fluids *via* lymphatic and blood circulations.

Tolerance to nominal antigens is demonstrable when, after exposing the organism to antigen, an immune response to that antigen cannot be induced on re-challenge. In some circumstances, an immune response can be elicited on re-challenge but is attenuated or modified: the latter circumstance is known as immune deviation. Tolerance is an active process involving T cell proliferation followed by apoptosis (activation-induced cell death, AICD) and clearance of cell debris by resident tissue scavenger cells. However, if the initial encounter with antigen involves cell necrosis and/or "adjuvant" effects of microorganisms *via* PRR activation and induction of IL-1/IL-18 *via* the inflammasome or other mechanisms (van de Veerdonk et al., 2011), an active pathogenic immune response with concomitant inflammation and bystander tissue damage may occur.

There are several routes of antigen (Ag) administration for inducing tolerance: intravenous (iv), subcutaneous (s/c), mucosal (oral/nasal/conjunctival), intraperitoneal (ip), and intraocular (io). Io injection involves antigen uptake by APC (Dullforce et al., 2004) and induction of antigen-specific T cells, followed by T cell and APC apoptosis *via* Fas/FasL (Green and Ferguson, 2001). Systemic tolerance to that antigen can then be demonstrated by antigen re-challenge in a delayed-type hypersensitivity (DTH) skin test (as originally shown for alloantigens by Medawar, 1948). This response is antigen-specific and thus has "memory," requires a minimum period of 3 days to develop, and is dependent on an intact oculo-splenic axis (Streilein and Niederkorn, 1981). The tolerizing effect can be transferred to naïve mice by serum (Griffith et al., 1995), and by circulating mononuclear cells (PBMC; Wilbanks and Streilein, 1992), from mice injected io with antigen some days previously. Some of these PBMC express the F4/80 antigen (Wilbanks and Streilein, 1992).

Trafficking studies using tagged molecules in mice injected io, or after application to the abraded cornea, indicate that soluble antigen rapidly reaches the eye-DLN within 30 min (Camelo et al., 2004, 2006) and then circulates widely to other lymph nodes (LNs; including the mesenteric) and to the spleen within several hours. Cell-associated antigen, after injection into the anterior chamber, can be detected in the DLN at 6 h, and in the spleen after 16–24 h indicating that cellular traffic from the eye to these sites is possible (Kuffová et al., 2008). However, data from experiments which involve intraocular injection require cautious interpretation since io penetration necessarily involves some backflow of antigen from the eye directly into the blood and lymphatic circulations (see below).

Irrespective of how the antigen reaches the spleen, i.e., as soluble, or later as cell-associated antigen, most groups agree that DTH-testable antigen-specific tolerance can be transferred to naïve mice by splenic $CD8^+$ Tregs from mice injected io with antigen. The nature of the APC which promotes this tolerance is unclear, but mice deficient in cells expressing the macrophage surface marker F4/80, fail to generate tolerance after io injection (Lin et al., 2005). Intuitively, however, it is unlikely that cells from the eye directly mediate this effect since the number of ocular $F4/80^+$ cells is limited; furthermore, previous studies failed to demonstrate APC migration from the iris after io antigen administration (Dullforce et al., 2004). An alternative possibility is that ocular fluids, perhaps containing material shed as microparticles or exosomes, or more conventionally as soluble proteins, from incoming inflammatory cells (including T cells undergoing Fas/FasL-mediated apoptosis and expressing TRAIL or shedding sTRAIL (Green and Ferguson, 2001; Griffith et al., 2011)), enter the blood circulation and arrive at the spleen where further amplification of the NKT cell/F4/80 spleen cell-mediated process of T cell apoptosis occurs (Faunce and Stein-Streilein, 2002). Since tolerance *via* this route is transferable by serum and has been shown to involve TCR components (Griffith et al., 1995), a possible explanation for T cell antigen specificity *via* cell surface particle shedding in this model arises.

The main flaw in these studies, going back to Medawar, is that the technical procedure of inoculating antigens into the eye causes breakdown of the blood–ocular barrier, however transiently, with leakage of antigen into the periocular blood and lymphatics, sufficient to permit rapid tracking of antigen to the spleen either directly *via* the blood or after a first pass through the DLN and thence to the spleen *via* the LN conduit system, high endothelial venules (HEV) and efferent lymphatics (Plskova et al., 2002; Kuffová et al., 2008). Tolerance induced by io injection is therefore not, in substance, different from tolerance induced by iv injection (antigen tracks directly to spleen), or by sc or ip injection (antigen tracks *via* DLN to the spleen). Models which suggest that TGFβ-treated $F4/80^+$ peritoneal macrophages preferentially mirror ocular IP overstate the case (Hara et al., 1992; Niederkorn, 2009), and are probably not mechanistically different from models of myeloid suppressor cell activity (Gabrilovich and Nagaraj, 2009). Indeed, a recent study confirmed the role of splenic red pulp $F4/80^{hi}$Mac-1^{lo} macrophages as immunosuppressive cells and showed that they act by inducing $CD4^+CD25^+$ Tregs in a CSF-1-dependent manner (Kurotaki et al., 2011). Gregerson et al. (2009) in an attempt to eliminate this technical flaw, showed that $CD4^+CD25^+$ Treg-mediated tolerance to endogenous retinal antigen, tested *via* retinal antigen-expressing viral infection, was reduced when the source of retinal antigen had been removed by enucleation of the eyes. However, the trauma of enucleation is likely to induce a systemic concomitant "Danger" signal which is difficult to control for (Miller et al., 2011; van de Veerdonk et al., 2011). In contrast, intact peripheral T cell anergy as well as $CD4^+CD25^+$ Treg generation (Lambe et al., 2007) seemed necessary to avoid spontaneous uveitis in a transgenic neoantigen model despite considerable central deletion (Lambe et al., 2007).

The time differential for soluble antigen (minutes) vs cell-associated antigen (hours) trafficking from the eye or indeed from any LN drainage site is important in the context of induction of tolerance vs immunity. LN resident APC, which will preferentially capture fast-tracking soluble antigen as it percolates along the conduits, present low levels of specific antigenic peptide–MHC Class II (p-MHCII) complexes, while migratory DCs entering the DLN several hours later present high levels of p-MHCII (Itano et al.,

2003; Sixt et al., 2005). Both resident and migratory DCs have the capacity to induce T cell proliferation, but only migratory DC, through prolonged T cell/DC interactions, induce T cells which mediate DTH (for review, see Catron et al., 2004). Thus the earlier arrival of soluble antigen to the secondary lymphoid tissues will promote tolerance rather than DTH-style T cell responses. This underappreciated concept may explain many aspects of ocular IP and the essential nature of the time-dependent oculo-splenic axis since it may be indirectly accessed via rapid transit of "tolerizing" antigenic signals by a first pass through eye-DLN as well as directly through the bloodstream.

SUBVERTING PRIVILEGE-PROMOTING CELLS

Despite the above caveats to what IP actually is and how it functions, there is little doubt that the intraocular microenvironment is immunomodulatory. Some of this is attributable to ocular-specific cells such as the iris–ciliary body epithelium and the RPE as shown in many *in vitro* studies, through mediators such as NO, PGE2, and retinoids (Forrester et al., 2008b; see Immunological Properties of the Eye). However, the microenvironment is altered by mediators introduced, e.g., *via* a disrupted BRB. Cytokines such as IFNγ, generated systemically during viral infections, can activate RPE cells to up-regulate immunosuppressive activity *via* PDL-1 (Ke et al., 2010) or to produce pro-inflammatory chemokines and cytokines locally, as well as induce MHC Class II expression on normally negative RPE (Liversidge et al., 1988, 1994; Mesri et al., 1994) and endothelial cells. Secretion of IL-6 by RPE cells in an environment rich in TGFβ, may be sufficient to convert $CD4^+$ Tregs to Th17 cells and completely alter the immunosuppressive microenvironment to a pro-inflammatory one (Crane et al., 1999).

BREACHES OF PRIVILEGE

Immune privilege comes at a cost – if the privileged status of the eye is compromised, the ensuing disease can be devastating (Caspi, 2006; Forrester et al., 2008b). Several ocular conditions occur in which IP fails.

NON-INFECTIOUS INTRAOCULAR INFLAMMATION (UVEITIS)

Uveitis is a common disease (Darrell et al., 1962; Gritz and Wong, 2004; Williams et al., 2007) and comes in several varieties (**Box 1**). Direct infectious uveitis is less common while non-infectious uveitis is presumed to be (auto)immune in character. Despite their low expression on ocular cells, certain forms of uveitis have strong links with MHC Class I antigens (HLA B27: acute anterior uveitis; HLA B51: Behcet's disease; HLA A29: birdshot retinochoroiditis; Levinson, 2007). Acute anterior uveitis may be self-limiting, which has been attributed to Fas/FasL-induced T cell apoptosis, demonstrating IP in action in human disease (Dick et al., 1999).

Non-infectious uveitis is $CD4^+$ T cell-mediated (Th1 and Th17) in humans and mice (Amadi-Obi et al., 2007; Caspi, 2010). Ocular tissues contain many potential autoantigenic targets, especially retina, apparently sequestered from the immune system (Gregerson et al., 2009). Consequently, escape of antigen during damage (trauma, infection, inherited degenerations) is one route to activate rare autoreactive T cells in the periphery (Caspi, 2010). However, most uveitis occurs in virgin tissue, raising the question: how do activated T cells cross the BRB? Systemic signals, from chemokines and other molecules, appear to "prepare the way" across the endothelium (Xu et al., 2004, 2008; Crane et al., 2006).

"Systemic signals" generated during infections, involving mechanisms such as molecular mimicry and bystander activation (Benoist and Mathis, 2001; Ji et al., 2010), do not disguise the fact that, once activated, antigen-specific T cells enter the retina and cornea with as much ease as into any tissue, accumulate *in situ*, and attract pro-inflammatory macrophages which cause tissue damage (Forrester et al., 1998). In EAU, inflammation declines inversely with an increase in $CD4^+CD25^+$FoxP3 Tregs, which accumulate and promote resolution. However, EAU in the C57/BL6 mouse does not completely resolve but persists with a macrophage-mediated choroidoretinal angiogenic response (Chen et al., 2012) much as occurs in some uncommon human uveitides (PIC, see **Box 1**; Atan et al., 2011).

Spontaneous models of EAU more closely represent non-infectious human disease, which occurs without a recognizable trigger. Limited T cell anergy as well as possible antigen escape from sequestration (immunological ignorance) have been suggested to underpin breakdown of tolerance in this model (Lambe et al., 2007; Gregerson et al., 2009). Interestingly, early cells entering the tissues in this model are IL-17-secreting γδ-like T cells (Makinen, 2006), which have been implicated in the early pathogenesis of conventional IRBP-induced EAU (Nian et al., 2011). However, γδ T cells may have a regulatory role (Girardi and Hayday, 2005; Pennington et al., 2005; Nian et al., 2010) as well as a pathogenic role (Nian et al., 2011) in autoimmune uveoretinitis.

Immune privilege appears not to afford much protection against the damaging effects of uveitis possibly because IP works at the level of tissue homeostasis, mainly keeping healthy tissue free of random migrants which might provoke inflammation. However, when faced with a serious challenge, IP dismally fails to prevent severe destruction: in uncontrolled sight-threatening uveitis, both infection and the immune response to infection, can cause permanent structural damage. Therapies such as anti-TNFα disable the destructive effects of inflammation while permitting harmless monocytes to traverse the tissues without causing damage (reviewed in Khera et al., 2010).

TRANSPLANTATION

Several types of ocular allografts are performed in humans, including corneal, limbal (stem cells), scleral, and retinal grafts. However, IP does not protect against allograft rejection: even artificial corneas constructed from pig collagen, are rejected *via* antibody-mediated mechanisms (Liu et al., 2007).

Corneal grafts

Despite a long-established reputation for high rates of acceptance, long-term corneal graft survival rates actually lag behind vascularized solid-organ grafts (Williams and Coster, 1997). Corneal grafts in humans are normally performed without tissue matching, and graft acceptance was historically attributed to the absence of passenger leukocytes. However, as indicated above, corneal tissue contains both resident MHC Class II^+ macrophages and

peripheral lymphatics (Brissette-Storkus et al., 2002; Sosnova et al., 2005; Xu et al., 2007a; Ecoiffier et al., 2010). Despite the presence of MHC Class I and II leukocytes in the donor cornea, corneal graft rejection occurs at a slower tempo than comparably unmatched solid-organ allografts indicating a degree of "privilege"; in part this is due to the fact that corneal graft rejection is mediated *via* indirect allorecognition, and direct alloresponses do not contribute to this process (Boisgerault et al., 2009). Corneal graft rejection thus resembles chronic indirect allorecognition of vascularized grafts which is also of a considerably slower tempo, is mediated by CD4 T cells, and is greatly accelerated when innate immune activation is at high levels, e.g., in cases of infectious or atopic ocular surface disease, in both of which vascularization is prominent (Niederkorn, 2010). CD4 Th1 cells are the main pathogenic T cells which induce corneal allograft rejection, although recent evidence has implicated Th2 cells; intriguingly there is a suggestion that IL-17A, whether derived from Th17 cells or from other sources, is necessary for allograft survival (reviewed in Cunnusamy et al., 2010). The evidence for IP preventing corneal graft rejection is therefore not strong, its main effect probably being to blunt direct allorecognition. Perhaps the most intriguing possibility is that the absence of a strong $CD8^+$ T cell cytolytic alloresponse in orthotopic corneal graft rejection (Boisgerault et al., 2009) may not simply be attributable to low levels of donor MHC antigens, but that donor leukocytes migrating to the host DLN, arrive there as "privileged" cells from a healthy thrombospondin (TSP)-, RA-, and TGFβ-rich donor immunoregulatory microenvironment (Saban et al., 2010) and are more liable to promote tolerance rather than immunity. The balance will be decided by the level of inflammation and innate immunity at the site of the graft and thus the degree of surgical manipulation and associated trauma itself will influence the outcome. In addition, corneal expression of immunoregulatory molecules such as FasL and TRAIL help to promote this IP/TCT (Ferguson and Griffith, 2007). One major contributor to the immunoregulatory microenvironment appears to be DAF (CD55) expressed by both donor and host corneal cells (Esposito et al., 2010). However, this is insufficient to fully prevent the indirect alloresponse, which continues to fuel the rejection process.

Retinal transplants

Several attempts have been made to transplant retinal tissue (Anosova et al., 2001) or cells (photoreceptor cells, West et al., 2010; Gust and Reh, 2011; RPE cells, Tezel et al., 2007) to repair damaged or degenerating retina (West et al., 2009), but only occasionally has survival and, importantly neural integration, been reported (MacLaren et al., 2006). The erroneous notion that ocular IP will promote acceptance of such grafts has been exposed by the need for immunosuppression to assist graft acceptance (West et al., 2010). Very recently, photoreceptor transplantation, neural integration, and even evidence of visual function in mice have been demonstrated, indicating the feasibility of such an approach although information on the duration of survival of the photoreceptors was not detailed (Pearson et al., 2012). In a similar study, photoreceptor survival was found to extend for 4 weeks after which there was progressive apoptosis of the grafted cells in the absence of significant inflammation. Survival could be extended by prior transfection of the precursor photoreceptor cells with XIAP, an anti-apoptotic gene (Yao et al., 2011).

Intraocular stem cell transplants have been used to encourage retinal cell differentiation in the appropriate microenvironment, but also to capitalize on the immunosuppressive properties of certain cells, such as mesenchymal stem cells (MSCs). It is too early to decide whether these approaches are fanciful, but systemic immunosuppression is required to delay rejection of such grafts even for a short time, and accepted grafts do not appear to integrate with retinal neuronal circuits (Hill et al., 2009; West et al., 2010).

OCULAR INFECTIONS: LESSONS LEARNT FROM AIDS

Infectious uveitis is unusual in the absence of systemic immunodeficiency. Many infectious organisms such as *Toxoplasma gondii* and herpes simplex virus, reside latently in privileged sites such as the eye and brain (Kinchington et al., 2012); however, the relative sanctuary provided by these sites is still under some degree of systemic immune control since it is not until the CD4 T cell is disabled, as in untreated AIDS patients, that these organisms can "reactivate" and replicate, thereby causing severe damage (Scholz et al., 2003). Organisms include toxoplasma, mycobacteria, pneumocystis, candida, and other fungi, and several herpes viruses such as CMV, HSV, and HTLV-1. Such organisms survive and persist but "hide" from the immune system by hijacking privilege (Jones et al., 2006; Lepisto et al., 2007). Interestingly, the new phenomenon of "immune recovery uveitis" in HAART-treated AIDS patients indicates that once the eye's guard is lowered, it is susceptible to immune-mediated damage (Jabs, 2011), thus revealing that the privileged status of the eye is an active process requiring continued maintenance. Immune-mediated damage in these circumstances can also be the result of reactivated infection, thus complicating the issue further (Moorthy et al., 2012).

These developments in the context of AIDS have relevance for non-AIDS-associated infectious uveitis in immunocompetent patients. For instance much of the sight-threatening effects of infectious disease (including the full panoply of viral, (myco)bacterial, parasitic, and fungal infections such as trachoma, onchocerca, toxoplasmosis, herpes stromal keratitis in which there can be extensive tissue damage), is caused by a robust or even exaggerated immune response. Thus although the host survives, blindness may be the cost. The corollary is also true, as evidenced by the failure of IP in an aging immune system to protect against the re-awakening of latent infectious disease, much of which was contracted in the neonatal and early childhood years. Interestingly, those rare fatal cases of CNS HSV infection in children appear to be linked to a mutation in TLR3, providing an example from Nature in which defective innate immunity tips the balance in this precarious struggle between immunity and infection (Zhang et al., 2007; Guo et al., 2011).

PRIVILEGE IN THE BALANCE – PARA-INFLAMMATION

It is clear from the above that ocular IP is a finely judged act which can readily tip. This applies to many physiological processes and Medzhitov (2008) described it neatly as para-inflammation when considering the body's response to predictable perturbations such as aging.

DEGENERATIVE RETINAL DISEASE AND THE OCULAR IMMUNE RESPONSE

Removal of dead and dying cells is homeostatic tissue husbandry. Ferguson has championed this process to explain systemic tolerance induced after io injection of antigen, and regards it as a general phenomenon (Ferguson and Griffith, 2007; Griffith et al., 2011). Dead-cell scavenging occurs throughout life, and depends on innate immune mechanisms involving resident macrophage and parenchymal cell activity. AMD is considered a disease in which para-inflammatory mechanisms fail either for genetic (defects in complement genes) or environmental (smoking, diet, metabolic disturbance) or even chronic infective (chlamydia, HSV) reasons (reviewed in Xu et al., 2009). AMD is characterized by the accumulation of waste products (drusen) presumably from retinal photoreceptor cells, both inside (increased lipofuscin accumulation) and below the RPE cell (**Figure 4A**), in part due to impaired complement Factor H binding to sulfated glycosaminoglycans (Clark et al., 2010). Resident F4/80$^+$ macrophages and DCs in the sub-RPE/choroid (**Figure 1**) assist in clearing drusen (Forrester et al., 2010); however, when drusen accumulate, there is deposition of complement components and other acute phase proteins, leading to a low grade pro-inflammatory macrophage response and eventual subretinal neovascularization (Skeie and Mullins, 2009; **Figure 4B**). RPE cells normally produce anti-angiogenic factors (pigment epithelial cell-derived factor, PEDF and TSP), but can switch to produce pro-inflammatory factors such as VEGF, which promote wet AMD (Ablonczy et al., 2009). Prior to this (aberrant) reparative angiogenic response however, chronic age-related degenerative changes in the RPE occur and atrophic (Guo et al., 2011) AMD ensues, possibly as a consequence of a defect in intracellular microRNA regulation via DICER1 (Kaneko et al., 2011). In fact recent evidence suggests that whether AMD develops as the dry or wet form reflects how the "privileged" retina responds to specific mediators. In dry AMD, DICER appears to act via induction of the inflammasome through NLRP3 and secretion of IL-18, leading to RPE cell death, the hallmark of geographic atrophy or dry AMD (Tarallo et al., 2012). In contrast, cells which phagocytose drusen, the characteristic waste product produced by the RPE cell, also secrete increased amounts of IL-18 again through NLRP-mediated activation of the inflammasome, but under these circumstances IL-18 may protect against retinal angiogenic responses, the hallmark of wet AMD and the obverse of geographic atrophy (Doyle et al., 2012). Thus it appears, in this specific instance, that IL-18, which is constitutively produced by the RPE cell (Jiang et al., 2001), acts as a regulator of RPE function and determines whether the RPE cell succumbs to aging stress (para-inflammation) and dies (geographic atrophy) while attempting to prevent an angiogenic response to an increasing age-related pro-inflammatory microenvironment.

The angiogenic response in AMD is similar to other intraocular angiogenic responses such as the neovascular membranes which occur in low grade chronic uveitis, and may be promoted by arginase$^+$ macrophages, as opposed to immunoregulatory (F4/80$^+$) macrophages associated with IP. Thus IP can be breached not only by severe overwhelming inflammatory disease, but by chronic, low grade angiogenesis-associated inflammation as in chronic uveitis and AMD. Moreover, pathological subretinal neovascularization can be reversed by utilizing IP-promoting properties of FasL (Roychoudhury et al., 2010) and it may also be true that IL-18 produced by the RPE is a further IP-associated mediator of a constitutive anti-angiogenic response (Jiang et al., 2001; Doyle et al., 2012).

Thus there is a spectrum of responses from homeostasis and health (intact IP) through aging and chronic disease (disabled IP) to fulminant (infectious) retinal necrosis (absent IP). Much of this activity is considered "auto-inflammatory" involving activation of innate immune receptors on various subsets of macrophages, but an adaptive immune response in AMD, involving lipid or proteolipid antigen and CD1a may also contribute to AMD-like disease (Hollyfield et al., 2008). This has implications for potential stem cell therapies currently being mooted for treatment of inherited retinal disease, and even AMD, since the assumption that IP protects intraocular stem cell inocula from scrutiny by the immune system is clearly not true. As discussed below for glaucoma, the ultimate test of an immunological basis for AMD will depend on whether an immunologically based therapy will modify the disease.

GLAUCOMA AND RETINAL NERVE DAMAGE

Chronic open angle glaucoma is a disease where cellular waste accumulates in the trabecular meshwork (aqueous drainage pathway, **Figure 1**) and may initiate an inflammatory response (Wax, 2011). While the evidence for this is scanty, the outcome of glaucoma is neuroretinal damage which, when mediated by the amino acid glutamate, is accompanied by a prominent inflammatory response. Remarkably, however, the retinal response to glutamate damage appears to be one of enhanced neuroprotection mediated by recruitment of myeloid-derived suppressor cells (MDSCs) (London et al., 2011). Indeed glutamate has been shown to have IP properties at least in the brain (Fallarino et al., 2010; Hansen and Caspi, 2010). There is a need for good experimental models of glaucoma which truly reflect the human disease. A popular current model involves thermal or hypertonic occlusion of episcleral veins, thus preventing aqueous drainage. The associated raised intraocular pressure leads to ganglion cell damage in which activated CD200R$^+$ retinal microglia are implicated (Taylor et al., 2011).

In addition to the need for a suitable model of experimental glaucoma which would reflect the human disease, the complex role of IP-mediating factors is revealed. In a spontaneous model of glaucoma, the absence of sFasL, which is a cleavage product of membrane bound FasL, was shown to be associated with increased retinal ganglion cell death while administration of sFasL to the same mice, protected them from damage (Gregory et al., 2011).

The evidence for involvement of the immune system in glaucoma is tenuous but suggestive. Numerous studies in humans both of systemic factors and of ocular tissue obtained from patients, show several features such as potential serum indicators of disease as well as signs of innate immune activation of glial cells with increased expression of MHC class II and other activation markers (reviewed in Tezel, 2011). However, whether this is genuine evidence for an adaptive immune response in glaucoma,

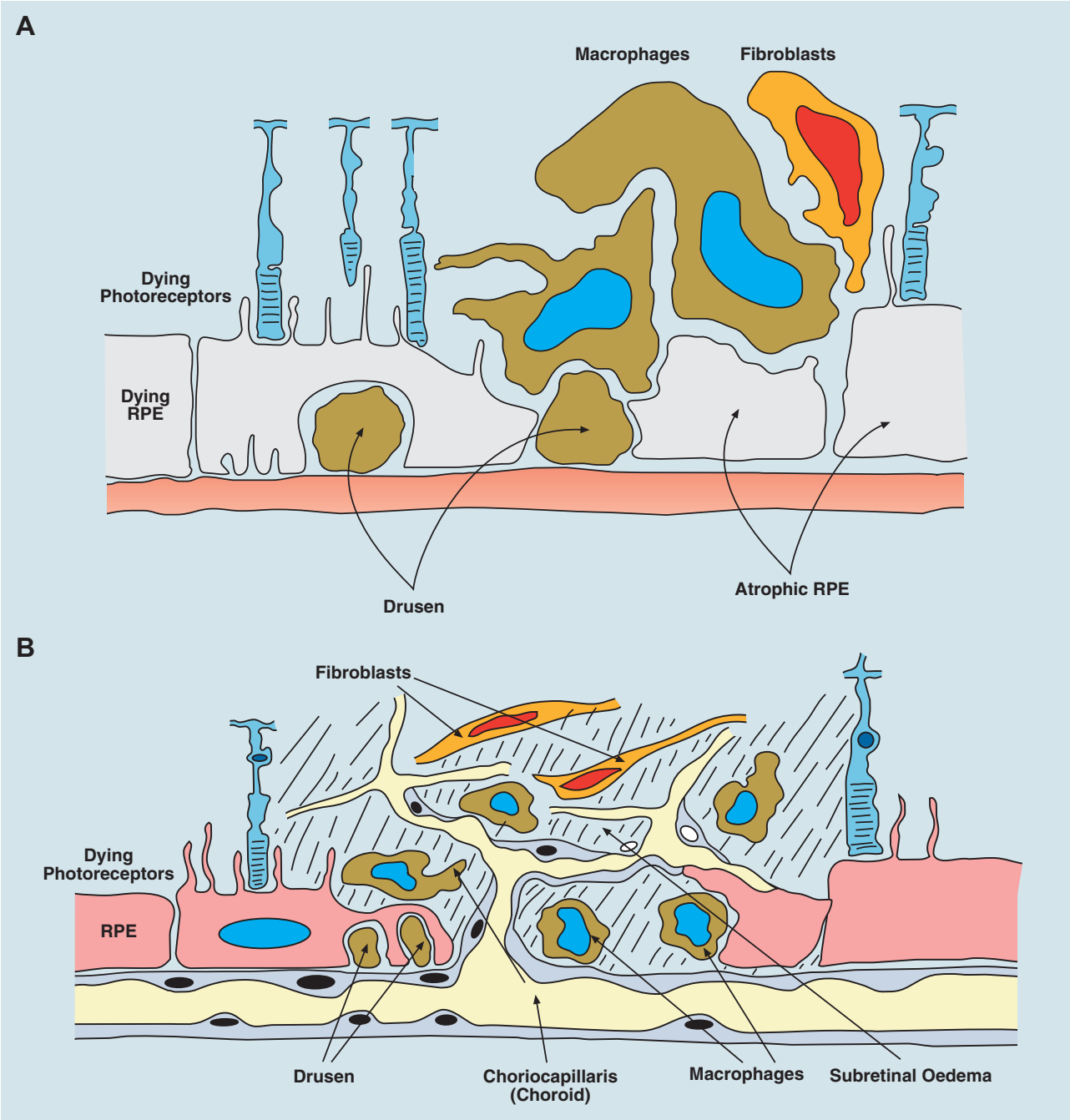

FIGURE 4 | Age-related macular degeneration (AMD) occurs in two forms: dry, atrophic damage to the retinal photoreceptor and RPE cells with chronic progressive loss of visual acuity; and wet AMD in which new vessels originating from the choroidal layer, penetrate the basement membrane of the RPE cells and leak fluid or blood into the subretinal space with rapid loss of vision. (A) Diagram of dry AMD showing drusen under the RPE layer, and extensive atrophic damage to RPE cells. **(B)** Diagram of wet AMD showing in-growth of new blood vessels into the subretinal space, causing extensive destruction of RPE and photoreceptor layers.

or simply an up-regulated innate immune response to damage as occurs in para-inflammation, is a moot point. As for AMD, the test will come when an immunologically based therapy shows an effective protection against the progressive vision-destroying consequences of glaucoma.

DIABETIC RETINOPATHY

Diabetic retinopathy (DR) is the fourth commonest cause of world-wide blindness, potentially increasing as the epidemic of diabetes expands (Suttorp-Schulten and Rothova, 1996). DR is a microvascular endotheliopathy, affecting small capillaries and

post-capillary venules leading to occlusion and expanding areas of retinal ischemia (Grant et al., 2004; Busik et al., 2009; Bhatwadekar et al., 2010). Like aging, diabetes leads to increasing levels of intravascular leukocyte activation and adhesion, contributing to capillary occlusion (MacKinnon et al., 2004). This probably involves platelet–monocyte interactions (Ogata et al., 2006) since there is evidence that $CCR5^{+}CD11b^{+}$ monocytes are the culprit leukocytes (Serra et al., 2012). Trapped monocytes, as well as activated retinal microglial and other retinal cells are sources of VEGF driven by HIF1α (Wang et al., 2010; You et al., 2010), and activate retinal angiogenesis, producing the vision-destroying late disease, proliferative DR (PDR). Thus, drugs such as statins act not only on metabolic lipid pathways (Klein, 2010) but also as inhibitors of leukocyte adhesion to ameliorate or delay disease (Greenwood and Mason, 2007) which has been confirmed in a model of DR (Serra et al., 2012). The ocular pathology is not specific but reflects a general process, also affecting kidneys and peripheral nerves; indeed direct effects on bone marrow-derived hemopoietic stem cell precursors accounts for the poor overall wound healing response in diabetes (Busik et al., 2009). PDR in fact represents a last-ditch but misguided attempt by the retina to repair itself.

An interesting convergence of dysregulated metabolism and activation of immune cells has emerged through the discovery of the succinate receptor (SUCNR1; reviewed in Ariza et al., 2012) which is likely to impact on the pathogenesis of diabetes and its complications. Succinate is a normal metabolite involved in the citric acid cycle and, in times of stress, accumulates in the mitochondria and finds its way to the extracellular space through a series of transporters and porins in the various cell membranes. Extracellular succinate levels above a certain concentration activate SUCNR1 on immature DCs and macrophages and lead to induction of the inflammasome via accumulation of HIF1α even in normoxic conditions (Wen et al., 2012).

The SUCNR1 receptor is expressed in the retina, specifically in the retinal ganglion cells and in the RPE cells and has been identified as having a major role in angiogenesis in the developing retina and also possibly in the retinal ischemia associated with DR (Sapieha et al., 2008). The role of immune cells, particularly inflammatory macrophages in retinal angiogenesis is well-known (Chen et al., 2012) and the possibility that succinate may facilitate, if not drive, theses responses in pathological conditions such as diabetes and perhaps in inflammation generally is an intriguing one.

WHEN PRIVILEGE IS DANGEROUS

Immune privilege allows the eye "to keep a clean house," but may compromise survival of the host, for instance through unchecked growth of tumors or through uncontrolled viral replication in the CNS.

INTRAOCULAR MELANOMA

The commonest primary tumor of the eye is melanoma but tumor growth is slower than in other tissues and the risk of tumor spread is less; ocular melanoma occurs in older patients and late metastases are commoner than in cutaneous melanoma (Kujala et al., 2003; Rigel et al., 2010). The mode of spread in part determines metastatic risk, ocular melanoma spread being predominantly hematogenous, while cutaneous melanoma risk is determined by local invasive depth (Rigel et al., 2010). Unchecked growth of non-ocular tumors correlates with immune suppression, also described as a form of IP (Kandalaft et al., 2010), and ascribed in ocular tumors to tumor-infiltrating Tregs (Mougiakakos et al., 2010). IP-related mechanisms may not only allow active growth of allogeneic tumors grafted intraocularly, but eventually facilitates tumor metastasis, leading to death (Niederkorn, 1997). However, experimental models of intraocular tumors do not precisely mirror the condition in humans, in particular by the important fact that the experiment necessarily requires breakdown of the ocular barrier which is the critical factor determining metastases in humans. Despite or perhaps in the light of this caveat, it is somewhat surprising that intraocular melanomas in humans fail to grow as rapidly as skin melanomas. The behavior of intraocular tumors is related to the density of intratumoral macrophages (reviewed in Jager et al., 2011) and depends on their type and angiogenic properties: ocular melanoma pathogenicity is predicated on its vascularity both clinically and experimentally (Foss et al., 1996; McKenna and Kapp, 2006) while removal of M2-type macrophages almost completely prevents tumor growth (Ly et al., 2010). It is interesting that IP of non-ocular tumors is also attributed to vascularity and to VEGF expression (Kandalaft et al., 2010). In addition, intratumoral macrophages may belong to the myeloid-derived suppressor cells (MDSCs) variety (Natarajan and Thomson, 2010; Bronkhorst et al., 2011; Chioda et al., 2011) and directly suppress anti-tumor T cell responses. These tumor-IP related cells have been identified in the spleen as GM-CSF-dependent, $CD11b^{+}Gr1^{lo/int}$ myeloid cells (Dolcetti et al., 2010; Ugel et al., 2012) and it is interesting to speculate whether they are equivalent to the spleen $F4/80^{+}$ macrophages identified as potential mediators of ocular IP (see above). In contrast, in other ocular tumors, at least experimentally, pro-inflammatory macrophages promote tumor regression (McKenna and Chen, 2010) and are recruited from a population of $CD11b^{+}CD15^{+}$ granulocytic cells in the circulation (McKenna et al., 2009). However, these cells are associated with Treg induction and their precise role is unclear. Interestingly, Tregs in some non-ocular tumors may have a beneficial effect by regulating MDSCs, revealing the complexity of cellular interactions within the tumor microenvironment (Zhang et al., 2010). The "privileged" tumor microenvironment may actually co-operate with ocular IP. However, while a synergistic "immunoregulatory" double-dose of IP may be operative, tumor IP and ocular IP may alternatively cancel each other out, allowing either an uncontrolled profound pro-inflammatory immune response, with spontaneous regression of the tumor and severe collateral intraocular inflammation, or a super-suppressed, profoundly anergic anti-tumor response with metastases and rapid extraocular spread (i.e., a failure of response). The former is less likely than the latter but is well-documented clinically and experimentally (Knisely and Niederkorn, 1990; Shields et al., 1999). In addition, several mechanisms exist for tumor rejection or evasion by the immune system. In one series of experiments, rejection of tumors in the mouse anterior chamber of the eye was dependent on $CD4^{+}$ T cells and TRAIL, the same molecule which is considered to underpin ocular IP-related

systemic immune tolerance (see above; Wang et al., 2003). A further regulatory element in IP-mediated growth of intraocular tumors is *via* the sympathetic nervous system which in one experiment was seen to be closely linked to intraocular production of TGFβ (Vega et al., 2009).

Not all intraocular tumors behave similarly even when derived from similar cells: many follow the pattern of growth underpinned by privileged immunity while others are rejected. Recently it has been shown that a specific tumor in mice, Ad5E1 can follow two patterns of rejection one of which involves severe rejection and tumor necrosis mediated by IFNγ with severe destructive bystander damage to the eye which is dependent on TNFα, while the second pattern also leads to tumor rejection but there is minimal ocular damage. In the latter case, tumor killing was mediated by an arginase^{+} population of macrophages (Coursey et al., 2012). Thus direct killing via CTLs or by different subsets of macrophages appears to determine the outcome both for the tumor and for the eye (for review, see McKenna and Chen, 2010). Similar cellular diversity has been found in intraocular melanomas in humans (Bronkhorst et al., 2012).

It can be seen from this discussion that the immune response to ocular tumors hangs in the balance, as does the survival of the host. This precarious condition is dictated by the strength of the ocular IP effect vs the desirable but impaired, anti-tumor response. For tumors arising in the eye such as melanoma, the combined effect of these "privileged" responses is beneficial both to the eye and the host provided the tumor is contained within the eye, but once it breaks free the desired systemic immune response is inadequate to control the tumor with fatal consequences in many cases.

PRIMARY INTRAOCULAR LYMPHOMA

The eye contains few B and T cells outside the vasculature, occasional cells passing through the fenestrated walls of the uveal vessels (**Figure 1**). Primary intraocular lymphoma is grouped with CNS lymphoma, a rare extra-nodal variant of non-Hodgkin's lymphoma (Algazi et al., 2009) arising from post-germinal center B cells (Coupland et al., 2009) and occurs by seeding of privileged sites by hemopoietic progenitor cells after variable lineage differentiation. The precise location of the lymphoma predicts its behavior with retinal lymphomas being aggressive and choroidal lymphomas being more "indolent," while iris and ciliary body lymphomas are very rare (Coupland and Damato, 2008; Mashayekhi et al., 2012). This behavior may reflect the relative IP status of the tissue, retina being likely to posses greater privilege than choroid, perhaps determined by specific subsets of resident myeloid cells (see Cells and Tissues).

Primary intraocular tumors may represent one aspect of "an experiment of Nature." The paradoxically beneficial effect of Treg cells (see above) in some tumors of the head and neck is clearly demonstrated by the development of tertiary lymphoid structures (TLS) surrounding the tumor. TLS express intense immunoregulatory activity directed toward reducing chronic inflammation, a recognized poor prognostic sign, and have been likened to sites of induced IP. Constitutive IP in the eye may behave like a "TLS" to protect the host in cases of intraocular lymphomas but is a dangerous strategy (Fridman et al., 2010).

However, due to the rarity of primary intraocular lymphoma, their biology is poorly understood and animal models have not been very informative so far, thus the call for multicenter studies to investigate these tumors is timely (Chan et al., 2011).

CANCER-ASSOCIATED RETINOPATHY AND THE PARA-NEOPLASTIC SYNDROME

Another experiment of Nature is revealed by cancer-associated retinopathy (CAR). Retinal antigens aberrantly expressed in extra-ocular tumors induce serum antibodies and T cell responses, invoking a para-neoplastic syndrome of progressive retinal degeneration and eventual blindness (Bazhin et al., 2007). Early studies revealed that antigens such as the photoreceptor visual cycle regulatory protein, recoverin, were responsible and many further retinal antigens including the potent autoantigen, IRBP (see above) have been implicated (Bianciotto et al., 2010). These observations spawned more intense investigations into (auto)immune-mediated causes of common retinal degenerations such as AMD, and prompted much of the immunological studies which came in the wake of knowledge concerning the role of innate immune genes such as complement. Interestingly, an alternative theory involving secretion of VEGF by the tumor and expression of VEGFR1 by retinal neural and vascular cells has been proposed to explain CAR syndrome which involves specifically loss of pericytes and increased retinal vascular leakage (Cao and Cao, 2010).

Cancer-associated retinopathy also provides insight into the nature of ocular IP and supports the notion of immunological ignorance (antigen sequestration) as one form of IP. However, the discussion above clearly shows that "all roads lead to Rome" and that ocular IP, just like immunological tolerance generally, is subject to many checkpoints. It is not intrinsically different from other forms of immune tolerance (Matzinger and Kamala, 2011).

CONCLUSION

Immune regulation/tolerance induction occurs primarily *via* central and peripheral mechanisms. However, there is an evolving understanding that the target tissue and its microenvironment can also modify the immune response (Matzinger and Kamala, 2011) and how this develops depends on the nature and properties of the tissue. The eye provides an excellent paradigm for the concept of tissue regulation of immune responses and how the same "danger signals" might not be recognized in the eye as they are, for instance, in the lung or skin. Thus, the violent immune response to toxoplasma in the gut is much more subdued in the eye, giving the parasite time to sequester itself from the patrolling killer immune cells. However, the process is not failsafe and when it fails, it fails gloriously, with irreparable damage to ocular structures and loss of sight (e.g., HSV-induced acute retinal necrosis).

The ocular immune response encapsulates the full range of classical and non-classical immune responses and demonstrates many features which are reflected in other tissues, but eye tissues by modifying these responses reveal unexpected and novel features, which are relevant to immune responses generally. In addition,

IP involves many recognized immunoregulatory processes, including induction of Tregs, and is inducible and transferable. This has therapeutic potential, particularly for devising ways to restore tolerance in ocular inflammatory disease, and for preventing rejection of cells and tissues, such as stem cells, currently being considered for treatment of world-wide blinding diseases such as AMD.

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REFERENCES

Ablonczy, Z., Prakasam, A., Fant, J., Fauq, A., Crosson, C., and Sambamurti, K. (2009). Pigment epithelium-derived factor maintains retinal pigment epithelium function by inhibiting vascular endothelial growth factor-R2 signaling through gamma-secretase. *J. Biol. Chem.* 284, 30177–30186.

Algazi, A. P., Kadoch, C., and Rubenstein, J. L. (2009). Biology and treatment of primary central nervous system lymphoma. *Neurotherapeutics* 6, 587–597.

Amadi-Obi, A., Yu, C. R., Liu, X., Mahdi, R. M., Clarke, G. L., Nussenblatt, R. B., et al. (2007). TH17 cells contribute to uveitis and scleritis and are expanded by IL-2 and inhibited by IL-27/STAT1. *Nat. Med.* 13, 711–718.

Anderson, M. S., Venanzi, E. S., Klein, L., Chen, Z., Berzins, S. P., Turley, S. J., et al. (2002). Projection of an immunological self shadow within the thymus by the aire protein. *Science* 298, 1395–1401.

Anosova, N. G., Illigens, B., Boisgérault, F., Fedoseyeva, E. V., Young, M. J., and Benichou, G. (2001). Antigenicity and immunogenicity of allogeneic retinal transplants. *J. Clin. Invest.* 108, 1175–1183.

Ariza, A. C., Deen, P. M., and Robben, J. H. (2012). The succinate receptor as a novel therapeutic target for oxidative and metabolic stress-related conditions. *Front. Endocrinol. (Lausanne)* 3, 22.

Aschenbrenner, K., D'Cruz, L. M., Vollmann, E. H., Hinterberger, M., Emmerich, J., Swee, L. K., et al. (2007). Selection of Foxp3+ regulatory T cells specific for self antigen expressed and presented by Aire+ medullary thymic epithelial cells. *Nat. Immunol.* 8, 351–358.

Atan, D., Fraser-Bell, S., Plskova, J., Kuffová, L., Hogan, A., Tufail, A., et al. (2011). Punctate inner choroidopathy and multifocal choroiditis with panuveitis share haplotypic associations with IL10 and TNF loci. *Invest. Ophthalmol. Vis. Sci.* 52, 3573–3581.

Bazhin, A. V., Schadendorf, D., Philippov, P. P., and Eichmuller, S. B. (2007). Recoverin as a cancer-retina antigen. *Cancer Immunol. Immunother.* 56, 110–116.

Benoist, C., and Mathis, D. (2001). Autoimmunity provoked by infection: how good is the case for T cell epitope mimicry? *Nat. Immunol.* 2, 797–801.

Bhatwadekar, A. D., Guerin, E. P., Jarajapu, Y. P., Caballero, S., Sheridan, C., Kent, D., et al. (2010). Transient inhibition of transforming growth factor-beta1 in human diabetic CD34+ cells enhances vascular reparative functions. *Diabetes* 59, 2010–2019.

Bianciotto, C., Shields, C. L., Thirkill, C. E., Materin, M. A., and Shields, J. A. (2010). Paraneoplastic retinopathy with multiple detachments of the neurosensory retina and autoantibodies against interphotoreceptor retinoid binding protein (IRBP) in cutaneous melanoma. *Br. J. Ophthalmol.* 94, 1684–1685, 1696.

Boisgerault, F., Liu, Y., Anosova, N., Dana, R., and Benichou, G. (2009). Differential roles of direct and indirect allorecognition pathways in the rejection of skin and corneal transplants. *Transplantation* 87, 16–23.

Brissette-Storkus, C. S., Reynolds, S. M., Lepisto, A. J., and Hendricks, R. L. (2002). Identification of a novel macrophage population in the normal mouse corneal stroma. *Invest. Ophthalmol. Vis. Sci.* 43, 2264–2271.

Bronkhorst, I. H., Ly, L. V., Jordanova, E. S., Vrolijk, J., Versluis, M., Luyten, G. P., et al. (2011). Detection of M2-macrophages in uveal melanoma and relation with survival. *Invest. Ophthalmol. Vis. Sci.* 52, 643–650.

Bronkhorst, I. H., Vu, T. H., Jordanova, E. S., Luyten, G. P., Burg, S. H., and Jager, M. J. (2012). Different subsets of tumor-infiltrating lymphocytes correlate with macrophage influx and monosomy 3 in uveal melanoma. *Invest. Ophthalmol. Vis. Sci.* 53, 5370–5378.

Busik, J. V., Tikhonenko, M., Bhatwadekar, A., Opreanu, M., Yakubova, N., Caballero, S., et al. (2009). Diabetic retinopathy is associated with bone marrow neuropathy and a depressed peripheral clock. *J. Exp. Med.* 206, 2897–2906.

Camelo, S., Kezic, J., Shanley, A., Rigby, P., and McMenamin, P. G. (2006). Antigen from the anterior chamber of the eye travels in a soluble form to secondary lymphoid organs via lymphatic and vascular routes. *Invest. Ophthalmol. Vis. Sci.* 47, 1039–1046.

Camelo, S., Shanley, A., Voon, A. S., and McMenamin, P. G. (2004). The distribution of antigen in lymphoid tissues following its injection into the anterior chamber of the rat eye. *J. Immunol.* 172, 5388–5395.

Cao, R., and Cao, Y. (2010). Cancer-associated retinopathy: a new mechanistic insight on vascular remodeling. *Cell Cycle* 9, 1882–1885.

Caspi, R. R. (2006). Ocular autoimmunity: the price of privilege? *Immunol. Rev.* 213, 23–35.

Caspi, R. R. (2010). A look at autoimmunity and inflammation in the eye. *J. Clin. Invest.* 120, 3073–3083.

Caspi, R. R., Roberge, F. G., and Nussenblatt, R. B. (1987). Organ-resident, nonlymphoid cells suppress proliferation of autoimmune T-helper lymphocytes. *Science* 237, 1029–1032.

Catron, D. M., Itano, A. A., Pape, K. A., Mueller, D. L., and Jenkins, M. K. (2004). Visualizing the first 50 hr of the primary immune response to a soluble antigen. *Immunity* 21, 341–347.

Cervantes-Barragan, L., Firner, S., Bechmann, I., Waisman, A., Lahl, K., Sparwasser, T., et al. (2012). Regulatory T cells selectively preserve immune privilege of self-antigens during viral central nervous system infection. *J. Immunol.* 188, 3678–3685.

Chan, C. C., Rubenstein, J. L., Coupland, S. E., Davis, J. L., Harbour, J. W., Johnston, P. B., et al. (2011). Primary vitreoretinal lymphoma: a report from an International Primary Central Nervous System Lymphoma Collaborative Group symposium. *Oncologist* 16, 1589–1599.

Chen, M., Copland, D. A., Zhao, J., Liu, J., Forrester, J. V., Dick, A. D., et al. (2012). Persistent inflammation subverts thrombospondin-1-induced regulation of retinal angiogenesis and is driven by CCR2 ligation. *Am. J. Pathol.* 180, 235–245.

Chioda, M., Peranzoni, E., Desantis, G., Papalini, F., Falisi, E., Solito, S., et al. (2011). Myeloid cell diversification and complexity: an old concept with new turns in oncology. *Cancer Metastasis Rev.* 30, 27–43.

Clark, S. J., Bishop, P. N., and Day, A. J. (2010). Complement factor H and age-related macular degeneration: the role of glycosaminoglycan recognition in disease pathology. *Biochem. Soc. Trans.* 38, 1342–1348.

Cobbold, S. P. (2009). Future therapeutics for the induction of peripheral immune tolerance in autoimmune disease and organ transplantation. *Immunotherapy* 1, 447–460.

Cong, Y., Wang, L., Konrad, A., Schoeb, T., and Elson, C. O. (2009). Curcumin induces the tolerogenic dendritic cell that promotes differentiation of intestine-protective regulatory T cells. *Eur. J. Immunol.* 39, 3134–3146.

Coupland, S. E., Chan, C. C., and Smith, J. (2009). Pathophysiology of retinal lymphoma. *Ocul. Immunol. Inflamm.* 17, 227–237.

Coupland, S. E., and Damato, B. (2008). Understanding intraocular lymphomas. *Clin. Exp. Ophthalmol.* 36, 564–578.

Coursey, T. G., Chen, P. W., and Niederkorn, J. Y. (2012). IFN-gamma-independent intraocular tumor rejection is mediated by a macrophage-dependent process that leaves the eye intact. *J. Leukoc. Biol.* 92, 939–950.

Crane, I. J., Kuppner, M. C., McKillop-Smith, S., Wallace, C. A., and Forrester, J. V. (1999). Cytokine regulation of granulocyte-macrophage colony-stimulating factor (GM-CSF) production by human retinal pigment epithelial cells. *Clin. Exp. Immunol.* 115, 288–293.

Crane, I. J., Xu, H., Wallace, C., Manivannan, A., Mack, M., Liversidge, J., et al. (2006). Involvement of CCR5 in the passage of Th1-type cells across the blood–retina barrier in experimental autoimmune uveitis. *J. Leukoc. Biol.* 79, 435–443.

Cunnusamy, K., Chen, P. W., and Niederkorn, J. Y. (2010). Paradigm shifts in the role of CD4+ T cells in keratoplasty. *Discov. Med.* 10, 452–461.

Darrell, R. W., Wagener, H. P., and Kurland, L. T. (1962). Epidemiology of uveitis. Incidence and prevalence in a small urban community. *Arch. Ophthalmol.* 68, 502–514.

DeVoss, J., Hou, Y., Johannes, K., Lu, W., Liou, G. I., Rinn, J., et al. (2006). Spontaneous autoimmunity prevented by thymic expression of a single self-antigen. *J. Exp. Med.* 203, 2727–2735.

Dick, A. D., Siepmann, K., Dees, C., Duncan, L., Broderick, C., Liversidge, J., et al. (1999). Fas-Fas ligand-mediated apoptosis within aqueous during idiopathic acute anterior uveitis. *Invest. Ophthalmol. Vis. Sci.* 40, 2258–2267.

Do, M. T., and Yau, K. W. (2010). Intrinsically photosensitive retinal ganglion cells. *Physiol. Rev.* 90, 1547–1581.

Dolcetti, L., Peranzoni, E., Ugel, S., Marigo, I., Fernandez Gomez, A., Mesa, C., et al. (2010). Hierarchy of immunosuppressive strength among myeloid-derived suppressor cell subsets is determined by GM-CSF. *Eur. J. Immunol.* 40, 22–35.

Doyle, S. L., Campbell, M., Ozaki, E., Salomon, R. G., Mori, A., Kenna, P. F., et al. (2012). NLRP3 has a protective role in age-related macular degeneration through the induction of IL-18 by drusen components. *Nat. Med.* 18, 791–798.

Dullforce, P. A., Garman, K. L., Seitz, G. W., Fleischmann, R. J., Crespo, S. M., Planck, S. R., et al. (2004). APCs in the anterior uveal tract do not migrate to draining lymph nodes. *J. Immunol.* 172, 6701–6708.

Ecoiffier, T., Yuen, D., and Chen, L. (2010). Differential distribution of blood and lymphatic vessels in the murine cornea. *Invest. Ophthalmol. Vis. Sci.* 51, 2436–2440.

Esposito, A., Suedekum, B., Liu, J., An, F., Lass, J., Strainic, M. G., et al. (2010). Decay accelerating factor is essential for successful corneal engraftment. *Am. J. Transplant.* 10, 527–534.

Fallarino, F., Volpi, C., Fazio, F., Notartomaso, S., Vacca, C., Busceti, C., et al. (2010). Metabotropic glutamate receptor-4 modulates adaptive immunity and restrains neuroinflammation. *Nat. Med.* 16, 897–902.

Faunce, D. E., and Stein-Streilein, J. (2002). NKT cell-derived RANTES recruits APCs and CD8+ T cells to the spleen during the generation of regulatory T cells in tolerance. *J. Immunol.* 169, 31–38.

Faure, J. P. (1980). Autoimmunity and the retina. *Curr. Top. Eye Res.* 2, 215–302.

Ferguson, T. A., and Griffith, T. S. (2007). The role of Fas ligand and TNF-related apoptosis-inducing ligand (TRAIL) in the ocular immune response. *Chem. Immunol. Allergy* 92, 140–154.

Forrester, J. V., Dick, A. D., McMenamin, P. G., and Roberts, F. (2008a). *The Eye: Basic Science in Practice.* Edinburgh: Saunders Elsevier.

Forrester, J. V., Xu, H., Lambe, T., and Cornall, R. (2008b). Immune privilege or privileged immunity? *Mucosal Immunol.* 1, 372–381.

Forrester, J. V., Huitinga, I., Lumsden, L., and Dijkstra, C. D. (1998). Marrow-derived activated macrophages are required during the effector phase of experimental autoimmune uveoretinitis in rats. *Curr. Eye Res.* 17, 426–437.

Forrester, J. V., Lee, W. R., Kerr, P. R., and Dua, H. S. (1990). The uveal effusion syndrome and trans-scleral flow. *Eye (Lond.)* 4(Pt 2), 354–365.

Forrester, J. V., Xu, H., Kuffova, L., Dick, A. D., and McMenamin, P. G. (2010). Dendritic cell physiology and function in the eye. *Immunol. Rev.* 234, 282–304.

Foss, A. J., Alexander, R. A., Jefferies, L. W., Hungerford, J. L., Harris, A. L., and Lightman, S. (1996). Microvessel count predicts survival in uveal melanoma. *Cancer Res.* 56, 2900–2903.

Francisco, L. M., Sage, P. T., and Sharpe, A. H. (2010). The PD-1 pathway in tolerance and autoimmunity. *Immunol. Rev.* 236, 219–242.

Frank, G. M., Buela, K. A., Maker, D. M., Harvey, S. A., and Hendricks, R. L. (2012). Early responding dendritic cells direct the local NK response to control herpes simplex virus 1 infection within the cornea. *J. Immunol.* 188, 1350–1359.

Fridman, W. H., Galon, J., Dieu-Nosjean, M. C., Cremer, I., Fisson, S., Damotte, D., et al. (2010). Immune infiltration in human cancer: prognostic significance and disease control. *Curr. Top. Microbiol. Immunol.* 344, 1–24.

Fujisaki, J., Wu, J., Carlson, A. L., Silberstein, L., Putheti, P., Larocca, R., et al. (2011). In vivo imaging of Treg cells providing immune privilege to the haematopoietic stem-cell niche. *Nature* 474, 216–219.

Gabrilovich, D. I., and Nagaraj, S. (2009). Myeloid-derived suppressor cells as regulators of the immune system. *Nat. Rev. Immunol.* 9, 162–174.

Geissmann, F., Revy, P., Brousse, N., Lepelletier, Y., Folli, C., Durandy, A., et al. (2003). Retinoids regulate survival and antigen presentation by immature dendritic cells. *J. Exp. Med.* 198, 623–634.

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., et al. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330, 841–845.

Girardi, M., and Hayday, A. C. (2005). Immunosurveillance by gammadelta T cells: focus on the murine system. *Chem. Immunol. Allergy* 86, 136–150.

Grant, M. B., Afzal, A., Spoerri, P., Pan, H., Shaw, L. C., and Mames, R. N. (2004). The role of growth factors in the pathogenesis of diabetic retinopathy. *Expert Opin. Investig. Drugs* 13, 1275–1293.

Green, D. R., and Ferguson, T. A. (2001). The role of Fas ligand in immune privilege. *Nat. Rev. Mol. Cell Biol.* 2, 917–924.

Greenwood, J., and Mason, J. C. (2007). Statins and the vascular endothelial inflammatory response. *Trends Immunol.* 28, 88–98.

Gregerson, D. S., Heuss, N. D., Lehmann, U., and McPherson, S. W. (2009). Peripheral induction of tolerance by retinal antigen expression. *J. Immunol.* 183, 814–822.

Gregory, M. S., Hackett, C. G., Abernathy, E. F., Lee, K. S., Saff, R. R., Hohlbaum, A. M., et al. (2011). Opposing roles for membrane bound and soluble Fas ligand in glaucoma-associated retinal ganglion cell death. *PLoS ONE* 6, e17659. doi: 10.1371/journal.pone.0017659

Griffith, T. S., Brincks, E. L., Gurung, P., Kucaba, T. A., and Ferguson, T. A. (2011). Systemic immunological tolerance to ocular antigens is mediated by TRAIL-expressing CD8+ T cells. *J. Immunol.* 186, 791–798.

Griffith, T. S., Herndon, J. M., Lima, J., Kahn, M., and Ferguson, T. A. (1995). The immune response and the eye. TCR alpha-chain related molecules regulate the systemic immunity to antigen presented in the eye. *Int. Immunol.* 7, 1617–1625.

Gritz, D. C., and Wong, I. G. (2004). Incidence and prevalence of uveitis in Northern California; the Northern California Epidemiology of Uveitis Study. *Ophthalmology* 111, 491–500; discussion 500.

Guo, Y., Audry, M., Ciancanelli, M., Alsina, L., Azevedo, J., Herman, M., et al. (2011). Herpes simplex virus encephalitis in a patient with complete TLR3 deficiency: TLR3 is otherwise redundant in protective immunity. *J. Exp. Med.* 208, 2083–2098.

Gust, J., and Reh, T. A. (2011). Adult donor rod photoreceptors integrate into the mature mouse retina. *Invest. Ophthalmol. Vis. Sci.* 52, 5266–5272.

Hamrah, P., and Dana, M. R. (2007). Corneal antigen-presenting cells. *Chem. Immunol. Allergy* 92, 58–70.

Hansen, A. M., and Caspi, R. R. (2010). Glutamate joins the ranks of immunomodulators. *Nat. Med.* 16, 856–858.

Hara, Y., Caspi, R. R., Wiggert, B., Dorf, M., and Streilein, J. W. (1992). Analysis of an in vitro-generated signal that induces systemic immune deviation similar to that elicited by antigen injected into the anterior chamber of the eye. *J. Immunol.* 149, 1531–1538.

Hattori, T., Saban, D. R., Emami-Naeini, P., Chauhan, S. K., Funaki, T., Ueno, H., et al. (2012). Donor-derived, tolerogenic dendritic cells suppress immune rejection in the indirect allosensitization-dominant setting of corneal transplantation. *J. Leukoc. Biol.* 91, 621–627.

Heuss, N. D., Lehmann, U., Norbury, C. C., McPherson, S. W., and Gregerson, D. S. (2012). Local activation of dendritic cells alters the pathogenesis of autoimmune disease in the retina. *J. Immunol.* 188, 1191–1200.

Hill, A. J., Zwart, I., Tam, H. H., Chan, J., Navarrete, C., Jen, L. S., et al. (2009). Human umbilical cord blood-derived mesenchymal stem cells do not differentiate into neural cell types or integrate into the retina after intravitreal grafting in neonatal rats. *Stem Cells Dev.* 18, 399–409.

Hollyfield, J. G., Bonilha, V. L., Rayborn, M. E., Yang, X., Shadrach, K. G., Lu, L., et al. (2008). Oxidative damage-induced inflammation initiates age-related macular degeneration. *Nat. Med.* 14, 194–198.

Huang, L., Baban, B., Johnson, B. A. III, and Mellor, A. L. (2010). Dendritic cells, indoleamine 2,3 dioxygenase and acquired immune privilege. *Int. Rev. Immunol.* 29, 133–155.

Itano, A. A., McSorley, S. J., Reinhardt, R. L., Ehst, B. D., Ingulli, E., Rudensky, A. Y., et al. (2003). Distinct dendritic cell populations sequentially present antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity. *Immunity* 19, 47–57.

Jabs, D. A. (2011). Cytomegalovirus retinitis and the acquired immunodeficiency syndrome – bench to bedside: LXVII Edward Jackson Memorial Lecture. *Am. J. Ophthalmol.* 151, 198–216.e1.

Jabs, D. A., Nussenblatt, R. B., and Rosenbaum, J. T. (2005). Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. *Am. J. Ophthalmol.* 140, 509–516.

Jager, M. J., Ly, L. V., El Filali, M., and Madigan, M. C. (2011). Macrophages in uveal melanoma and

in experimental ocular tumor models: friends or foes? *Prog. Retin. Eye Res.* 30, 129–146.

Jeon, E. J., Yoon, B. Y., Lim, J. Y., Oh, H. J., Park, H. S., Park, M. J., et al. (2012). Adoptive transfer of all-trans-retinal-induced regulatory T cells ameliorates experimental autoimmune arthritis in an interferon-gamma knockout model. *Autoimmunity* 45, 460–469.

Ji, Q., Perchellet, A., and Goverman, J. M. (2010). Viral infection triggers central nervous system autoimmunity via activation of CD8+ T cells expressing dual TCRs. *Nat. Immunol.* 11, 628–634.

Jiang, H. R., Wei, X., Niedbala, W., Lumsden, L., Liew, F. Y., and Forrester, J. V. (2001). IL-18 not required for IRBP peptide-induced EAU: studies in gene-deficient mice. *Invest. Ophthalmol. Vis. Sci.* 42, 177–182.

Jones, L. A., Alexander, J., and Roberts, C. W. (2006). Ocular toxoplasmosis: in the storm of the eye. *Parasite Immunol.* 28, 635–642.

Kandalaft, L. E., Motz, G. T., Busch, J., and Coukos, G. (2010). Angiogenesis and the tumor vasculature as antitumor immune modulators: the role of vascular endothelial growth factor and endothelin. *Curr. Top. Microbiol. Immunol.* 344, 129–148.

Kaneko, H., Dridi, S., Tarallo, V., Gelfand, B. D., Fowler, B. J., Cho, W. G., et al. (2011). DICER1 deficit induces Alu RNA toxicity in age-related macular degeneration. *Nature* 471, 325–330.

Kawazoe, Y., Sugita, S., Keino, H., Yamada, Y., Imai, A., Horie, S., et al. (2012). Retinoic acid from retinal pigment epithelium induces T regulatory cells. *Exp. Eye Res.* 94, 32–40.

Ke, Y., Sun, D., Jiang, G., Kaplan, H. J., and Shao, H. (2010). PD-L1(hi) retinal pigment epithelium (RPE) cells elicited by inflammatory cytokines induce regulatory activity in uveitogenic T cells. *J. Leukoc. Biol.* 88, 1241–1249.

Khera, T. K., Dick, A. D., and Nicholson, L. B. (2010). Mechanisms of TNFα regulation in uveitis: focus on RNA-binding proteins. *Prog. Retin. Eye Res.* 29, 610–621.

Kim, H. T., and Kim, J. W. (2012). Compartmentalization of vertebrate optic neuroephithelium: external cues and transcription factors. *Mol. Cells* 33, 317–324.

Kinchington, P. R., St Leger, A. J., Guedon, J. M., and Hendricks, R. L. (2012). Herpes simplex virus and varicella zoster virus, the house guests who never leave. *Herpesviridae* 3, 5.

Klein, B. E. (2010). Reduction in risk of progression of diabetic retinopathy. *N. Engl. J. Med.* 363, 287–288.

Knisely, T. L., and Niederkorn, J. Y. (1990). Immunologic evaluation of spontaneous regression of an intraocular murine melanoma. *Invest. Ophthalmol. Vis. Sci.* 31, 247–257.

Kuffová, L., Netuková, M., Duncan, L., Porter, A., Stockinger, B., and Forrester, J. V. (2008). Cross presentation of antigen on MHC class II via the draining lymph node after corneal transplantation in mice. *J. Immunol.* 180, 1353–1361.

Kujala, E., Makitie, T., and Kivela, T. (2003). Very long-term prognosis of patients with malignant uveal melanoma. *Invest. Ophthalmol. Vis. Sci.* 44, 4651–4659.

Kumar, H., Kawai, T., and Akira, S. (2011). Pathogen recognition by the innate immune system. *Int. Rev. Immunol.* 30, 16–34.

Kurotaki, D., Kon, S., Bae, K., Ito, K., Matsui, Y., Nakayama, Y., et al. (2011). CSF-1-dependent red pulp macrophages regulate CD4 T cell responses. *J. Immunol.* 186, 2229–2237.

Kushwah, R., and Hu, J. (2011). Role of dendritic cells in the induction of regulatory T cells. *Cell Biosci.* 1, 20–30.

Lambe, T., Leung, J. C., Ferry, H., Bouriez-Jones, T., Makinen, K., Crockford, T. L., et al. (2007). Limited peripheral T cell anergy predisposes to retinal autoimmunity. *J. Immunol.* 178, 4276–4283.

Lepisto, A. J., Frank, G. M., and Hendricks, R. L. (2007). How herpes simplex virus type 1 rescinds corneal privilege. *Chem. Immunol. Allergy* 92, 203–212.

Levinson, R. D. (2007). Immunogenetics of ocular inflammatory disease. *Tissue Antigens* 69, 105–112.

Lin, H. H., Faunce, D. E., Stacey, M., Terajewicz, A., Nakamura, T., Zhang-Hoover, J., et al. (2005). The macrophage F4/80 receptor is required for the induction of antigen-specific efferent regulatory T cells in peripheral tolerance. *J. Exp. Med.* 201, 1615–1625.

Liu, L., Kuffová, L., Griffith, M., Dang, Z., Muckersie, E., Liu, Y., et al. (2007). Immunological responses in mice to full-thickness corneal grafts engineered from porcine collagen. *Biomaterials* 28, 3807–3814.

Liversidge, J., Grabowski, P., Ralston, S., Benjamin, N., and Forrester, J. V. (1994). Rat retinal pigment epithelial cells express an inducible form of nitric oxide synthase and produce nitric oxide in response to inflammatory cytokines and activated T cells. *Immunology* 83, 404–409.

Liversidge, J. M., Sewell, H. F., and Forrester, J. V. (1988). Human retinal pigment epithelial cells differentially express MHC class II (HLA, DP, DR and DQ) antigens in response to in vitro stimulation with lymphokine or purified IFN-gamma. *Clin. Exp. Immunol.* 73, 489–494.

London, A., Itskovich, E., Benhar, I., Kalchenko, V., Mack, M., Jung, S., et al. (2011). Neuroprotection and progenitor cell renewal in the injured adult murine retina requires healing monocyte-derived macrophages. *J. Exp. Med.* 208, 23–39.

Ly, L. V., Baghat, A., Versluis, M., Jordanova, E. S., Luyten, G. P., van Rooijen, N., et al. (2010). In aged mice, outgrowth of intraocular melanoma depends on proangiogenic M2-type macrophages. *J. Immunol.* 185, 3481–3488.

Mackay, I. R. (2010). Travels and travails of autoimmunity: a historical journey from discovery to rediscovery. *Autoimmun. Rev.* 9, A251–A258.

Mackenzie, W. (1830). *A Practical Treatise on the Diseases of the Eye.* London: Longman, Rees, Orme, Brown & Green.

MacKinnon, J. R., Knott, R. M., and Forrester, J. V. (2004). Altered L-selectin expression in lymphocytes and increased adhesion to endothelium in patients with diabetic retinopathy. *Br. J. Ophthalmol.* 88, 1137–1141.

MacLaren, R. E., Pearson, R. A., MacNeil, A., Douglas, R. H., Salt, T. E., Akimoto, M., et al. (2006). Retinal repair by transplantation of photoreceptor precursors. *Nature* 444, 203–207.

Maeda, A., Maeda, T., Golczak, M., Chou, S., Desai, A., Hoppel, C. L., et al. (2009). Involvement of all-trans-retinal in acute light-induced retinopathy of mice. *J. Biol. Chem.* 284, 15173–15183.

Makinen, K. (2006). *Spontaneous Model of Experimental Autoimmune Uveoretinitis: IRBP-HEL Transgenic Mice.* PhD thesis, University of Aberdeen, Aberdeen.

Mashayekhi, A., Shields, C. L., and Shields, J. A. (2012). Iris involvement by lymphoma: a review of 13 cases. *Clin. Exp. Ophthalmol.* doi: 10.1111/j.1442-9071.2012.02811.x [Epub ahead of print].

Matzinger, P. (2001). Essay 1: the Danger model in its historical context. *Scand. J. Immunol.* 54, 4–9.

Matzinger, P. (2007). Friendly and dangerous signals: is the tissue in control? *Nat. Immunol.* 8, 11–13.

Matzinger, P., and Kamala, T. (2011). Tissue-based class control: the other side of tolerance. *Nat. Rev. Immunol.* 11, 221–230.

Maynard, C. L., Hatton , R. D., Helms, W. S., Oliver, J. R., Stephensen, C. B., and Weaver, C. T. (2009). Contrasting roles for all-trans retinoic acid in TGF-beta-mediated induction of Foxp3 and Il10 genes in developing regulatory T cells. *J. Exp. Med.* 206, 343–357.

McKenna, K. C., Beatty, K. M., Bilonick, R. A., Schoenfield, L., Lathrop, K. L., and Singh, A. D. (2009). Activated CD11b+ CD15+ granulocytes increase in the blood of patients with uveal melanoma. *Invest. Ophthalmol. Vis. Sci.* 50, 4295–4303.

McKenna, K. C., and Chen, P. W. (2010). Influence of immune privilege on ocular tumor development. *Ocul. Immunol. Inflamm.* 18, 80–90.

McKenna, K. C., and Kapp, J. A. (2006). Accumulation of immunosuppressive CD11b+ myeloid cells correlates with the failure to prevent tumor growth in the anterior chamber of the eye. *J. Immunol.* 177, 1599–1608.

Medawar, P. B. (1948). Immunity to homologous grafted skin; the fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. *Br. J. Exp. Pathol.* 29, 58–69.

Medawar, P. B. (1961). Immunological tolerance. *Nature* 189, 14–17.

Medzhitov, R. (2008). Origin and physiological roles of inflammation. *Nature* 454, 428–435.

Mellor, A. L., and Munn, D. H. (2008). Creating immune privilege: active local suppression that benefits friends, but protects foes. *Nat. Rev. Immunol.* 8, 74–80.

Mesri, M., Liversidge, J., and Forrester, J. V. (1994). ICAM-1/LFA-1 interactions in T-lymphocyte activation and adhesion to cells of the blood–retina barrier in the rat. *Immunology* 83, 52–57.

Metzger, T. C., and Anderson, M. S. (2011). Control of central and peripheral tolerance by Aire. *Immunol. Rev.* 241, 89–103.

Miller, Y. I., Choi, S. H., Wiesner, P., Fang, L., Harkewicz, R., Hartvigsen, K., et al. (2011). Oxidation-specific epitopes are danger-associated molecular patterns recognized by pattern recognition receptors of innate immunity. *Circ. Res.* 108, 235–248.

Moorthy, R. S., Valluri, S., and Rao, N. A. (2012). Nontuberculous mycobacterial ocular and adnexal infections. *Surv. Ophthalmol.* 57, 202–235.

Mougiakakos, D., Johansson, C. C., Trocme, E., All-Ericsson, C., Economou, M. A., Larsson, O., et al. (2010). Intratumoral forkhead box P3-positive regulatory T cells predict poor survival in cyclooxygenase-2-positive uveal melanoma. *Cancer* 116, 2224–2233.

Nagy, L., Szanto, A., Szatmari, I., and Szeles, L. (2012). Nuclear hormone receptors enable macrophages and dendritic cells to sense their lipid environment and shape their immune response. *Physiol. Rev.* 92, 739–789.

Natarajan, S., and Thomson, A. W. (2010). Tolerogenic dendritic cells and myeloid-derived suppressor cells: potential for regulation and therapy of liver auto- and alloimmunity. *Immunobiology* 215, 698–703.

Nian, H., Shao, H., O'Brien, R. L., Born, W. K., Kaplan, H. J., and Sun, D. (2011). Activated gammadelta T cells promote the activation of uveitogenic T cells and exacerbate EAU development. *Invest. Ophthalmol. Vis. Sci.* 52, 5920–5927.

Nian, H., Shao, H., Zhang, G., Born, W. K., O'Brien, R. L., Kaplan, H. J., et al. (2010). Regulatory effect of gammadelta T cells on IL-17+ uveitogenic T cells. *Invest. Ophthalmol. Vis. Sci.* 51, 4661–4667.

Niederkorn, J. Y. (1997). Immunoregulation of intraocular tumours. *Eye (Lond.)* 11(Pt 2), 249–254.

Niederkorn, J. Y. (2006). See no evil, hear no evil, do no evil: the lessons of immune privilege. *Nat. Immunol.* 7, 354–359.

Niederkorn, J. Y. (2009). Role of NKT cells in anterior chamber-associated immune deviation. *Expert Rev. Clin. Immunol.* 5, 137–144.

Niederkorn, J. Y. (2010). High-risk corneal allografts and why they lose their immune privilege. *Curr. Opin. Allergy Clin. Immunol.* 10, 493–497.

Ogata, N., Nomura, S., Shouzu, A., Imaizumi, M., Arichi, M., and Matsumura, M. (2006). Elevation of monocyte-derived microparticles in patients with diabetic retinopathy. *Diabetes Res. Clin. Pract.* 73, 241–248.

Pearson, R. A., Barber, A. C., Rizzi, M., Hippert, C., Xue, T., West, E. L., et al. (2012). Restoration of vision after transplantation of photoreceptors. *Nature* 485, 99–103.

Pennington, D. J., Vermijlen, D., Wise, E. L., Clarke, S. L., Tigelaar, R. E., and Hayday, A. C. (2005). The integration of conventional and unconventional T cells that characterizes cell-mediated responses. *Adv. Immunol.* 87, 27–59.

Plskova, J., Duncan, L., Holán, V., Filipec, M., Kraal, G., and Forrester, J. V. (2002). The immune response to corneal allograft requires a site-specific draining lymph node. *Transplantation* 73, 210–215.

Rigel, D. S., Russak, J., and Friedman, R. (2010). The evolution of melanoma diagnosis: 25 years beyond the ABCDs. *CA Cancer J. Clin.* 60, 301–316.

Robertson, N. J., Brook, F. A., Gardner, R. L., Cobbold, S. P., Waldmann, H., and Fairchild, P. J. (2007). Embryonic stem cell-derived tissues are immunogenic but their inherent immune privilege promotes the induction of tolerance. *Proc. Natl. Acad. Sci. U.S.A.* 104, 20920–20925.

Roychoudhury, J., Herndon, J. M., Yin, J., Apte, R. S., and Ferguson, T. A. (2010). Targeting immune privilege to prevent pathogenic neovascularization. *Invest. Ophthalmol. Vis. Sci.* 51, 3560–3566.

Saban, D. R., Bock, F., Chauhan, S. K., Masli, S., and Dana, R. (2010). Thrombospondin-1 derived from APCs regulates their capacity for allosensitization. *J. Immunol.* 185, 4691–4697.

Sapieha, P., Sirinyan, M., Hamel, D., Zaniolo, K., Joyal, J. S., Cho, J. H., et al. (2008). The succinate receptor GPR91 in neurons has a major role in retinal angiogenesis. *Nat. Med.* 14, 1067–1076.

Scholz, M., Doerr, H. W., and Cinatl, J. (2003). Human cytomegalovirus retinitis: pathogenicity, immune evasion and persistence. *Trends Microbiol.* 11, 171–178.

Serra, A. M., Serra, A. M., Waddell, J., Manivannan, A., Xu, H., Cotter, M., and Forrester, J. V. (2012). CD11b+ bone marrow-derived monocytes are the major leukocyte subset responsible for retinal capillary leukostasis in experimental diabetes in mouse and express high levels of CCR5 in the circulation. *Am. J. Pathol.* 181, 719–727.

Sheridan, B. S., Knickelbein, J. E., and Hendricks, R. L. (2007). CD8 T cells and latent herpes simplex virus type 1: keeping the peace in sensory ganglia. *Expert Opin. Biol. Ther.* 7, 1323–1331.

Shields, C. L., Shields, C. L., Shields, J. A., Santos, M. C., Gündüz, K., Singh, A. D., and Othmane, I. (1999). Incomplete spontaneous regression of choroidal melanoma associated with inflammation. *Arch. Ophthalmol.* 117, 1245–1247.

Sixt, M., Kanazawa, N., Selg, M., Samson, T., Roos, G., Reinhardt, D. P., et al. (2005). The conduit system transports soluble antigens from the afferent lymph to resident dendritic cells in the T cell area of the lymph node. *Immunity* 22, 19–29.

Skeie, J. M., and Mullins, R. F. (2009). Macrophages in neovascular age-related macular degeneration: friends or foes? *Eye (Lond.)* 23, 747–755.

Sosnova, M., Bradl, M., and Forrester, J. V. (2005). CD34+ corneal stromal cells are bone marrow-derived and express hemopoietic stem cell markers. *Stem Cells* 23, 507–515.

Stein-Streilein, J. (2008). Immune regulation and the eye. *Trends Immunol.* 29, 548–554.

Streilein, J. W., and Niederkorn, J. Y. (1981). Induction of anterior chamber-associated immune deviation requires an intact, functional spleen. *J. Exp. Med.* 153, 1058–1067.

Sugita, S., Horie, S., Nakamura, O., Futagami, Y., Takase, H., Keino, H., et al. (2008). Retinal pigment epithelium-derived CTLA-2alpha induces TGFbeta-producing T regulatory cells. *J. Immunol.* 181, 7525–7536.

Sugita, S., Horie, S., Yamada, Y., Keino, H., Usui, Y., Takeuchi, M., et al. (2010). Suppression of bystander T helper 1 cells by iris pigment epithelium-inducing regulatory T cells via negative costimulatory signals. *Invest. Ophthalmol. Vis. Sci.* 51, 2529–2536.

Sugita, S., Keino, H., Futagami, Y., Takase, H., Mochizuki, M., Stein-Streilein J., et al. (2006). B7+ iris pigment epithelial cells convert T cells into CTLA-4+, B7-expressing CD8+ regulatory T cells. *Invest. Ophthalmol. Vis. Sci.* 47, 5376–5384.

Suttorp-Schulten, M. S., and Rothova, A. (1996). The possible impact of uveitis in blindness: a literature survey. *Br. J. Ophthalmol.* 80, 844–848.

Tarallo, V., Hirano, Y., Gelfand, B. D., Dridi, S., Kerur, N., Kim, Y., et al. (2012). DICER1 loss and Alu RNA induce age-related macular degeneration via the NLRP3 inflammasome and MyD88. *Cell* 149, 847–859.

Taylor, A. W., Alard, P., Yee, D. G., and Streilein, J. W. (1997). Aqueous humor induces transforming growth factor-beta (TGF-beta)-producing regulatory T-cells. *Curr. Eye Res.* 16, 900–908.

Taylor, S., Calder, C. J., Albon, J., Erichsen, J. T., Boulton, M. E., and Morgan, J. E. (2011). Involvement of the CD200 receptor complex in microglia activation in experimental glaucoma. *Exp. Eye Res.* 92, 338–343.

Tezel, G. (2011). The immune response in glaucoma: a perspective on the roles of oxidative stress. *Exp. Eye Res.* 93, 178–186.

Tezel, T. H., Del Priore, L. V., Berger, A. S., and Kaplan, H. J. (2007). Adult retinal pigment epithelial transplantation in exudative age-related macular degeneration. *Am. J. Ophthalmol.* 143, 584–595.

Ugel, S., Peranzoni, E., Desantis, G., Chioda, M., Walter, S., Weinschenk, T., et al. (2012). Immune tolerance to tumor antigens occurs in a specialized environment of the spleen. *Cell Rep.* 2, 628–639.

Usui, Y., Okunuki, Y., Hattori, T., Kezuka, T., Keino, H., Ebihara, N., et al. (2008). Functional expression of B7H1 on retinal pigment epithelial cells. *Exp. Eye Res.* 86, 52–59.

van de Veerdonk, F. L., Netea, M. G., Dinarello, C. A., and Joosten, L. A. (2011). Inflammasome activation and IL-1beta and IL-18 processing during infection. *Trends Immunol.* 32, 110–116.

Vega, J. L., Keino, H., and Masli, S. (2009). Surgical denervation of ocular sympathetic afferents decreases local transforming growth factor-beta and abolishes immune privilege. *Am. J. Pathol.* 175, 1218–1225.

Wacker, W. B. (1991). Proctor Lecture. Experimental allergic uveitis. Investigations of retinal autoimmunity and the immunopathologic responses evoked. *Invest. Ophthalmol. Vis. Sci.* 32, 3119–3128.

Waldmann, H. (2010). Tolerance: an overview and perspectives. *Nat. Rev. Nephrol.* 6, 569–576.

Wang, J., Xu, X., Elliott, M. H., Zhu, M., and Le, Y. Z. (2010). Muller cell-derived VEGF is essential for diabetes-induced retinal inflammation and vascular leakage. *Diabetes* 59, 2297–2305.

Wang, S., Boonman, Z. F., Li, H. C., He, Y., Jager, M. J., Toes, R. E., et al. (2003). Role of TRAIL and IFN-gamma in CD4+ T cell-dependent tumor rejection in the anterior chamber of the eye. *J. Immunol.* 171, 2789–2796.

Wax, M. B. (2011). The case for autoimmunity in glaucoma. *Exp. Eye Res.* 93, 187–190.

Wekerle, H. (2006). Breaking ignorance: the case of the brain. *Curr. Top. Microbiol. Immunol.* 305, 25–50.

Wen, H., Ting, J. P., and O'Neill, L. A. (2012). A role for the NLRP3 inflammasome in metabolic diseases – did Warburg miss inflammation? *Nat. Immunol.* 13, 352–357.

West, E. L., Pearson, R. A., Barker, S. E., Luhmann, U. F., Maclaren, R. E., Barber, A. C., et al. (2010). Long-term survival of photoreceptors transplanted into the adult murine neural retina requires immune modulation. *Stem Cells* 28, 1997–2007.

West, E. L., Pearson, R. A., MacLaren, R. E., Sowden, J. C., and Ali, R. R. (2009). Cell transplantation strategies for retinal repair. *Prog. Brain Res.* 175, 3–21.

Wilbanks, G. A., and Streilein, J. W. (1992). Macrophages capable of inducing anterior chamber associated immune deviation demonstrate spleen-seeking migratory properties. *Reg. Immunol.* 4, 130–137.

Williams, G. J., Brannan, S., Forrester, J. V., Gavin, M. P., Paterson-Brown, S. P., Purdie, A. T., et al. (2007). The prevalence of sight-threatening uveitis in Scotland. *Br. J. Ophthalmol.* 91, 33–36.

Williams, K. A., and Coster, D. J. (1997). Rethinking immunological privilege: implications for corneal and limbal stem cell transplantation. *Mol. Med. Today* 3, 495–501.

Xu, H., Chen, M., and Forrester, J. V. (2009). Para-inflammation in the aging retina. *Prog. Retin. Eye Res.* 28, 348–368.

Xu, H., Chen, M., Reid, D. M., and Forrester, J. V. (2007a). LYVE-1-positive macrophages are present in normal murine eyes. *Invest. Ophthalmol. Vis. Sci.* 48, 2162–2171.

Xu, H., Dawson, R., Forrester, J. V., and Liversidge, J. (2007b). Identification of novel dendritic cell populations in normal mouse retina. *Invest. Ophthalmol. Vis. Sci.* 48, 1701–1710

Xu, H., Manivannan, A., Crane, I., Dawson, R., and Liversidge, J. (2008). Critical but divergent roles for CD62L and CD44 in directing blood monocyte trafficking in vivo during inflammation. *Blood* 112, 1166–1174.

Xu, H., Manivannan, A., Goatman, K. A., Jiang, H. R., Liversidge, J., Sharp, P. F., et al. (2004). Reduction in shear stress, activation of the endothelium, and leukocyte priming are all required for leukocyte passage across the blood–retina barrier. *J. Leukoc. Biol.* 75, 224–232.

Yao, J., Feathers, K. L., Khanna, H., Thompson, D., Tsilfidis, C., Hauswirth, W. W., et al. (2011). XIAP therapy increases survival of transplanted rod precursors in a degenerating host retina. *Invest. Ophthalmol. Vis. Sci.* 52, 1567–1572.

Yoshida, M., Kezuka, T., and Streilein, J. W. (2000). Participation of pigment epithelium of iris and ciliary body in ocular immune privilege. 2. Generation of TGF-beta-producing regulatory T cells. *Invest. Ophthalmol. Vis. Sci.* 41, 3862–3870.

You, J. J., Yang, C. M., Chen, M. S., and Yang, C. H. (2010). Regulation of Cyr61/CCN1 expression by hypoxia through cooperation of c-Jun/AP-1 and HIF-1alpha in retinal vascular endothelial cells. *Exp. Eye Res.* 91, 825–836.

Zamiri, P., Sugita, S., and Streilein, J. W. (2007). Immunosuppressive properties of the pigmented epithelial cells and the subretinal space. *Chem. Immunol. Allergy* 92, 86–93.

Zhang, B., Jia, H., Liu, J., Yang, Z., Jiang, T., Tang, K., et al. (2010). Depletion of regulatory T cells facilitates growth of established tumors: a mechanism involving the regulation of myeloid-derived suppressor cells by lipoxin A4. *J. Immunol.* 185, 7199–7206.

Zhang, S. Y., Jouanguy, E., Ugolini, S., Smahi, A., Elain, G., Romero, P., et al. (2007). TLR3 deficiency in patients with herpes simplex encephalitis. *Science* 317, 1522–1527.

Zhou, R., and Caspi, R. R. (2010). Ocular immune privilege. *F1000 Biol. Rep.* 2, pii: 3.

Zhou, R., Horai, R., Silver, P. B., Mattapallil, M. J., Zárate-Bladés, C. R., Chong, W. P., et al. (2012). The living eye "disarms" uncommitted autoreactive T cells by converting them to Foxp3(+) regulatory cells following local antigen recognition. *J. Immunol.* 188, 1742–1750.

ZIrm, E. (1906). Eine erfolgreich totlae Keratoplastik. *Arch. Ophthalmol.* 64, 580.

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Ocular immune privilege and ocular melanoma: parallel universes or immunological plagiarism?

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Evidence of immune privilege in the eye was recorded almost 140 years ago, yet interest in immune privilege languished for almost a century. However, the past 35 years have witnessed a plethora of research and a rekindled interest in the mechanisms responsible for immune privilege in the anterior chamber of the eye. This research has demonstrated that multiple anatomical, structural, physiological, and immunoregulatory processes contribute to immune privilege and remind us of the enormous complexity of this phenomenon. It is widely accepted that immune privilege is an adaptation for reducing the risk of immune-mediated inflammation in organs such as the eye and brain whose tissues have a limited capacity to regenerate. Recent findings suggest that immune privilege also occurs in sites where stem cells reside and raise the possibility that immune privilege is also designed to prevent the unwitting elimination of stem cells by immune-mediated inflammation at these sites. Uveal melanoma arises within the eye and as such, benefits from ocular immune privilege. A significant body of research reveals an intriguing parallel between the mechanisms that contribute to immune privilege in the eye and those strategies used by uveal melanoma cells to evade immune elimination once they have disseminated from the eye and establish metastatic foci in the liver. Uveal melanoma metastases seem to have "plagiarized" the blueprints used for ocular immune privilege to create "*ad hoc* immune privileged sites" in the liver.

Keywords: anterior chamber, eye, immune privilege, stem cells, uveal melanoma

INTRODUCTION

The roots of immune privilege reach back two centuries to an observation made by the Dutch ophthalmologist van Dooremaal (1873). In an attempt to identify the etiology of cataracts van Dooremaal inserted a variety of foreign objects and tissues into the eyes of rabbits and dogs. Although he failed to discover the cause of cataracts, he observed a significant prolongation in the survival of mouse skin grafts placed into the anterior chamber (AC) of the dog eye (van Dooremaal, 1873). Another 75 years would pass before Medawar "rediscovered" the prolonged survival of foreign tissue grafts placed into the AC of rabbits and coined the term "immune privilege" to describe this phenomenon (Medawar, 1948). Medawar concluded that the apparent absence of lymphatic drainage from the AC resulted in a sequestration of antigens in the eye resulting in a condition that contemporary immunologists might call "immunological ignorance." The late 1970s ushered in a new era in immune privilege research that was led by Streilein and colleagues who made the remarkable observation that antigens introduced into the AC of the eye not only gained access to the peripheral lymphoid tissues, but in the process, induced a systemic down regulation of antigen-specific cell-mediated immunity (Kaplan et al., 1975; Kaplan and Streilein, 1977, 1978). This immunoregulatory phenomenon was termed AC-associated immune deviation (ACAID; Streilein and Niederkorn, 1981). The discovery of ACAID kindled a renewed interest in immune privilege research that has led to numerous insights over the past 35 years. This body of work has revealed that immune privilege is the product of multiple anatomical, physiological, and immunoregulatory processes that share a common feature – restriction of immune inflammation in an organ whose tissues have a severely limited capacity to regenerate.

In addition to the AC of the eye, there are other notable immune privileged sites in the body including the testis, hair follicle, placenta, and brain. The eye is part of the brain, both anatomically and embryologically, and like the brain, has a severely limited capacity to regenerate its tissues. Thus, immune privilege is believed to be an adaptation to protect the eye and the brain from injury inflicted by immune-mediated inflammation. By contrast, the testis, hair follicle, and placenta are sites where stem cells reside. Protecting stem cells from immune-mediated elimination has obvious survival benefits for the host and in the case of the placenta, for the survival of the species. It is noteworthy that many of the mechanisms that sustain immune privilege in the central nervous system (i.e., eye and brain) are also employed in sites where stem cells reside (e.g., testis, hair follicle, and placenta) and by stem cells themselves (Arck et al., 1997; Moffett-King, 2002; Aluvihare et al., 2004, 2005; Niederkorn, 2006; Robertson et al., 2007; Fujisaki et al., 2011; Kinori et al., 2011; Meinhardt and Hedger, 2011; Mital et al., 2011).

The immune system is functionally divided into two basic components: (a) innate immunity and (b) adaptive immunity. The innate immune response is characterized by its rapid activation

and its conspicuous absence of antigen specificity and memory. Components of the innate immune apparatus include natural killer (NK) cells, macrophages, granulocytes, and the alternative pathway for complement activation. Elements of the innate immune response serve as "first responders" to infections and provide a nimble, albeit limited level of protection, which is replaced by the adaptive immune response. The adaptive immune system is characterized by its exquisite antigen specificity and memory. Although slower to develop, the adaptive immune response provides a comprehensive protection that persists longer than the innate immune response and possesses memory that allows for a rapid reactivation to future encounters with pathogens. Both innate and adaptive immune responses are capable of inflicting irreparable injury to ocular tissues and stem cells.

IMMUNE PRIVILEGE AND THE INNATE IMMUNE RESPONSE

Innate immune responses have the potential to inflict significant irreparable damage to the eye. Granulocytes and macrophages elaborate a variety of proteases and reactive oxygen species (ROS) that are known to damage innocent bystander cells. However, the aqueous humor (AH) that fills the AC is endowed with a variety of anti-inflammatory and immunosuppressive cytokines, as well as free radical scavengers that buffer or neutralize proinflammatory cytokines and ROS (Taylor, 2007). Moreover, AH contains factors that induce apoptosis of neutrophils and macrophages (D'Orazio et al., 1999). The complement cascade can be activated through the alternative pathway through exposure to bacterial products and under such conditions it functions as a component of the innate immune system. Complement activation culminates in the generation of a membrane attack complex (MAC) that punches holes in the plasma membrane, which leads to osmotic lysis of both bacterial and mammalian cells. Activation of the complement cascade also generates soluble factors that recruit and activate neutrophils. However, injury inflicted by complement activation is minimized by complement regulatory proteins (CRPs) that are present in the AH and that also decorate the membranes of cells lining the AC (Lass et al., 1990; Bora et al., 1993; Goslings et al., 1996, 1998; Sohn et al., 2000a).

Natural killer cells are members of the innate immune system and are believed to protect against viral infections and neoplasms while the adaptive immune response is still being generated. Cytotoxic T lymphocytes (CTLs) are important elements of the adaptive immune response to viral infections and neoplasms. CTLs recognize viral antigens and tumor antigens that are displayed on major histocompatibility complex (MHC) class I molecules. MHC class I molecules act as the "docking station" that facilitates the binding and cytolytic activity of CTLs. However, to evade CTL-mediated killing many tumors and viruses down-regulate MHC class I molecules and thereby render the cancer cells and virus-infected cells invisible to CTLs. To compensate for this evasive strategy, the immune system enlists the aid of NK cells, which are programmed to kill any cell failing to express MHC class I molecules. However, the corneal endothelial cells that line the AC of the eye and cells in the various layers of the retina express little or no MHC class I molecules and are therefore potentially vulnerable to NK cell-mediated cytolysis. Moreover, corneal endothelial cells and cells of the retina are amitotic and cannot regenerate. However, the AH contains two cytokines that inhibit NK cell-mediated cytolytic activity. Macrophage migration inhibitory factor (MIF) produces an immediate inhibition of NK cell-mediated cytolysis of corneal endothelial cells (Apte and Niederkorn, 1996). Transforming growth factor-β (TGF-β) also inhibits NK cell-mediated cytolysis, but does not produce maximal inhibition for 22–24 h (Apte and Niederkorn, 1996). Thus, the AH is endowed with molecules that produce both immediate and delayed inhibition of NK cell activity. In addition to soluble inhibitory factors, the cells lining the AC express non-classical MHC class Ib molecules such as HLA-E in humans and Qa-2 in mice, which can transmit "off" signals to NK cells (Niederkorn et al., 1999; Le Discorde et al., 2003). The importance of intraocular inhibition of NK cell activity was confirmed in studies in which NK-sensitive human uveal melanoma cells were transplanted either subcutaneously (SC) or into the AC of nude mice. Although nude mice lack a functional T cell repertoire, they display potent NK cell activity. Human uveal melanoma cells were briskly rejected by an NK cell-dependent process when they were transplanted SC in nude mice, but grew progressively in the eyes, even at doses that were 50-times lower than the doses that were rejected following SC transplantation (Apte et al., 1997).

Immune privilege of innate immune responses is also present in sites where stem cells reside such as the hair follicle, placenta, and testis. MIF is expressed in the hair follicle (Ito et al., 2008), Leydig cells of the testis (Meinhardt et al., 1996; Okuma et al., 2005), and in the placenta (Vigano et al., 2007). Moreover, there is a close association between reduced MIF in the hair follicle and the development of alopecia areata, an autoimmune disease of the skin (Ito et al., 2008). MIF produced by human decidual cells of the uterus inhibits the cytolytic activity of uterine NK cells and is believed to contribute to the immune privilege of the allogeneic fetus (Arcuri et al., 2006; Vigano et al., 2007). As mentioned earlier, non-classical class Ib MHC molecules are expressed in the eye and are believed to be important inhibitors of NK cell-mediated cytolysis. It is noteworthy that non-classical class Ib molecules are also expressed in the testis (Slukvin et al., 1999; Ryan et al., 2002) and in the placenta (Kovats et al., 1990; Ishitani and Geraghty, 1992; Rouas-Freiss et al., 1997, 1999). Thus, both soluble and cell membrane-bound molecules that inhibit innate immune responses are expressed in both the eye and in sites where stem cells reside (**Table 1**).

IMMUNE PRIVILEGE AND THE ADAPTIVE IMMUNE RESPONSE

The adaptive immune response is characterized by exquisite antigen specificity and memory. T cells and antibodies are the central players in adaptive immune responses and each has the capacity to produce injury to the eye. Many of the strategies employed to buffer injurious innate immune responses in the eye and in sites of stem cell residence are effective in blocking adaptive immune responses that have the potential to damage stem cells and ocular tissues that cannot regenerate.

ANTI-INFLAMMATORY AND IMMUNOSUPPRESSIVE FACTORS

The AH contains at least five factors that inhibit the expression of T cell-mediated inflammation: (a) TGF-β; (b) α-melanocyte stimulating hormone (α-MSH); (c) vasoactive intestinal peptide (VIP);

Table 1 | Factors that contribute to immune privilege in the eye and in sites of stem cell residence.

Molecule	Mode of action	Eye	Placenta	BM	Testis	Hair follicle
TGF-β	Inhibit NK cell activity and promote generation of Tregs	Y	Y	Y	Y	Y
MIF	Inhibit NK cell activity	Y	Y	?	Y	Y
MHC class Ib	Inhibit NK cell activity	Y	Y	?	Y	Y
IDO	Deplete tryptophan and induce T cell apoptosis	Y	Y	?	Y	Y
FasL	Induce apoptosis of T cells	Y	Y	?	Y	Y/N
TRAIL	Inhibit T cell proliferation	Y	Y	?	?	?
CRPs	Inactivate complement	Y	Y	?	?	?
PD-L1 (B7-H1)	Inhibit T cell proliferation	Y	?	?	Y	?
Tregs	Inhibit T cell immunity	Y	Y	Y	Y	?

BM, bone marrow; Y, yes; Y/N, indirect evidence; ?, not determined.

(d) calcitonin gene-related protein (CGRP); and (e) somatostatin (Granstein et al., 1990; Cousins et al., 1991; Taylor et al., 1994a,b; Taylor and Yee, 2003). Cells lining the AC also produce indoleamine dioxygenase (IDO), an enzyme that catabolizes tryptophan, which is a key amino acid that is necessary for T lymphocyte survival (Beutelspacher et al., 2006; Ryu and Kim, 2007). CRPs are present in the AH and are also expressed on the cell membranes of many cells lining the interior of the eye. CRPs are effective in maintaining immunological homeostasis within the eye. It is believed that a low level of complement activation is always present in the body, including the eye but under normal homeostatic conditions, CRPs act to restrain the untoward effects of complement activation. As evidence in support this, Sohn et al. (2000b) reported that administration of neutralizing antibody to CRPs in rats resulted in spontaneous ocular inflammation.

Immune privileged sites in which stem cells reside express many of the soluble factors that are found in the AH of the eye and are effective in suppressing adaptive immune responses (**Table 1**). TGF-β is produced by Sertoli cells in the testis (Meinhardt and Hedger, 2011), in the hair follicle (Kinori et al., 2011), and by the placenta (Niederkorn, 2006). Moreover, murine embryonic stem cells (ESCs) themselves upregulate TGF-β2 and create an "*ad hoc*" immune privileged niche in the bone marrow (Robertson et al., 2007). The hair follicle, placenta, and Leydig cells of the testis elaborate α-MSH, which also suppresses T cell immunity *in situ* (Niederkorn, 2006; Kinori et al., 2011; Meinhardt and Hedger, 2011). Although it is not a secreted soluble factor, IDO acts to locally suppress T cell-mediated inflammation by depleting tryptophan and starving T cells. Interestingly, IDO is present in the anterior segment of the eye, in Sertoli cells of the testis, and in the placenta (Mellor et al., 2001; Beutelspacher et al., 2006; Fallarino et al., 2009).

Whether stem cells themselves contribute to the immune privilege in sites where they reside or whether they are beneficiaries of the local immunosuppressive properties of these regions remains unresolved. Numerous studies have reported that ESCs express very low levels of MHC class I molecules and virtually no class II and enjoy a significant degree of immune privilege (Drukker et al., 2002, 2006; Li et al., 2004a; Menard et al., 2005; Bonde and Zavazava, 2006). Moreover, adult stem cells (e.g., either mesenchymal or amniotic origin) themselves display immune privilege (Uccelli et al., 2008). By contrast, there is equally compelling evidence that ESCs are not inherently endowed with immune privilege and can undergo immune rejection (Nussbaum et al., 2007; Robertson et al., 2007; Chidgey and Boyd, 2008; Swijnenburg et al., 2008; Wu et al., 2008). Nonetheless, it is clear that many of the niches in which stem cells reside are classical immune privileged sites that provide a milieu that diminishes the likelihood of inflammation and immune-mediated injury.

CELL MEMBRANE-BOUND MOLECULES

Cells that line the interior of the eye express cell membrane-bound molecules that either induce apoptosis or inhibit proliferation of T cells entering the eye. FasL is expressed throughout the eye and purges activated T cells and neutrophils that enter the eye in response to viral infections or corneal transplants (Griffith et al., 1995; Stuart et al., 1997; Yamagami et al., 1997). PD-L1 is another member of the B7 family of membrane proteins that induce down regulation of T cell proliferation and cytokine production and promote apoptosis of inflammatory cells (Dong et al., 2002; Ding et al., 2005; Saunders et al., 2005; Okazaki and Honjo, 2007). PD-L1 is expressed in both the mouse and human eye (Hori et al., 2006; Shen et al., 2007; Yang et al., 2009) and is necessary for the survival of corneal allografts (Hori et al., 2006; Shen et al., 2007). PD-L1 is upregulated in the eyes of patients with sympathetic ophthalmia and in ocular cells exposed to the proinflammatory cytokines such as TNF-α and IFN-γ, which suggests that PD-L1 serves as a buffer for dampening immune-mediated inflammation of the eye (Yang et al., 2009). Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the TNF family and is expressed on cells lining the interior of the eye and is believed to contribute to ocular immune privilege in a manner similar to that invoked by PD-L1 (Lee et al., 2002; Wang et al., 2003).

Cell membrane-bound molecules are also expressed in stem cell niches. FasL is expressed on Sertoli cells of the testis (Bellgrau et al., 1995) and on the cells of the placenta (Niederkorn, 2006). FasL message is down-regulated in the hair follicles in patients with the autoimmune disease alopecia areata, which suggests that FasL is involved in maintaining immune privilege in the hair follicle (Kang et al., 2010). PD-L1 is found on Sertoli cells of the testis (Dal Secco et al., 2008) and on cells of the placenta (Petroff et al., 2003; Petroff, 2005; Holets et al., 2009). Thus, there is an interesting parallel in the mechanisms and molecules that

sustain immune privilege in the eye and that shield stem cells from immune-mediated elimination.

DYNAMIC IMMUNOREGULATORY PROCESSES THAT SUSTAIN IMMUNE PRIVILEGE

In addition to soluble and cell membrane-bound molecules that dampen immune-mediated inflammation, immune privileged sites are designed to promote the generation of dynamic immunoregulatory processes that down-regulate adaptive immune responses. The seminal studies by Streilein and colleagues in the late 1970s introduced the paradigm of ACAID and the concept that immune privilege was also sustained by regulatory cells that deflected the adaptive immune response away from effector mechanisms that imposed extensive injury to collateral tissues. A plethora of studies have revealed the complex nature of ACAID and have demonstrated that at least four organ systems contribute to the generation of ACAID: (a) eye; (b) thymus; (c) spleen, and (d) sympathetic nervous system (Niederkorn, 2006, 2009; Niederkorn and Stein-Streilein, 2010). The induction of ACAID is initiated when antigens are introduced into the AC. Although it was originally believed that injecting antigens into the AC was tantamount to an intravenous (IV) injection, which is a well-known method for inducing immune tolerance (Asherson and Stone, 1965). However, the eye is not a passive participant in this process and the immune deviation induced by AC injection of antigens is fundamentally different from IV-induced immune deviation (Wilbanks and Streilein, 1990, 1991; Kosiewicz et al., 1998; Sonoda et al., 2001). Removal of the eye within 3 days of AC injection of antigen prevents the induction of ACAID. During this obligatory 3-day period, it is believed that antigen is captured by F4/80$^+$ antigen presenting cells (APCs), which under the influence of cytokines in the AH are imprinted to preferentially produce IL-10 and down-regulate the production of IL-12. The ocular APC emigrate from the eye to the thymus and spleen. Within the thymus the ocular APCs evoke the generation of CD4$^-$,CD8$^-$, NK1.1$^+$ T cells (NKT cells), which subsequently emerge from the thymus and migrate to the spleen via the blood vascular route (Wang et al., 1997, 2001). Like the eye and spleen, the thymus is an active participant in the induction of ACAID. Removing the thymus within 3 days of AC injection of antigen prevents the induction of ACAID (Wang et al., 1997). The sympathetic nervous system is also an active player in the induction of AC AID, as chemical sympathectomy prevents the induction of ACAID (Li et al., 2004b). Although it is not clear how the sympathetic nervous system participates in the induction of ACAID, it appears that the generation of ocular F4/80$^+$ APCs is not affected by the sympathetic nervous system (Li et al., 2004b). However, chemical sympathectomy prevents the generation of thymic NK1.1$^+$ T cells and splenic CD8$^+$ T regulatory cells (T regs; Li et al., 2004b). A population of F4/80$^+$ ocular APCs is also believed to migrate from the eye to the spleen where the APCs secrete MIP-2, which attracts CD4$^+$ NKT cells, which in turn interact with the ocular APCs and secrete RANTES. RANTES recruits other cells into the marginal zone of the spleen. Within the marginal zone of the spleen, F4/80$^+$ APCs, NKT cells, B cells, and CD4$^+$ T cells, under the influence of the third component of complement, collaborate to generate antigen-specific CD8$^+$ T (**Figure 1**).

Pigmented epithelial cells of the iris and ciliary body line a portion of the AC and exert important immunoregulatory effects. The ciliary body cells secrete AH, which contains multiple immunosuppressive and anti-inflammatory molecules (Taylor et al., 1992, 1994a,b, 1997; Taylor and Yee, 2003; Taylor, 2007). In addition to secreting AH, iris and ciliary body cells directly suppress T lymphocyte proliferation and block production of IFN-γ by a contact-dependent process that is independent of the AH-borne soluble factors (Yoshida et al., 2000a). *In vitro* studies have shown that T lymphocytes co-cultured with iris and ciliary body cells acquire Treg activity that inhibits T lymphocyte proliferation and antigen-specific DTH (Yoshida et al., 2000b). The *in situ* generation of T regs requires direct contact between T lymphocytes and the iris and ciliary body cells. The locally generated T regs suppress inflammation by secreting active and latent forms of TGF-β. The only blood vessels in the anterior segment of the eye reside in the iris and ciliary body. Thus, inflammatory cells such as T lymphocytes that enter the AC of the eye extravasate via the iris and ciliary body blood vessels and as a result are in direct contact with the pigmented cells of the iris and ciliary body and thus, are subject to *in situ* induction of T reg activity. The *in situ*-generated T regs greet subsequent waves of inflammatory cells that enter the AC and impose their suppressive effects to further dampen inflammation in the AC.

The AC possesses a second pathway for the *in situ* generation of T regs. Soluble factors in the AH, namely α-MSH, can convert T lymphocytes into CD4$^+$CD25$^+$ T regs that suppress DTH and extinguish immune-mediated inflammation such as experimental autoimmune uveitis (EAU; Nishida and Taylor, 1999; Taylor and Namba, 2001; Namba et al., 2002).

Local induction of T reg activity also occurs in sites where stem cells reside. The allogeneic fetus confronts the mother with alien histocompatibility antigens of paternal origin and thus, is at considerable risk for immune rejection. However, a wide array of anatomical, physiological, and immunoregulatory adaptations protect the fetus from immune rejection. The allogeneic fetus induces a form of immune deviation with striking parallels with ACAID. One might even argue that maternal immune privilege is initiated even before fertilization of the ovum. Seminal fluid contains one of the highest concentrations of TGF-β of any bodily fluid (Robertson et al., 2002) and the TGF-β concentration in the uterine luminal fluid increases over threefold immediately after insemination (Tremellen et al., 1998). Semen, like AH, has the capacity to promote the development of immune tolerance and T regs (Lengerova and Vojtiskova, 1963; Robertson et al., 1997; James et al., 2003). In both humans and mice, there is a steep increase in the number of CD4$^+$CD25$^+$ T regs during pregnancy and depletion of CD4$^+$CD25$^+$ T regs induces abortion in mice (Aluvihare et al., 2004; Somerset et al., 2004). Induction of T regs also occurs in the testis. Soluble antigens injected into the testis induce a form of immune deviation that is reminiscent of ACAID (Li et al., 1997; Ditzian-Kadanoff, 1999; Verajankorva et al., 2002). A recent study demonstrated that allogeneic hematopoietic stem cell transplants reside in the bone marrow where immune reactivity exists, yet the stem cell transplants do not undergo immune rejection (Fujisaki et al., 2011). Allogeneic hematopoietic stem cells reside in the bone marrow in close proximity to CD4$^+$CD25$^+$ T regs. Interestingly,

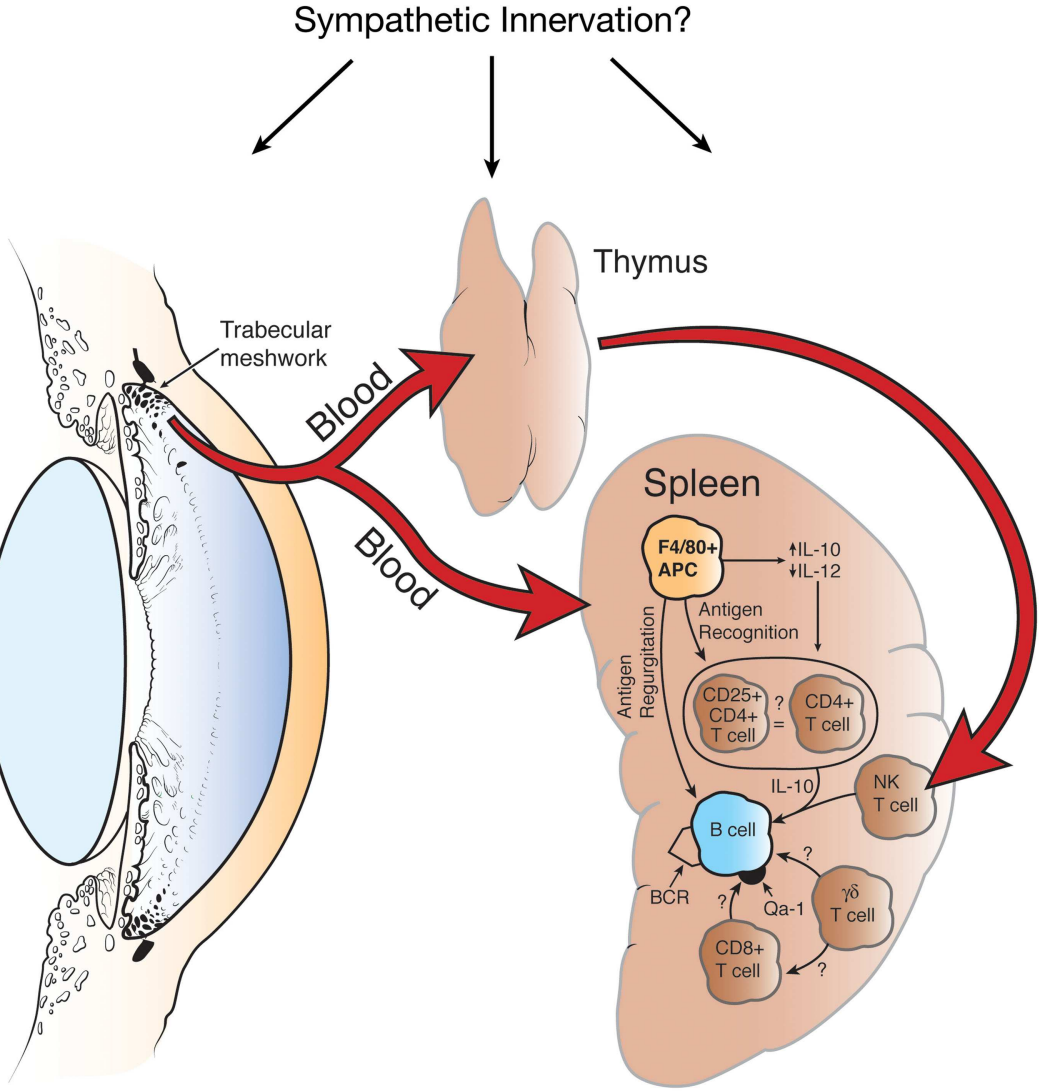

FIGURE 1 | Organ systems involved in the induction of ACAID. Removal of the eye, spleen, or thymus within 72 h of injecting antigen into the anterior chamber prevents the induction of ACAID. Chemical sympathectomy prior to injecting antigen into the anterior chamber also abrogates ACAID. BCR, B cell receptor. Reproduced from Niederkorn (2006), with permission from Nature Publishing Group.

the hematopoietic stem cells are lost if the hosts are depleted of $CD4^+CD25^+$ T regs (Fujisaki et al., 2011). To date no studies have examined if the hair follicle environment promotes the induction of immune deviation or the generation of T regs (Kinori et al., 2011).

OCULAR IMMUNE PRIVILEGE AND OCULAR MELANOMA: PARALLEL UNIVERSES OR IMMUNOLOGICAL PLAGIARISM?

Uveal melanoma is the most common intraocular malignancy in adults and occurs with a frequency of seven cases per million per year in the Western world (Singh and Topham, 2003). There is ample evidence that uveal melanomas are recognized by elements of both the innate and adaptive immune responses. Although uveal melanomas reside within an immunologically privileged site and escape immune elimination in the eye, they metastasize to other body sites, which do not provide the same immunological sanctuary that occurs in the eye. However, growing evidence suggests that uveal melanomas have adopted many of the strategies that contribute to immune privilege in the eye and use them to escape immune rejection after the tumor cells have disseminated from the eye to distant body sites (**Table 2**).

UVEAL MELANOMAS AND IMMUNE PRIVILEGE TO INNATE IMMUNE RESPONSES

Studies in both humans and experimental animals indicate that the innate immune responses, namely NK cells, have an important influence on the growth and metastasis of uveal melanomas (Niederkorn, 2010). *In vitro* studies have shown that human uveal melanoma cells are susceptible to *in vitro* cytolysis by NK cells (Ma and Niederkorn, 1995; Ma et al., 1995; He et al., 2004). However,

Table 2 | Mechanisms and molecules that maintain immune privilege in the eye and are "plagiarized" by uveal melanoma metastases to escape immune surveillance.

Mechanism/ molecule	Mode of action	Eye	Uveal melanoma
TGF-β	Inhibit NK cells	Y	Y
MIF	Inhibit NK cells	Y	Y
IDO	Deplete T cells	Y	Y
FasL	Deplete T cells	Y	Y
TRAIL	Inhibit T cell proliferation and induce T cell apoptosis	Y	Y
CRPs	Inactivate complement	Y	Y
PD-L1	Inhibit T cell proliferation and induce T cell apoptosis	Y	Y
Low level of MHC class Ia	Escape detection by CTLs	Y	Y
MHC class Ib	Inhibit NK cells	Y	Y
Tregs	Inhibit T cells	Y	?

Y, yes; ?, not determined.

the susceptibility of uveal melanoma cells to NK cell-mediated lysis is inversely correlated with the expression of MHC class I molecules (Ma and Niederkorn, 1995; Ma et al., 1995) and is consistent with the "missing self" hypothesis, which posits that NK cells are programmed to kill any cell, malignant or non-malignant, that fails to express MHC class I molecules (Ljunggren and Karre, 1990). However, within the eye uveal melanomas are shielded from NK cell-mediated cytolysis by the buffering effects of the AH, which contains two potent inhibitors of NK cell activity: TGF-β and MIF. Both of these cytokines are present in the AH at concentrations that strongly inhibit NK cell-mediated cytolysis (Apte and Niederkorn, 1996; Apte et al., 1997, 1998). Experiments in nude mice have provided compelling evidence that AH-borne factors prevent NK cell-mediated elimination of uveal melanomas in the eye. Nude mice lack a functional T lymphocyte repertoire, yet have a robust NK cell population. Uveal melanoma cells were briskly rejected following subcutaneous transplantation in nude mice, yet grew progressively if transplanted into the eye, even at doses 50-fold lower than the subcutaneous doses (Apte et al., 1997). Elimination of NK cells by intraperitoneal injection of anti-asialo GM1 antiserum prevented nude mice from rejecting subcutaneously injected uveal melanoma cells and confirmed that the rejection of the subcutaneously injected uveal melanoma cells was indeed mediated by NK cells and also indicated that NK cell-mediated rejection of uveal melanoma cells can occur outside of the eye (Apte et al., 1997).

Uveal melanomas have a propensity to metastasize to the liver and 95% of the patients who die from uveal melanoma have liver metastases (Einhorn et al., 1974; Donoso et al., 1985). Lymphocytes can infiltrate primary uveal melanomas and in some cases, as many as 40% of the tumor-infiltrating lymphocytes (TIL) express NK cell markers (Ksander et al., 1991; Meecham et al., 1992; de Waard-Siebinga et al., 1996). Moreover, NK cells isolated from uveal melanoma-containing eyes display NK cell-mediated cytolytic activity (Ksander et al., 1991). However, as mentioned earlier, the AH of the eye contains MIF and TGF-β, both of which inhibit NK cell-mediated cytolytic activity *in vitro* and *in situ*. However, once in the liver, uveal melanoma cells find themselves in an environment that has the highest concentration of NK cells of any organ in the body (Godfrey et al., 2000; Crispe, 2009; Gao et al., 2009; Nemeth et al., 2009). To compensate for this harsh new reality, uveal melanomas have adopted the strategies employed by the eye to block NK cell-mediated cytolysis. In one study, liver metastases of uveal melanomas produced approximately twice as much MIF as primary uveal melanoma cells (Repp et al., 2000). Uveal melanomas also express TGF-β2, the isoform of TGF-β that suppresses NK cell activity (Esser et al., 2001). Verbik et al. (1997) examined the expression of MHC class I molecules on primary uveal melanomas and liver metastases from the same patient and discovered that liver metastases expressed a 10-fold higher expression of MHC class I molecules compared to the primary melanoma. The susceptibility of tumor cells to NK cell-mediated cytolysis is also affected by the tumor cell's expression of NK cell activating ligands. NKG2D is an activating receptor that is expressed on NK cells and when it interacts with its ligand, MIC-A/B, which is expressed on NK-sensitive tumors, it transmits an activating signal that results in NK cell-mediated cytolysis of the tumor cells. An interesting recent study reported that MIC-A/B was expressed on 50% of primary uveal melanomas, but was undetectable on all 11 metastases specimens tested (Vetter et al., 2004). Thus, uveal melanomas appear to undergo a selection process once they leave the eye that favors the survival of cells that are resistant to NK cell-mediated cytolysis.

UVEAL MELANOMAS AND IMMUNE PRIVILEGE TO ADAPTIVE IMMUNE RESPONSES

Uveal melanomas have also high-jacked strategies used by the eye to create "*ad hoc*" immune privilege against adaptive immune responses in the liver.

IDO AND THE STARVATION OF T CELLS

T lymphocytes are incapable of generating tryptophan and perish if this amino acid is absent. The enzyme IDO catalyzes the degradation of tryptophan and thereby terminates T lymphocyte immune responses (Munn et al., 1999; Frumento et al., 2002; Mellor et al., 2002; Terness et al., 2002). IDO is expressed in many ocular tissues and is believed to contribute to the immune privilege of corneal allografts (Malina and Martin, 1993; Beutelspacher et al., 2006; Ryu and Kim, 2007). IDO is expressed by some tumors and is believed to be a strategy for evading immune surveillance (Uyttenhove et al., 2003). Chen et al. (2007) reported that neither primary uveal melanomas nor liver metastases constitutively expressed IDO. However uveal melanoma cells exposed to IFN-γ, a cytokine produced by both T lymphocytes and NK cells, rapidly upregulated biologically active IDO. Thus, uveal melanoma cells are poised to generate IDO if they perceive the presence of either adaptive or innate immune elements and thereby evade immune elimination in the liver.

COUNTER ATTACK BY PD-L1

PD-L1 is expressed throughout the eye and sustains immune privilege by down-regulating T lymphocyte proliferation and inducing

apoptosis of inflammatory cells expressing its receptor, PD-1. PD-L1 also contributes to the immune privilege of corneal allografts (Hori et al., 2006; Shen et al., 2007). In a recent study, approximately half of the primary uveal melanoma cell lines tested constitutively expressed PD-L1 and only 20% of the metastases cell lines were positive (Yang et al., 2009). However, exposure to the proinflammatory cytokine IFN-γ resulted in the expression of PD-L1 on primary and metastases cell lines. Thus, uveal melanomas have the capacity to sense the presence of an inflammatory response in the form of IFN-γ and respond by upregulating molecules such as IDO and PD-L1 that launch a counter attack that extinguishes immune-mediated inflammation directed against uveal melanomas. However, this escape mechanism can be circumvented. Uveal melanoma cells transfected with the T cell co-stimulatory molecule, CD80, do not upregulate PD-L1 when exposed to IFN-γ and instead, activate T lymphocytes (Haile et al., 2011). This finding suggests that unraveling the mysteries of immune privilege may have important implications for designing therapeutic modalities for managing malignancies such as uveal melanoma that have adopted immune privilege as a strategy for escaping immune surveillance.

BUFFERING EFFECTS CRPs

Complement regulatory proteins are expressed in both soluble and cell membrane-bound forms throughout the eye and act as buffers to limit spontaneous inflammation and complement-mediated cytolysis of ocular cells. Uveal melanomas have high-jacked this strategy and express all three categories of CRPs (CD46, CD55, and CD59), which protect melanoma cells from complement-mediated lysis *in vitro* and presumably *in vivo* (Goslings et al., 1996). There is evidence that at least one proinflammatory cytokine, TNF-α, up regulates CRPs on uveal melanoma cells (Blom et al., 1997). Thus, like PD-L1 and IDO, CRPs have the capacity to be upregulated when inflammation and possibly adaptive immune effector elements are perceived.

CONCLUSION AND PERSPECTIVES

Our understanding of immune privilege has changed significantly over the past 50 years. What was originally perceived as an anatomical anomaly in which the putative absence of lymphatic channels in the eye and brain acted to sequester antigens and create a state of immunological ignorance has evolved into a more complex and dynamic phenomenon that is the sum total of processes and molecules that prevent the induction and expression of both innate and adaptive immunity. It is now widely accepted that immune privilege is an adaptation to protect organs such as the eye and brain, which have limited capacities to regenerate, from immune-mediated injury. However, the same mechanisms and molecules that provide immune privilege to the eye and brain are also present in sites where stem cells reside and by stem cells themselves. Unwitting injury to stem cells by immune-mediated inflammation could have devastating consequences for the host's survival or in the case of the allogeneic fetus, for the survival of the species. Uveal melanomas have "plagiarized" the blueprints used by the eye to establish immune privilege and used them to escape immune surveillance once the tumors leave the eye and metastasize to the liver. Immune privilege in the eye is neither permanent nor absolute. A variety of maneuvers can ablate immune privilege in the eye. Perhaps the next phase of immune privilege research is to take the lessons we have learned in abrogating immune privilege and apply them to the treatment of uveal melanoma metastases.

REFERENCES

Aluvihare, V. R., Kallikourdis, M., and Betz, A. G. (2004). Regulatory T cells mediate maternal tolerance to the fetus. *Nat. Immunol.* 5, 266–271.

Aluvihare, V. R., Kallikourdis, M., and Betz, A. G. (2005). Tolerance, suppression and the fetal allograft. *J. Mol. Med.* 83, 88–96.

Apte, R. S., Mayhew, E., and Niederkorn, J. Y. (1997). Local inhibition of natural killer cell activity promotes the progressive growth of intraocular tumors. *Invest. Ophthalmol. Vis. Sci.* 38, 1277–1282.

Apte, R. S., and Niederkorn, J. Y. (1996). Isolation and characterization of a unique natural killer cell inhibitory factor present in the anterior chamber of the eye. *J. Immunol.* 156, 2667–2673.

Apte, R. S., Sinha, D., Mayhew, E., Wistow, G. J., and Niederkorn, J. Y. (1998). Cutting edge: role of macrophage migration inhibitory factor in inhibiting NK cell activity and preserving immune privilege. *J. Immunol.* 160, 5693–5696.

Arck, P. C., Ferrick, D. A., Steele-Norwood, D., Croitoru, K., and Clark, D. A. (1997). Murine T cell determination of pregnancy outcome: I. Effects of strain, alphabeta T cell receptor, gammadelta T cell receptor, and gammadelta T cell subsets. *Am. J. Reprod. Immunol.* 37, 492–502.

Arcuri, F., Cintorino, M., Carducci, A., Papa, S., Riparbelli, M. G., Mangioni, S., Di Blasio, A. M., Tosi, P., and Vigano, P. (2006). Human decidual natural killer cells as a source and target of macrophage migration inhibitory factor. *Reproduction* 131, 175–182.

Asherson, G. L., and Stone, S. H. (1965). Selective and specific inhibition of 24 hour skin reactions in the guinea-pig. I. Immune deviation: description of the phenomenon and the effect of splenectomy. *Immunology* 9, 205–217.

Bellgrau, D., Gold, D., Selawry, H., Moore, J., Franzusoff, A., and Duke, R. C. (1995). A role for CD95 ligand in preventing graft rejection. *Nature* 377, 630–632.

Beutelspacher, S. C., Pillai, R., Watson, M. P., Tan, P. H., Tsang, J., McClure, M. O., George, A. J., and Larkin, D. F. (2006). Function of indoleamine 2,3-dioxygenase in corneal allograft rejection and prolongation of allograft survival by over-expression. *Eur. J. Immunol.* 36, 690–700.

Blom, D. J., Goslings, W. R., De Waard-Siebinga, I., Luyten, G. P., Claas, F. H., Gorter, A., and Jager, M. J. (1997). Lack of effect of different cytokines on expression of membrane-bound regulators of complement activity on human uveal melanoma cells. *J. Interferon Cytokine Res.* 17, 695–700.

Bonde, S., and Zavazava, N. (2006). Immunogenicity and engraftment of mouse embryonic stem cells in allogeneic recipients. *Stem Cells* 24, 2192–2201.

Bora, N. S., Gobleman, C. L., Atkinson, J. P., Pepose, J. S., and Kaplan, H. J. (1993). Differential expression of the complement regulatory proteins in the human eye. *Invest. Ophthalmol. Vis. Sci.* 34, 3579–3584.

Chen, P. W., Mellon, J. K., Mayhew, E., Wang, S., He, Y. G., Hogan, N., and Niederkorn, J. Y. (2007). Uveal melanoma expression of indoleamine 2,3-deoxygenase: establishment of an immune privileged environment by tryptophan depletion. *Exp. Eye Res.* 85, 617–625.

Chidgey, A. P., and Boyd, R. L. (2008). Immune privilege for stem cells: not as simple as it looked. *Cell Stem Cell* 3, 357–358.

Cousins, S. W., McCabe, M. M., Danielpour, D., and Streilein, J. W. (1991). Identification of transforming growth factor-beta as an immunosuppressive factor in aqueous humor. *Invest. Ophthalmol. Vis. Sci.* 32, 2201–2211.

Crispe, I. N. (2009). The liver as a lymphoid organ. *Annu. Rev. Immunol.* 27, 147–163.

Dal Secco, V., Riccioli, A., Padula, F., Ziparo, E., and Filippini, A. (2008). Mouse Sertoli cells display phenotypical and functional traits of antigen-presenting cells in response to interferon gamma. *Biol. Reprod.* 78, 234–242.

de Waard-Siebinga, I., Hilders, C. G., Hansen, B. E., van Delft, J. L., and Jager, M. J. (1996). HLA expression and tumor-infiltrating immune cells in uveal melanoma. *Graefes Arch. Clin. Exp. Ophthalmol.* 234, 34–42.

Ding, H., Wu, X., and Gao, W. (2005). PD-L1 is expressed by human renal tubular epithelial cells and suppresses T cell cytokine synthesis. *Clin. Immunol.* 115, 184–191.

Ditzian-Kadanoff, R. (1999). Testicular-associated immune deviation and prevention of adjuvant-induced arthritis by three tolerization methods. *Scand. J. Immunol.* 50, 150–158.

Dong, H., Strome, S. E., Salomao, D. R., Tamura, H., Hirano, F., Flies, D. B., Roche, P. C., Lu, J., Zhu, G., Tamada, K., Lennon, V. A., Celis, E., and Chen, L. (2002). Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat. Med.* 8, 793–800.

Donoso, L. A., Shields, J. A., Augsburger, J. J., Orth, D. H., and Johnson, P. (1985). Metastatic uveal melanoma: diffuse hepatic metastasis in a patient with concurrent normal serum liver enzyme levels and liver scan. *Arch. Ophthalmol.* 103, 758.

D'Orazio, T. J., DeMarco, B. M., Mayhew, E. S., and Niederkorn, J. Y. (1999). Effect of aqueous humor on apoptosis of inflammatory cell types. *Invest. Ophthalmol. Vis. Sci.* 40, 1418–1426.

Drukker, M., Katchman, H., Katz, G., Even-Tov Friedman, S., Shezen, E., Hornstein, E., Mandelboim, O., Reisner, Y., and Benvenisty, N. (2006). Human embryonic stem cells and their differentiated derivatives are less susceptible to immune rejection than adult cells. *Stem Cells* 24, 221–229.

Drukker, M., Katz, G., Urbach, A., Schuldiner, M., Markel, G., Itskovitz-Eldor, J., Reubinoff, B., Mandelboim, O., and Benvenisty, N. (2002). Characterization of the expression of MHC proteins in human embryonic stem cells. *Proc. Natl. Acad. Sci. U.S.A.* 99, 9864–9869.

Einhorn, L. H., Burgess, M. A., and Gottlieb, J. A. (1974). Metastatic patterns of choroidal melanoma. *Cancer* 34, 1001–1004.

Esser, P., Grisanti, S., and Bartz-Schmidt, K. (2001). TGF-beta in uveal melanoma. *Microsc. Res. Tech.* 52, 396–400.

Fallarino, F., Luca, G., Calvitti, M., Mancuso, F., Nastruzzi, C., Fioretti, M. C., Grohmann, U., Becchetti, E., Burgevin, A., Kratzer, R., van Endert, P., Boon, L., Puccetti, P., and Calafiore, R. (2009). Therapy of experimental type 1 diabetes by isolated Sertoli cell xenografts alone. *J. Exp. Med.* 206, 2511–2526.

Frumento, G., Rotondo, R., Tonetti, M., Damonte, G., Benatti, U., and Ferrara, G. B. (2002). Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. *J. Exp. Med.* 196, 459–468.

Fujisaki, J., Wu, J., Carlson, A. L., Silberstein, L., Putheti, P., Larocca, R., Gao, W., Saito, T. I., Lo Celso, C., Tsuyuzaki, H., Sato, T., Cote, D., Sykes, M., Strom, T. B., Scadden, D. T., and Lin, C. P. (2011). In vivo imaging of Treg cells providing immune privilege to the haematopoietic stem-cell niche. *Nature* 474, 216–219.

Gao, B., Radaeva, S., and Park, O. (2009). Liver natural killer and natural killer T cells: immunobiology and emerging roles in liver diseases. *J. Leukoc. Biol.* 86, 513–528.

Godfrey, D. I., Hammond, K. J., Poulton, L. D., Smyth, M. J., and Baxter, A. G. (2000). NKT cells: facts, functions and fallacies. *Immunol. Today* 21, 573–583.

Goslings, W. R., Blom, D. J., de Waard-Siebinga, I., van Beelen, E., Claas, F. H., Jager, M. J., and Gorter, A. (1996). Membrane-bound regulators of complement activation in uveal melanomas. CD46, CD55, and CD59 in uveal melanomas. *Invest. Ophthalmol. Vis. Sci.* 37, 1884–1891.

Goslings, W. R., Prodeus, A. P., Streilein, J. W., Carroll, M. C., Jager, M. J., and Taylor, A. W. (1998). A small molecular weight factor in aqueous humor acts on C1q to prevent antibody-dependent complement activation. *Invest. Ophthalmol. Vis. Sci.* 39, 989–995.

Granstein, R. D., Staszewski, R., Knisely, T. L., Zeira, E., Nazareno, R., Latina, M., and Albert, D. M. (1990). Aqueous humor contains transforming growth factor-beta and a small (less than 3500 daltons) inhibitor of thymocyte proliferation. *J. Immunol.* 144, 3021–3027.

Griffith, T. S., Brunner, T., Fletcher, S. M., Green, D. R., and Ferguson, T. A. (1995). Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science* 270, 1189–1192.

Haile, S. T., Bosch, J. J., Agu, N. I., Zeender, A. M., Somasundaram, P., Srivastava, M. K., Britting, S., Wolf, J. B., Ksander, B. R., and Ostrand-Rosenberg, S. (2011). Tumor cell programmed death ligand 1-mediated T cell suppression is overcome by coexpression of CD80. *J. Immunol.* 186, 6822–6829.

He, Y. G., Mayhew, E., Mellon, J., and Niederkorn, J. Y. (2004). Expression and possible function of IL-2 and IL-15 receptors on human uveal melanoma cells. *Invest. Ophthalmol. Vis. Sci.* 45, 4240–4246.

Holets, L. M., Carletti, M. Z., Kshirsagar, S. K., Christenson, L. K., and Petroff, M. G. (2009). Differentiation-induced post-transcriptional control of B7-H1 in human trophoblast cells. *Placenta* 30, 48–55.

Hori, J., Wang, M., Miyashita, M., Tanemoto, K., Takahashi, H., Takemori, T., Okumura, K., Yagita, H., and Azuma, M. (2006). B7-H1-induced apoptosis as a mechanism of immune privilege of corneal allografts. *J. Immunol.* 177, 5928–5935.

Ishitani, A., and Geraghty, D. E. (1992). Alternative splicing of HLA-G transcripts yields proteins with primary structures resembling both class I and class II antigens. *Proc. Natl. Acad. Sci. U.S.A.* 89, 3947–3951.

Ito, T., Ito, N., Saatoff, M., Hashizume, H., Fukamizu, H., Nickoloff, B. J., Takigawa, M., and Paus, R. (2008). Maintenance of hair follicle immune privilege is linked to prevention of NK cell attack. *J. Invest. Dermatol.* 128, 1196–1206.

James, E., Chai, J. G., Dewchand, H., Macchiarulo, E., Dazzi, F., and Simpson, E. (2003). Multiparity induces priming to male-specific minor histocompatibility antigen, HY, in mice and humans. *Blood* 102, 388–393.

Kang, H., Wu, W. Y., Lo, B. K., Yu, M., Leung, G., Shapiro, J., and McElwee, K. J. (2010). Hair follicles from alopecia areata patients exhibit alterations in immune privilege-associated gene expression in advance of hair loss. *J. Invest. Dermatol.* 130, 2677–2680.

Kaplan, H. J., Stevens, T. R., and Streilein, J. W. (1975). Transplantation immunology of the anterior chamber of the eye. I. An intra-ocular graft-vs-host reaction (immunogenic anterior uveitis). *J. Immunol.* 115, 800–804.

Kaplan, H. J., and Streilein, J. W. (1977). Immune response to immunization via the anterior chamber of the eye. I.F. lymphocyte-induced immune deviation. *J. Immunol.* 118, 809–814.

Kaplan, H. J., and Streilein, J. W. (1978). Immune response to immunization via the anterior chamber of the eye. II. An analysis of F1 lymphocyte-induced immune deviation. *J. Immunol.* 120, 689–693.

Kinori, M., Kloepper, J. E., and Paus, R. (2011). Can the hair follicle become a model for studying selected aspects of human ocular immune privilege? *Invest. Ophthalmol. Vis. Sci.* 52, 4447–4458.

Kosiewicz, M. M., Alard, P., and Streilein, J. W. (1998). Alterations in cytokine production following intraocular injection of soluble protein antigen: impairment in IFN-gamma and induction of TGF- beta and IL-4 production. *J. Immunol.* 161, 5382–5390.

Kovats, S., Main, E. K., Librach, C., Stubblebine, M., Fisher, S. J., and DeMars, R. (1990). A class I antigen, HLA-G, expressed in human trophoblasts. *Science* 248, 220–223.

Ksander, B. R., Rubsamen, P. E., Olsen, K. R., Cousins, S. W., and Streilein, J. W. (1991). Studies of tumor-infiltrating lymphocytes from a human choroidal melanoma. *Invest. Ophthalmol. Vis. Sci.* 32, 3198–3208.

Lass, J. H., Walter, E. I., Burris, T. E., Grossniklaus, H. E., Roat, M. I., Skelnik, D. L., Needham, L., Singer, M., and Medof, M. E. (1990). Expression of two molecular forms of the complement decay-accelerating factor in the eye and lacrimal gland. *Invest. Ophthalmol. Vis. Sci.* 31, 1136–1148.

Le Discorde, M., Moreau, P., Sabatier, P., Legeais, J. M., and Carosella, E. D. (2003). Expression of HLA-G in human cornea, an immune-privileged tissue. *Hum. Immunol.* 64, 1039–1044.

Lee, H. O., Herndon, J. M., Barreiro, R., Griffith, T. S., and Ferguson, T. A. (2002). TRAIL: a mechanism of tumor surveillance in an immune privileged site. *J. Immunol.* 169, 4739–4744.

Lengerova, A., and Vojtiskova, M. (1963). Prolonged survival of syngeneic male skin grafts in parous C57B1 mice. *Folia Biol. (Praha)* 9, 72–74.

Li, H., Ren, J., Dhabuwala, C. B., and Shichi, H. (1997). Immunotolerance induced by intratesticular antigen priming: expression of TGF-beta, Fas and Fas ligand. *Ocul. Immunol. Inflamm.* 5, 75–84.

Li, L., Baroja, M. L., Majumdar, A., Chadwick, K., Rouleau, A., Gallacher, L., Ferber, I., Lebkowski, J., Martin, T., Madrenas, J., and Bhatia, M. (2004a). Human embryonic stem cells possess immune-privileged properties. *Stem Cells* 22, 448–456.

Li, X., Taylor, S., Zegarelli, B., Shen, S., O'Rourke, J., and Cone, R. E. (2004b). The induction of splenic suppressor T cells through an immune-privileged site requires an

intact sympathetic nervous system. *J. Neuroimmunol.* 153, 40–49.

Ljunggren, H. G., and Karre, K. (1990). In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol. Today* 11, 237–244.

Ma, D., Luyten, G. P., Luider, T. M., and Niederkorn, J. Y. (1995). Relationship between natural killer cell susceptibility and metastasis of human uveal melanoma cells in a murine model. *Invest. Ophthalmol. Vis. Sci.* 36, 435–441.

Ma, D., and Niederkorn, J. Y. (1995). Transforming growth factor-beta down-regulates major histocompatibility complex class I antigen expression and increases the susceptibility of uveal melanoma cells to natural killer cell-mediated cytolysis. *Immunology* 86, 263–269.

Malina, H. Z., and Martin, X. D. (1993). Indoleamine 2,3-dioxygenase activity in the aqueous humor, iris/ciliary body, and retina of the bovine eye. *Graefes Arch. Clin. Exp. Ophthalmol.* 231, 482–486.

Medawar, P. B. (1948). Immunity to homologous grafted skin. III. The fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. *Br. J. Exp. Pathol.* 29, 58–69.

Meecham, W. J., Char, D. H., and Kaleta-Michaels, S. (1992). Infiltrating lymphocytes and antigen expression in uveal melanoma. *Ophthalmic Res.* 24, 20–26.

Meinhardt, A., Bacher, M., McFarlane, J. R., Metz, C. N., Seitz, J., Hedger, M. P., de Kretser, D. M., and Bucala, R. (1996). Macrophage migration inhibitory factor production by Leydig cells: evidence for a role in the regulation of testicular function. *Endocrinology* 137, 5090–5095.

Meinhardt, A., and Hedger, M. P. (2011). Immunological, paracrine and endocrine aspects of testicular immune privilege. *Mol. Cell. Endocrinol.* 335, 60–68.

Mellor, A. L., Keskin, D. B., Johnson, T., Chandler, P., and Munn, D. H. (2002). Cells expressing indoleamine 2,3-dioxygenase inhibit T cell responses. *J. Immunol.* 168, 3771–3776.

Mellor, A. L., Sivakumar, J., Chandler, P., Smith, K., Molina, H., Mao, D., and Munn, D. H. (2001). Prevention of T cell-driven complement activation and inflammation by tryptophan catabolism during pregnancy. *Nat. Immunol.* 2, 64–68.

Menard, C., Hagege, A. A., Agbulut, O., Barro, M., Morichetti, M. C., Brasselet, C., Bel, A., Messas, E., Bissery, A., Bruneval, P., Desnos, M., Puceat, M., and Menasche, P. (2005). Transplantation of cardiac-committed mouse embryonic stem cells to infarcted sheep myocardium: a preclinical study. *Lancet* 366, 1005–1012.

Mital, P., Hinton, B. T., and Dufour, J. M. (2011). The blood-testis and blood-epididymis barriers are more than just their tight junctions. *Biol. Reprod.* 84, 851–858.

Moffett-King, A. (2002). Natural killer cells and pregnancy. *Nat. Rev. Immunol.* 2, 656–663.

Munn, D. H., Shafizadeh, E., Attwood, J. T., Bondarev, I., Pashine, A., and Mellor, A. L. (1999). Inhibition of T cell proliferation by macrophage tryptophan catabolism. *J. Exp. Med.* 189, 1363–1372.

Namba, K., Kitaichi, N., Nishida, T., and Taylor, A. W. (2002). Induction of regulatory T cells by the immunomodulating cytokines alpha-melanocyte-stimulating hormone and transforming growth factor-beta2. *J. Leukoc. Biol.* 72, 946–952.

Nemeth, E., Baird, A. W., and O'Farrelly, C. (2009). Microanatomy of the liver immune system. *Semin. Immunopathol.* 31, 333–343.

Niederkorn, J. Y. (2006). See no evil, hear no evil, do no evil: the lessons of immune privilege. *Nat. Immunol.* 7, 354–359.

Niederkorn, J. Y. (2009). Immune escape mechanisms of intraocular tumors. *Prog. Retin. Eye Res.* 28, 329–347.

Niederkorn, J. Y. (2010). "NK cells in the eye," in *Natural Killer Cells*, eds M. T. Lotze and A. W. Thomson (Amsterdam: Academic Press), 385–401.

Niederkorn, J. Y., Chiang, E. Y., Ungchusri, T., and Stroynowski, I. (1999). Expression of a nonclassical MHC class Ib molecule in the eye. *Transplantation* 68, 1790–1799.

Niederkorn, J. Y., and Stein-Streilein, J. (2010). History and physiology of immune privilege. *Ocul. Immunol. Inflamm.* 18, 19–23.

Nishida, T., and Taylor, A. W. (1999). Specific aqueous humor factors induce activation of regulatory T cells. *Invest. Ophthalmol. Vis. Sci.* 40, 2268–2274.

Nussbaum, J., Minami, E., Laflamme, M. A., Virag, J. A., Ware, C. B., Masino, A., Muskheli, V., Pabon, L., Reinecke, H., and Murry, C. E. (2007). Transplantation of undifferentiated murine embryonic stem cells in the heart: teratoma formation and immune response. *FASEB J.* 21, 1345–1357.

Okazaki, T., and Honjo, T. (2007). PD-1 and PD-1 ligands: from discovery to clinical application. *Int. Immunol.* 19, 813–824.

Okuma, Y., Saito, K., O'Connor, A. E., Phillips, D. J., de Kretser, D. M., and Hedger, M. P. (2005). Reciprocal regulation of activin A and inhibin B by interleukin-1 (IL-1) and follicle-stimulating hormone (FSH) in rat Sertoli cells in vitro. *J. Endocrinol.* 185, 99–110.

Petroff, M. G. (2005). Immune interactions at the maternal-fetal interface. *J. Reprod. Immunol.* 68, 1–13.

Petroff, M. G., Chen, L., Phillips, T. A., Azzola, D., Sedlmayr, P., and Hunt, J. S. (2003). B7 family molecules are favorably positioned at the human maternal-fetal interface. *Biol. Reprod.* 68, 1496–1504.

Repp, A. C., Mayhew, E. S., Apte, S., and Niederkorn, J. Y. (2000). Human uveal melanoma cells produce macrophage migration-inhibitory factor to prevent lysis by NK cells. *J. Immunol.* 165, 710–715.

Robertson, N. J., Brook, F. A., Gardner, R. L., Cobbold, S. P., Waldmann, H., and Fairchild, P. J. (2007). Embryonic stem cell-derived tissues are immunogenic but their inherent immune privilege promotes the induction of tolerance. *Proc. Natl. Acad. Sci. U.S.A.* 104, 20920–20925.

Robertson, S. A., Ingman, W. V., O'Leary, S., Sharkey, D. J., and Tremellen, K. P. (2002). Transforming growth factor beta – a mediator of immune deviation in seminal plasma. *J. Reprod. Immunol.* 57, 109–128.

Robertson, S. A., Mau, V. J., Hudson, S. N., and Tremellen, K. P. (1997). Cytokine-leukocyte networks and the establishment of pregnancy. *Am. J. Reprod. Immunol.* 37, 438–442.

Rouas-Freiss, N., Goncalves, R. M., Menier, C., Dausset, J., and Carosella, E. D. (1997). Direct evidence to support the role of HLA-G in protecting the fetus from maternal uterine natural killer cytolysis. *Proc. Natl. Acad. Sci. U.S.A.* 94, 11520–11525.

Rouas-Freiss, N., Khalil-Daher, I., Marchal-Bras Goncalves, R., Menier, C., Dausset, J., and Carosella, E. D. (1999). Role of HLA-G in maternal-fetal immune tolerance. *Transplant. Proc.* 31, 724–725.

Ryan, A. F., Grendell, R. L., Geraghty, D. E., and Golos, T. G. (2002). A soluble isoform of the rhesus monkey nonclassical MHC class I molecule Mamu-AG is expressed in the placenta and the testis. *J. Immunol.* 169, 673–683.

Ryu, Y. H., and Kim, J. C. (2007). Expression of indoleamine 2,3-dioxygenase in human corneal cells as a local immunosuppressive factor. *Invest. Ophthalmol. Vis. Sci.* 48, 4148–4152.

Saunders, P. A., Hendrycks, V. R., Lidinsky, W. A., and Woods, M. L. (2005). PD-L2:PD-1 involvement in T cell proliferation, cytokine production, and integrin-mediated adhesion. *Eur. J. Immunol.* 35, 3561–3569.

Shen, L., Jin, Y., Freeman, G. J., Sharpe, A. H., and Dana, M. R. (2007). The function of donor versus recipient programmed death-ligand 1 in corneal allograft survival. *J. Immunol.* 179, 3672–3679.

Singh, A. D., and Topham, A. (2003). Incidence of uveal melanoma in the United States: 1973–1997. *Ophthalmology* 110, 956–961.

Slukvin, I. I., Watkins, D. I., and Golos, T. G. (1999). Tissue distribution of the mRNA for a rhesus monkey major histocompatibility class Ib molecule, Mamu-AG. *Tissue Antigens* 53, 282–291.

Sohn, J. H., Kaplan, H. J., Suk, H. J., Bora, P. S., and Bora, N. S. (2000a). Complement regulatory activity of normal human intraocular fluid is mediated by MCP, DAF, and CD59. *Invest. Ophthalmol. Vis. Sci.* 41, 4195–4202.

Sohn, J. H., Kaplan, H. J., Suk, H. J., Bora, P. S., and Bora, N. S. (2000b). Chronic low level complement activation within the eye is controlled by intraocular complement regulatory proteins. *Invest. Ophthalmol. Vis. Sci.* 41, 3492–3502.

Somerset, D. A., Zheng, Y., Kilby, M. D., Sansom, D. M., and Drayson, M. T. (2004). Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T-cell subset. *Immunology* 112, 38–43.

Sonoda, K. H., Faunce, D. E., Taniguchi, M., Exley, M., Balk, S., and Stein-Streilein, J. (2001). NK T cell-derived IL-10 is essential for the differentiation of antigen- specific T regulatory cells in systemic tolerance. *J. Immunol.* 166, 42–50.

Streilein, J. W., and Niederkorn, J. Y. (1981). Induction of anterior chamber-associated immune deviation requires an intact, functional spleen. *J. Exp. Med.* 153, 1058–1067.

Stuart, P. M., Griffith, T. S., Usui, N., Pepose, J., Yu, X., and Ferguson, T. A. (1997). CD95 ligand (FasL)-induced apoptosis is necessary for corneal allograft survival. *J. Clin. Invest.* 99, 396–402.

Swijnenburg, R. J., Schrepfer, S., Govaert, J. A., Cao, F., Ransohoff, K., Sheikh, A. Y., Haddad, M., Connolly, A. J., Davis, M. M., Robbins, R. C.,

and Wu, J. C. (2008). Immunosuppressive therapy mitigates immunological rejection of human embryonic stem cell xenografts. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12991–12996.

Taylor, A., and Namba, K. (2001). In vitro induction of CD25 + CD4+ regulatory T cells by the neuropeptide alpha-melanocyte stimulating hormone (alpha-MSH). *Immunol. Cell Biol.* 79, 358–367.

Taylor, A. W. (2007). Ocular immunosuppressive microenvironment. *Chem. Immunol.* 92, 71–85.

Taylor, A. W., Alard, P., Yee, D. G., and Streilein, J. W. (1997). Aqueous humor induces transforming growth factor-beta (TGF-beta)- producing regulatory T-cells. *Curr. Eye Res.* 16, 900–908.

Taylor, A. W., Streilein, J. W., and Cousins, S. W. (1992). Identification of alpha-melanocyte stimulating hormone as a potential immunosuppressive factor in aqueous humor. *Curr. Eye Res.* 11, 1199–1206.

Taylor, A. W., Streilein, J. W., and Cousins, S. W. (1994a). Alpha-melanocyte-stimulating hormone suppresses antigen-stimulated T cell production of gamma-interferon. *Neuroimmunomodulation* 1, 188–194.

Taylor, A. W., Streilein, J. W., and Cousins, S. W. (1994b). Immunoreactive vasoactive intestinal peptide contributes to the immunosuppressive activity of normal aqueous humor. *J. Immunol.* 153, 1080–1086.

Taylor, A. W., and Yee, D. G. (2003). Somatostatin is an immunosuppressive factor in aqueous humor. *Invest. Ophthalmol. Vis. Sci.* 44, 2644–2649.

Terness, P., Bauer, T. M., Rose, L., Dufter, C., Watzlik, A., Simon, H., and Opelz, G. (2002). Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. *J. Exp. Med.* 196, 447–457.

Tremellen, K. P., Seamark, R. F., and Robertson, S. A. (1998). Seminal transforming growth factor beta1 stimulates granulocyte-macrophage colony-stimulating factor production and inflammatory cell recruitment in the murine uterus. *Biol. Reprod.* 58, 1217–1225.

Uccelli, A., Moretta, L., and Pistoia, V. (2008). Mesenchymal stem cells in health and disease. *Nat. Rev. Immunol.* 8, 726–736.

Uyttenhove, C., Pilotte, L., Theate, I., Stroobant, V., Colau, D., Parmentier, N., Boon, T., and Van den Eynde, B. J. (2003). Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. *Nat. Med.* 9, 1269–1274.

van Dooremaal, J. C. (1873). Die Entwicklung der in fremden Grund versetzten lebenden Geweba. *Albrecht Von Graefes Arch. Ophthalmol.* 19, 358–373.

Verajankorva, E., Setala, N., Teros, T., Salmi, A. A., and Pollanen, P. (2002). Testicular-associated immune deviation: flushing of the testicular lymph sinusoids induces immunosuppression and inhibits formation of EAE in SJL mice. *Scand. J. Immunol.* 55, 478–483.

Verbik, D. J., Murray, T. G., Tran, J. M., and Ksander, B. R. (1997). Melanomas that develop within the eye inhibit lymphocyte proliferation. *Int. J. Cancer* 73, 470–478.

Vetter, C. S., Lieb, W., Brocker, E. B., and Becker, J. C. (2004). Loss of nonclassical MHC molecules MIC-A/B expression during progression of uveal melanoma. *Br. J. Cancer* 91, 1495–1499.

Vigano, P., Cintorino, M., Schatz, F., Lockwood, C. J., and Arcuri, F. (2007). The role of macrophage migration inhibitory factor in maintaining the immune privilege at the fetal-maternal interface. *Semin. Immunopathol.* 29, 135–150.

Wang, S., Boonman, Z. F., Li, H. C., He, Y., Jager, M. J., Toes, R. E., and Niederkorn, J. Y. (2003). Role of TRAIL and IFN-gamma in CD4+ T cell-dependent tumor rejection in the anterior chamber of the eye. *J. Immunol.* 171, 2789–2796.

Wang, Y., Goldschneider, I., Foss, D., Wu, D. Y., O'Rourke, J., and Cone, R. E. (1997). Direct thymic involvement in anterior chamber-associated immune deviation: evidence for a nondeletional mechanism of centrally induced tolerance to extrathymic antigens in adult mice. *J. Immunol.* 158, 2150–2155.

Wang, Y., Goldschneider, I., O'Rourke, J., and Cone, R. E. (2001). Blood mononuclear cells induce regulatory NK T thymocytes in anterior chamber-associated immune deviation. *J. Leukoc. Biol.* 69, 741–746.

Wilbanks, G. A., and Streilein, J. W. (1990). Characterization of suppressor cells in anterior chamber-associated immune deviation (ACAID) induced by soluble antigen. Evidence of two functionally and phenotypically distinct T-suppressor cell populations. *Immunology* 71, 383–389.

Wilbanks, G. A., and Streilein, J. W. (1991). Studies on the induction of anterior chamber-associated immune deviation (ACAID). 1. Evidence that an antigen-specific, ACAID- inducing, cell-associated signal exists in the peripheral blood. *J. Immunol.* 146, 2610–2617.

Wu, D. C., Boyd, A. S., and Wood, K. J. (2008). Embryonic stem cells and their differentiated derivatives have a fragile immune privilege but still represent novel targets of immune attack. *Stem Cells* 26, 1939–1950.

Yamagami, S., Kawashima, H., Tsuru, T., Yamagami, H., Kayagaki, N., Yagita, H., Okumura, K., and Gregerson, D. S. (1997). Role of Fas-Fas ligand interactions in the immunorejection of allogeneic mouse corneal transplants. *Transplantation* 64, 1107–1111.

Yang, W., Li, H., Chen, P. W., Alizadeh, H., He, Y., Hogan, R. N., and Niederkorn, J. Y. (2009). PD-L1 expression on human ocular cells and its possible role in regulating immune-mediated ocular inflammation. *Invest. Ophthalmol. Vis. Sci.* 50, 273–280.

Yoshida, M., Kezuka, T., and Streilein, J. W. (2000a). Participation of pigment epithelium of iris and ciliary body in ocular immune privilege. 2. Generation of TGF-beta-producing regulatory T cells. *Invest. Ophthalmol. Vis. Sci.* 41, 3862–3870.

Yoshida, M., Takeuchi, M., and Streilein, J. W. (2000b). Participation of pigment epithelium of iris and ciliary body in ocular immune privilege. 1. Inhibition of T-cell activation in vitro by direct cell-to-cell contact. *Invest. Ophthalmol. Vis. Sci.* 41, 811–821.

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Regulation of CD8$^+$ T cell responses to retinal antigen by local FoxP3$^+$ regulatory T cells

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While pathogenic CD4 T cells are well known mediators of autoimmune uveoretinitis, CD8 T cells can also be uveitogenic. Since preliminary studies indicated that C57BL/6 mice were minimally susceptible to autoimmune uveoretinitis induction by CD8 T cells, the basis of the retinal disease resistance was sought. Mice that express β-galactosidase (βgal) on a retina-specific promoter (arrβgal mice) were backcrossed to mice expressing green fluorescent protein (GFP) and diphtheria toxin (DTx) receptor (DTR) under control of the Foxp3 promoter (Foxp3-DTR/GFP mice), and to T cell receptor transgenic mice that produce βgal-specific CD8 T cells (BG1 mice). These mice were used to explore the role of regulatory T cells in the resistance to retinal autoimmune disease. Experiments with T cells from double transgenic BG1 × Foxp3-DTR/GFP mice transferred into Foxp3-DTR/GFP × arrβgal mice confirmed that the retina was well protected from attempts to induce disease by adoptive transfer of activated BG1 T cells. The successful induction of retinal disease following unilateral intraocular administration of DTx to deplete regulatory T cells showed that the protective activity was dependent on local, toxin-sensitive regulatory T cells; the opposite, untreated eye remained disease-free. Although there were very few Foxp3$^+$ regulatory T cells in the parenchyma of quiescent retina, and they did not accumulate in retina, their depletion by local toxin administration led to disease susceptibility. We propose that these regulatory T cells modulate the pathogenic activity of βgal-specific CD8 T cells in the retinas of arrβgal mice on a local basis, allowing immuno regulation to be responsive to local conditions.

Keywords: tregs, autoimmunity, retina, Foxp3, EAU

INTRODUCTION

The eye contains several tissues that are delicate and/or non-regenerative, rendering them susceptible to inflammatory or tissue destructive immune responses that can have serious consequences for visual function. In response, the eye has developed specialized anatomical features and physiological mechanisms that contribute to maintenance of protective immune functions while limiting non-specific, collateral tissue damage associated with immune responses (Gregerson, 1998; Streilein, 2003; Niederkorn and Wang, 2005; Caspi, 2006; Taylor, 2009; Hori et al., 2010).

Among ocular tissues, the retina is unique in that it is part of the central nervous system (CNS), but differs from brain and spinal cord by the absence of meninges and the lack of lymphatic drainage (Yamada et al., 1991; Cserr et al., 1992). The retina also contains high concentrations of tissue-specific proteins associated with visual transduction including interphotoreceptor retinoid-binding protein (IRBP), arrestin, and photoreceptor opsins. T cell recognition of retinal antigens (Ag) can lead to experimental autoimmune uveoretinitis (EAU) when retinal immune privilege is breached by effector T cell responses. Conversely, the recognition of these Ag by T cells also forms the basis for those mechanisms of retinal immune privilege that are based on Ag-specificity (McPherson and Gregerson, 1994; Egwuagu et al., 1997; Gery and Egwuagu, 2002; Gregerson, 2002; Avichezer et al., 2003a; Ham et al., 2004; Carson et al., 2006; DeVoss et al., 2006; Lambe et al., 2007; Heuss et al., 2012). The actions of regulatory T cells (Tregs) are increasingly recognized to contribute to the immune homeostasis of the eye, and retina (Wenkel and Streilein, 1998; Grajewski et al., 2006; Niederkorn, 2007; Silver et al., 2007; Stein-Streilein and Taylor, 2007; Caspi, 2008).

While multiple lineages of T cells are capable of immunosuppressive activity (Shevach et al., 2006; Jutel and Akdis, 2008), the CD4$^+$CD25$^+$Foxp3$^+$ T cell is the prototypical Treg. Many of these Tregs develop in the thymus in response to self-Ag (natural Tregs, nTregs), in part due to *aire* promotion of peripheral Ag expression by medullary thymic epithelial cells (mTEC; Sakaguchi, 2011). However, CD4$^+$CD25$^+$Foxp3$^+$ Tregs are also generated in the periphery from mature, naïve CD4$^+$ T cells (induced Tregs, iTregs), and are thought to be important in modulating immune responses to microorganisms and autoimmune inflammation (Thorstenson and Khoruts, 2001; Curotto de Lafaille et al., 2004; Lohr et al., 2006; Apostolou et al., 2008). Using CD4$^+$ T cell receptor transgenic mice (βgal TCR mice) specific for *E. coli* βgal, in conjunction with mice expressing βgal as a transgenic neo-self-Ag in the retina (arrβgal mice), we demonstrated that retinal expression of βgal led to regulation of systemic immune responses to βgal (Gregerson and Dou, 2002). This activity was subsequently attributed to the generation of Tregs in the periphery from naïve CD4$^+$ precursors,

especially in lymphopenic hosts (Gregerson et al., 2008, 2009; McPherson et al., 2009; Heuss et al., 2012).

Although it is clear that newly generated iTregs provide protection from retinal autoimmunity, it is not clear how and where these iTregs are made and exert their effects. The βgal antigen in arrβgal mice is of retinal origin, but the site of Treg generation and expression of regulatory activity of the βgal-specific Tregs remains uncertain. The interaction between Ag-bearing dendritic cells (DC) and T cells in draining LNs is a major mechanism for the generation of iTregs (DiPaolo et al., 2007). However, the highly restricted, tissue-specific expression of retinal βgal combined with the apparent lack of lymphatic drainage from retina allows for the possibility that iTregs to retina-specific Ag might be generated and/or act in a local, tissue-specific manner. Evidence for induction of iTregs from naive T cells, but not committed T cells, following their injection into the posterior segment of the eye was recently shown (Zhou et al., 2012). Such a mechanism was consistent with our recent evidence for retinal DC that promoted production of iTregs that were recovered from quiet retina, and correlated the local antigen presenting cell (APC) activity with EAU susceptibility (Heuss et al., 2012).

While many studies have examined the effects of $Foxp3^+$ Tregs on CD4 T cell mediated autoimmunity, relatively few have looked at Treg modulation of the activity of autoreactive CD8 T cells. In studies to investigate the origin and retinal-protective function of Tregs specific for retinal Ags, and establish their role in a CD8 T cell model of autoimmunity, we examined the activity of Tregs from βgal-specific, CD8 TCR Tg mice in conjunction with arrβgal mice, and mice expressing a diphtheria toxin (DTx) receptor (DTR) and/or green fluorescent protein (GFP) under control of the Foxp3 promoter. Although $Foxp3^+$ Tregs were rarely found in the parenchyma of the quiescent retina, local Treg activity was critical for protection of the retina from uveitogenic $CD8^+$ T cells. Our results suggest that immune privilege of the retina is substantially dependent on Ag-specific Tregs that act locally. Although they are present in small numbers, they are sufficient to control the pathogenic and delayed-type hypersensitivity (DTH) activity of the uveitogenic CD8 T cells.

MATERIALS AND METHODS

MICE

Rod photoreceptor cell expression of βgal on the arrestin promoter in arrβgal transgenic mice yields approximately 150 ng βgal/retina, <0.5 ng βgal/pineal gland, and rare, unidentified $βgal^+$ brain cells (Gregerson and Dou, 2002). No other sites of βgal expression have been found. Arrβgal on the B6 background mice were generated from B10.A-arrβgal mice by backcrosses with normal B6 mice for greater than 10 generations. BG1 mice produce CD8 T cells expressing a transgenic Vβ7 TCR specific for the H2-K^b-restricted epitope DAPIYTNV in βgal (Donohue et al., 2006; Tewalt et al., 2009). Foxp3-GFP and Foxp3-DTR/GFP transgenic mice on the B6 background, which express GFP only or GFP and DTR under control of the Foxp3 promoter, respectively, have been described (Fontenot et al., 2005; Kim et al., 2007). Breeding stock was kindly provided by Dr. S. S. Way (University of Minnesota). BG1 mice were backcrossed to the arrβgal mice, and the Foxp3-DTR/GFP mice. Mice were handled in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research, and the University of Minnesota institutional animal care and use guidelines. Mice were housed under specific pathogen-free conditions.

INJECTIONS INTO THE ANTERIOR CHAMBER OF THE EYE, OR SUBCUTANEOUS (S.C.)

Diphtheria toxin and saline injections into the eye were done by the trans-corneal route into the anterior chamber (AC) as previously described (Lehmann et al., 2010). One microliter doses containing saline or 25 ng/μl DTx were given as indicated. DTx injections into the cheek were given s.c. using 25 ng DTx in 10 μl saline as indicated.

CELL CULTURE AND PURIFICATION

Pooled spleen and lymph node (LN) cell suspensions were prepared by tissue homogenization followed by filtration through a 70-μM cell strainer. Lymphocytes were also prepared from whole blood. Red blood cells were lysed using 0.17 M NH_4Cl and the remaining cells were washed twice in PBS and resuspended in X-Vivo 15 media (Lonza) supplemented with sodium pyruvate (100 μg/ml), L-glutamine (784 μg/ml), penicillin (100 U/ml), streptomycin (0.1 mg/ml), β-mercaptoethanol (50 μM), 1× MEM non-essential amino acids, and 2% fetal calf serum (FCS; Sigma). All cultures were maintained at 37°C in an atmosphere of 6% CO_2. The BG1 CD8 T cells were isolated using a CD8 T cell isolation kit (MiltenyiBiotec) per manufacturer's protocols.

FLOW CYTOMETRY

Cells from spleen, LN, or peripheral blood were prepared as described above except that the final suspension was made in FACS buffer (PBS with 2% FCS and 0.02% sodium azide). Fluorescent-labeled antibodies (BD Biosciences or eBioscience) were added to the cell suspensions, from 0.25 to 2.0 μl/10^6 cells, and incubated on ice for 30 min. The cells were washed, resuspended in FACS buffer, and analyzed on a FACS Calibur flow cytometer using Cell Quest (BD Biosciences) or FlowJo (Tree Star) software. Sorting of purified CD4 T cells into regulatory (GFP^+) or effector (GFP^-) populations was done on a FACS Aria flow cytometer (BD Bioscience). For flow cytometry on the cells in the retina, mice were euthanized, perfused, and the retinas removed as described (Lehmann et al., 2010). The retinas were dissociated using a solution of 0.2 μg/ml Liberase/TM (Roche) and 0.05% DNase in DPBS, stained with indicated antibodies, and analyzed as described (Lehmann et al., 2010). Analysis of all the cells collected from a single retina comprised a single sample.

CYTOKINE ASSAYS

Cultures containing 5×10^5 unfractionated spleen/LN cells from BG1 mice were aliquoted in triplicate into 96-well plates in final volume of 200 μl with or without βgal protein (10 μg/ml). Supernatants were harvested 48 h post stimulation and assayed for cytokines by cytometric bead array (BD Bioscience) per manufacturer's protocol.

INDUCTION AND TRANSFER OF TREGS

Foxp3-GFP mice were injected i.v. with 100 μg of βgal or bovine serum albumin (BSA) solubilized in PBS at 1 mg/ml. At 8 days

post-injection, $CD4^+$ T cells were isolated from spleen and LN by negative selection, and sorted for $CD3^+4^+GFP^+$ cells. The cells were washed and resuspended in PBS to 5×10^6/ml. Recipient mice received 5×10^5 cells i.v.

CELL TRANSFERS AND INDUCTION AND ANALYSIS OF AUTOIMMUNE DISEASE

For transfer of activated T cells, purified lymphocytes from LN and spleen of BG1 mice, or BG1 × Foxp3-DTR/GFP transgenic mice, as indicated, were stimulated with their cognate peptide (0.5 μM) and irradiated (3000 R) B6 splenocytes at a 1:10 ratio in the presence of IL-6 (5 ng/ml), IL-1 (2.5 ng/ml), and TGFβ (1 ng/ml). IL-2 (10 U/ml) was added 48 h post stimulation and the cells were cultured an additional 6 h. The cultures were washed and resuspended in PBS to a concentration of 2×10^7 cells/ml. Recipient mice were inoculated i.p. with 5-20 $\times 10^6$ cells. Prior to transfer, the indicated recipients were irradiated (900 R) or depleted of $CD25^+$ cells by anti-CD25 antibody (BioXcell, clone PC61, 500 μg given i.p. three times at 3 day intervals with the last dose given 3 days prior to T cell transfer). βgal-specific T cells were also generated by s.c. immunization of mice with a single, hindlimb 200 μl dose containing 200 μg βgal emulsified in complete Freund's adjuvant (CFA) containing 5 mg/ml *M. tuberculosis* (H37Ra, Sigma) followed by 0.5 μg pertussis toxin (Sigma) per mouse given in 100 μl saline i.p. At the indicated times post-transfer or post-immunization, the eyes were harvested, fixed in 10% buffered formalin, paraffin embedded, sectioned (5 μM), and stained with H&E. The slides were examined in a masked fashion, and EAU severity was scored from 0 (no disease) to 5 (complete loss of photoreceptor cells plus damage to the inner layers of the retina) based on histopathological changes to the retina (Gregerson et al., 1993).

ANALYSIS OF THE DELAYED-TYPE HYPERSENSITIVITY RESPONSE

Analysis of the DTH response (ear swelling assay) was done by injection of βgal (50 μg in 10 μl) into the ear pinna as previously described (Gregerson and Dou, 2002).

GENERATION AND ANALYSIS OF RADIATION-BONE MARROW CHIMERAS

Tibias and femurs were harvested from euthanized donor mice. Bone marrow (BM) was flushed out with calcium, magnesium-free phosphate-buffered saline (CMF-PBS), passed through a 70-μm mesh filter, and resuspended in CMF-PBS. Red blood cells were lysed in 0.17 M NH_4Cl. The BM was washed with CMF-PBS and resuspended to 5×10^7 cells/ml. Recipient mice were given 1×10^7 BM cells via i.p. injection. Recipient mice were irradiated with 1200 R of total body irradiation, given as a split dose (^{137}Cs, 2 × 600 R with a 3-h interval), prior to BM transfer. Chimerism was assayed after 19 weeks by flow cytometry for $CD45.1^+$ and $CD45.2^+$ cells in blood. Activated BG1 T cells were injected at 4.5×10^6 per recipient, and the eyes harvested 21 days later.

RESULTS

ANALYSIS OF βGAL-SPECIFIC TCR Tg MICE

We previously described CD4 T cell TCR Tg mice specific for βgal (βgalTCR) on the B10.A background. When backcrossed to mice expressing βgal in retina (arrβgal × βgalTCR mice), no spontaneous EAU was observed nor did immunization with βgal in CFA produce EAU in the double transgenic mice (Gregerson et al., 2009; McPherson et al., 2009). $CD4^+25^+$ Tregs were found to contribute to the resistance to EAU. The potential participation of Tregs in modulating the pathogenesis of EAU mediated by CD8 T cells specific for the same target Ag in retina was sought using the βgal-specific, BG1 CD8 TCR mice.

Analysis of $CD3^+$ splenocytes from BG1 mice showed that BG1 T cells were highly enriched for $CD8^+V\beta7^+$ cells (**Figure 1A**). To learn if BG1 T cells maintained a naïve phenotype in mice expressing retinal βgal, we compared T cells from BG1 mice with T cells from BG1 × arrβgal double transgenic mice for cell surface markers associated with Ag recognition. FACS analysis of LN $CD8^+V\beta7^+$ T cells showed similar expression levels of CD44, CD45RB, CD62L, and CD69 between BG1 mice and their double Tg counterparts (**Figure 1B**). The small number of $CD8^+V\beta7^+$ T cells from non-Tg B6 mice had a higher frequency of $CD44^+45RB^+$ cells (**Figure 1B**). As a control for T cell activation, the BG1 mice were immunized with βgal in CFA adjuvant. The $CD8^+V\beta7^+$ T cells showed a substantial shift to expression levels associated with T cell activation, higher CD44, and CD69, and lower CD45RB and CD62L (**Figure 1B**). The level of $CD25^+FoxP3^+$ T cells sampled from spleen was also similar between BG1 mice and their double Tg counterparts (**Figure 1C**), showing that the retinal expression of βgal did not alter the levels of Tregs. The total number of Tregs in both strains was small, compared to the frequency found in normal B6 mice, possibly reflecting the limited repertoire in this mice (see below). Analysis of cytokines produced by Ag stimulation *in vitro* showed that BG1 T cells produced only small amounts of IL-6 and IL-10, and a moderate amount of TNFα (**Figure 1D**). No spontaneous autoimmune disease was found in the retinas of BG1 × arrβgal double Tg mice.

Analysis of Foxp3-GFP mice for the distribution of GFP^+ Tregs in PBL showed that approximately 3.5% of circulating T cells was GFP^+ (**Figure 2A**). Of these, greater than 97% of the GFP^+ cells was $CD4^+$. In the BG1 × Foxp3-GFP mice, approximately 1.7% of peripheral T cells was GFP^+, and greater than 96% was $CD4^+$. Most of the GFP^+CD4^+ T cells in the BG1 mice also expressed the Vβ7 TCR transgene (**Figure 2B**). Virtually all of the small number of $CD8^+GFP^+$ T cells were $V\beta7^+$. The Ag-specific activity of the BG1 T cells lies in the βgal-specific $V\beta7^+$ population that responds to the class I-restricted DAPIYTNV epitope (Donohue et al., 2006; Tewalt et al., 2009). Activation of the BG1 cells with this peptide conferred pathogenic activity on them (see below).

ACTIVATED BG1 T CELLS CAN BE PATHOGENIC IN TISSUES EXPRESSING βGAL

Several rodent models show EAU induction by adoptive transfer of activated $CD4^+$ T cells specific for endogenous and transgenic retinal antigens (Gregerson et al., 1986, 1999; Rizzo et al., 1996; Su et al., 2001; Lambe et al., 2007). Conversely, few studies of EAU induction by CD8 T cells have been reported (McPherson et al., 2003; Song et al., 2008). We showed that βgal^+ retinal photoreceptor cells in B10.A mice were targets for autoimmune disease mediated by an oligoclonal line of activated, βgal-specific $CD8^+$ T cells (McPherson et al., 2003, 2006). BG1 T cells activated *in vitro* with their cognate βgal peptide and transferred into arrβgal mice produced minimal autoimmune disease in the untreated arrβgal

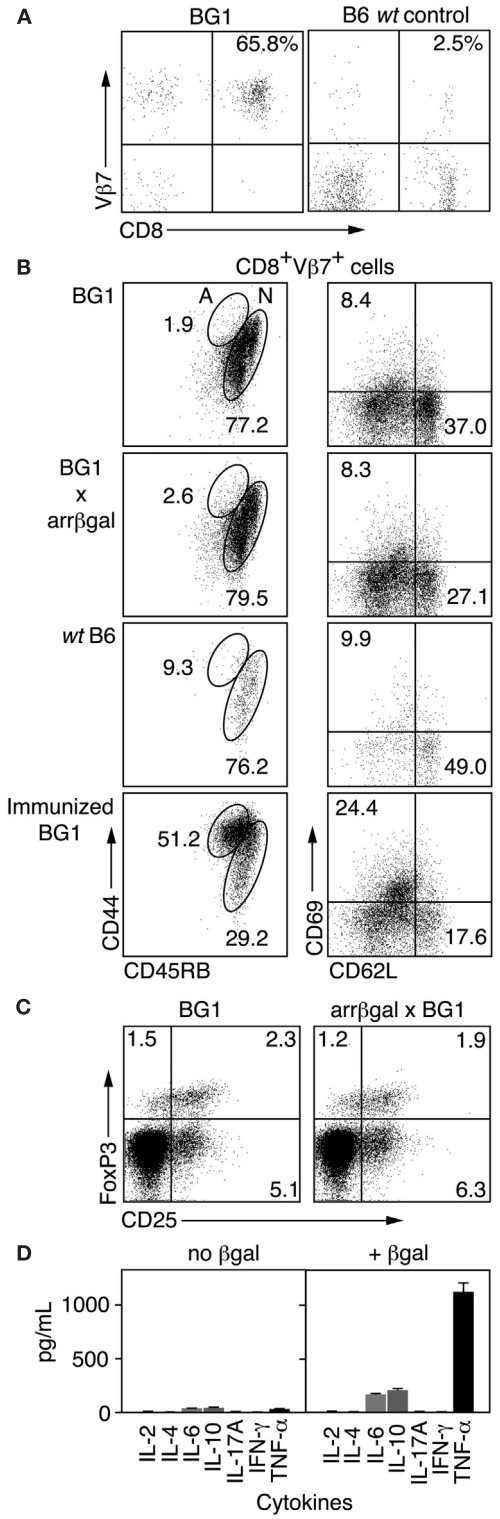

FIGURE 1 | Analysis of βgal-specific BG1 TCR Tg mice. (A) FACS analysis of $CD3^+$ splenocytes for CD8 and the clonotypic TCR βchain for the BG1 Tg mice. **(B)** FACS analysis for cell surface molecules associated with Ag recognition. Lymphocytes were gated on $CD8^+V\beta7^+$ and then analyzed for the indicated surface molecules. The percentage of cells having a naïve (N) *(Continued)*

FIGURE 1 | Continued
or activated (A) phenotype is indicated. **(C)** Comparison of Treg levels in BG1 vs. BG1 × arrβgal mice. Splenic lymphocytes were gated on $CD4^+CD90.2^+$ cells and then analyzed for CD25 and Foxp3. Representative FACS plots are shown. *P* values comparing the quadrants were determined by *t*-test, which showed that all were >0.05 ($n = 3$). **(D)** Cytokine production of βgal-specific BG1 TCR Tg splenocytes cultured with or without βgal stimulation.

recipient mice, relative to normal controls (**Figures 3A–D**). Recipients that were pretreated with sub lethal irradiation (900 R) to produce lymphopenia, or depleted of $CD25^+$ cells prior to BG1 T cell transfer showed a much higher incidence and severity of disease (**Table 1**; **Figures 3E–H**). The pathology seen in the retinas of BG1 recipients revealed minimal in filtrates and inflammation, even though the destruction of the retina ranged from limited loss of the βgal^+ photoreceptor cells to substantial destruction of the retina. The infiltration of autoreactive $CD8^+$ T cells was first seen in the βgal-expressing photoreceptor cells (ONL and OS, **Figure 3C**). More severe disease progressed to loss of the photoreceptor cells (**Figures 3E–G**) with minimal recruitment of other inflammatory cells. Disease in some mice progressed to substantial loss of the retinal cells making up the inner and outer nuclear layers, and the RGC (**Figure 3H**).

EXPRESSION OF βGAL IN THE RETINA INHIBITS THE BG1 DTH RESPONSE

Our previous studies showed that retinal Ag expression led to a reduction in the DTH response to that retinal Ag, whether the Ag-specific T cells were induced by immunization, adoptively transferred, or comprised the endogenous T cell repertoire in βgalTCR × B10.A-arrβgal double Tg mice (Gregerson and Dou, 2002; Gregerson et al., 2008, 2009; McPherson et al., 2009). The $CD8^+$ T cells in BG1 mice also mediated a DTH response to βgal, and retinal βgal expression in the double transgenic BG1 × arrβgal mice led to inhibition of the DTH response to βgal by the ear swelling assay (**Figure 4**). These results were consistent with previous experiments in the arrβgal mice showing that the inhibition of DTH in βgal-immunized mice was mediated by $CD3^+4^+25^+$ T cells.

ANTIGEN-SPECIFIC Foxp3$^+$ TREGS MODULATE CD8$^+$ T CELL RESPONSES *IN VIVO*

To determine if βgal-specific $Foxp3^+$ Tregs inhibit CD8 T cell mediated DTH in BG1 mice, Tregs were generated in Foxp3-GFP mice by i.v. administration of soluble βgal or BSA (Thorstenson and Khoruts, 2001; Zhang et al., 2001). GFP^+ cells were isolated 8 days later by flow sorting for transfer to BG1 recipients. Little difference was found in the frequency of GFP^+ Tregs in Ag injected donor mice compared to non-injected controls (**Figure 5A**). Tregs from βgal and BSA treated mice were transferred into naive BG1 mice and the DTH response to βgal was analyzed. Mice that received BSA-induced Tregs had no reduction in ear swelling compared to normal BG1 mice, while mice receiving βgal-induced Tregs showed a significant reduction in ear swelling (**Figure 5B**). Given that there was little, if any, difference in total Treg numbers between control and immunized mice, and that the

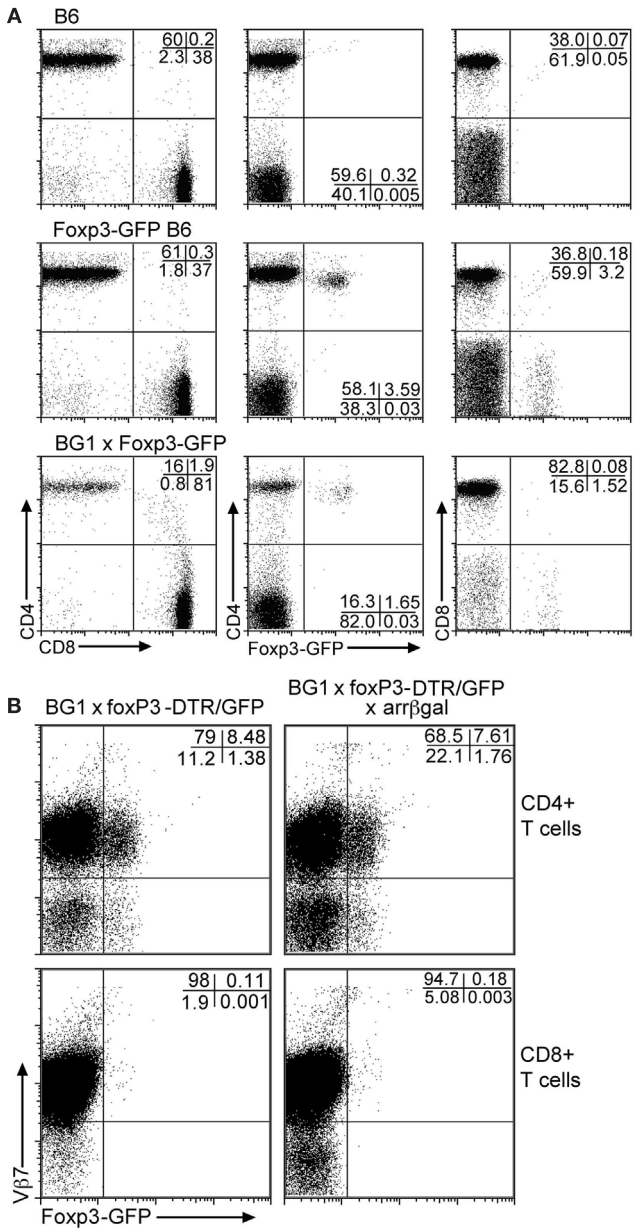

FIGURE 2 | Although the BG1 mice have a reduced population of CD4 T cells, the FoxP3-GFP$^+$ cells in BG1x Foxp3-DTR/GFP mice are concentrated in the CD4 population. (A) Comparison of Foxp3-GFP$^+$ T cells in PBL from mice with and without the TCR transgenes. **(B)** Association of the Foxp3-GFP$^+$ Tregs with the Vβ7$^+$ CD4 and CD8 T cells. The distribution of Tregs in the Vβ7$^+$ populations of T cells from peripheral blood was unaffected by retinal βgal expression. Analyses were gated on CD3$^+$ T cells. Representative plots are shown.

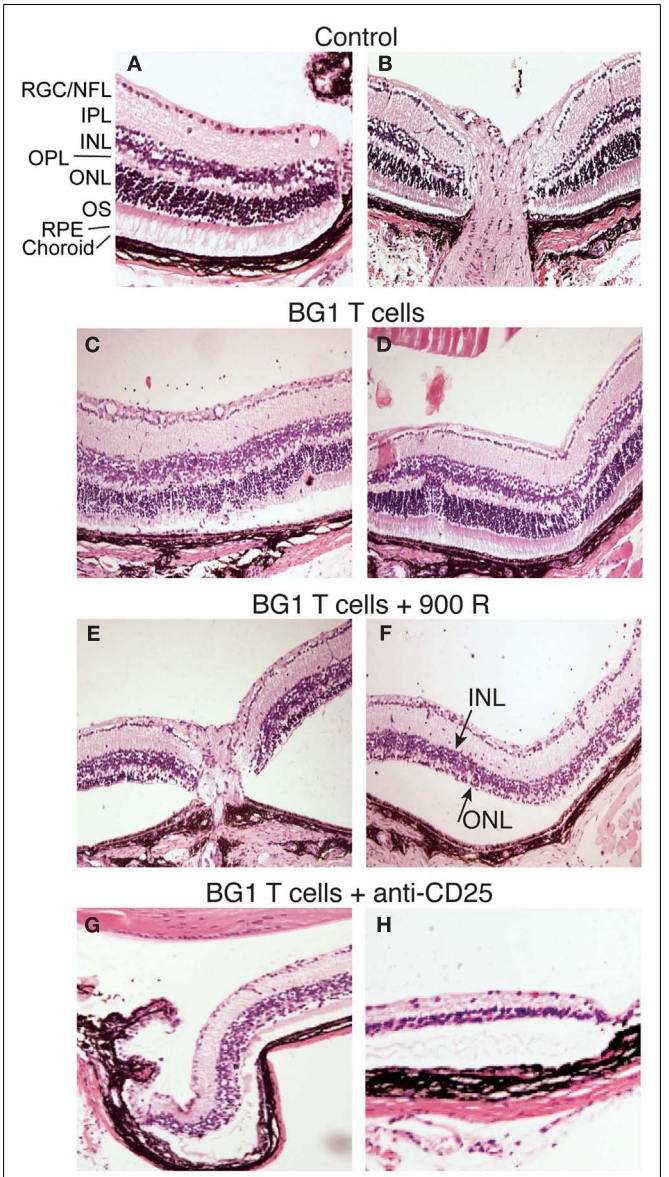

FIGURE 3 | Representative photomicrographs of autoimmune pathology in the eyes of arrβgal mice induced by transfer of activated BG1 T cells. (A) Control retina, peripheral edge. **(B)** Control retina, optic nerve head. **(C,D)**. Pathology in arrβgal mice induced by peptide-activated BG1 T cells. Eyes harvested 21 days post-transfer. **(E,F)** Pathology induced in arrβgal mice by the transfer of activated BG1 T cells after conditioning with 900 R TBI. Eyes harvested 21 days post-transfer. **(G,H)**. Pathology in the retinas of arrβgal mice that received anti-CD25 antibody prior to the transfer of activated BG1 T cells. Eyes harvested 25 days post-transfer. Abbreviations: RGC/NFL, retinal ganglion cell/nerve fiber layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; OS, outer segments; RPE, retinal pigment epithelium.

Tregs transferred contain both endogenous and Ag-specific Tregs induced by the i.v. βgal, the results suggested that even a small number of βgal-specific FoxP3$^+$ Tregs have a significant effect on the systemic response of βgal-specific CD8$^+$ T cells.

In reciprocal experiments, we examined whether the removal of Tregs would enhance the DTH response in BG1 × Foxp3-DTR/GFP double Tg mice. Tregs were substantially depleted by the i.p. administration of DTx. One day later, DTH was assayed by the ear swelling response to βgal. Depleted BG1 × Foxp3-DTR/GFP double Tg mice had elevated levels of ear swelling compared to similarly treated BG1 mice and non-DTx

Table 1 | Induction of EAU by adoptive transfer of activated BG1 T cells was enhanced by lymphopenia or Treg depletion.

	Retinal pathology	
T cells[1]/recipient[2]	Incidence[3]	Severity[4]
BG1$^-$ untreated	2/18	1.6 ± 2.0
BG1 + 900 R	14/20[5]	1.8 ± 1.4
BG1 + anti-CD25	3/4[5]	4.3 ± 1.2

[1] *BG1 T cells stimulated in vitro with DAPIYTNV.*

[2] *Arrβgal recipient mice were pretreated as shown.*

[3] *Number of mice with disease/total mice.*

[4] *Average score of diseased eyes only.*

[5] *$p < 0.05$ compared to BG1 T cells only.*

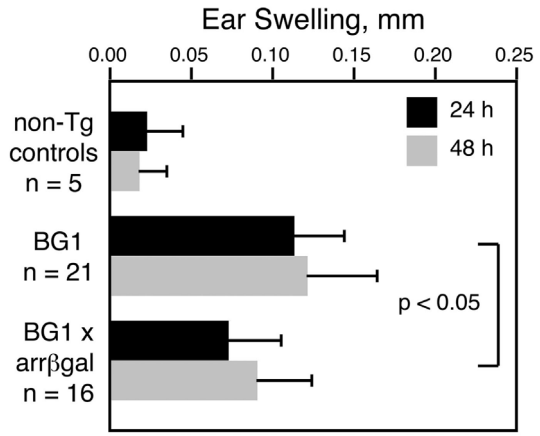

FIGURE 4 | Retinal expression of βgal in the BG1 × arrβgal mice leads to an inhibited DTH response to βgal. None of the mice were immunized; all were naive and unmanipulated. Ear swelling was induced as described in the Section "Materials and Methods." All results are given as mean ± SD with *P* values determined by *t*-test.

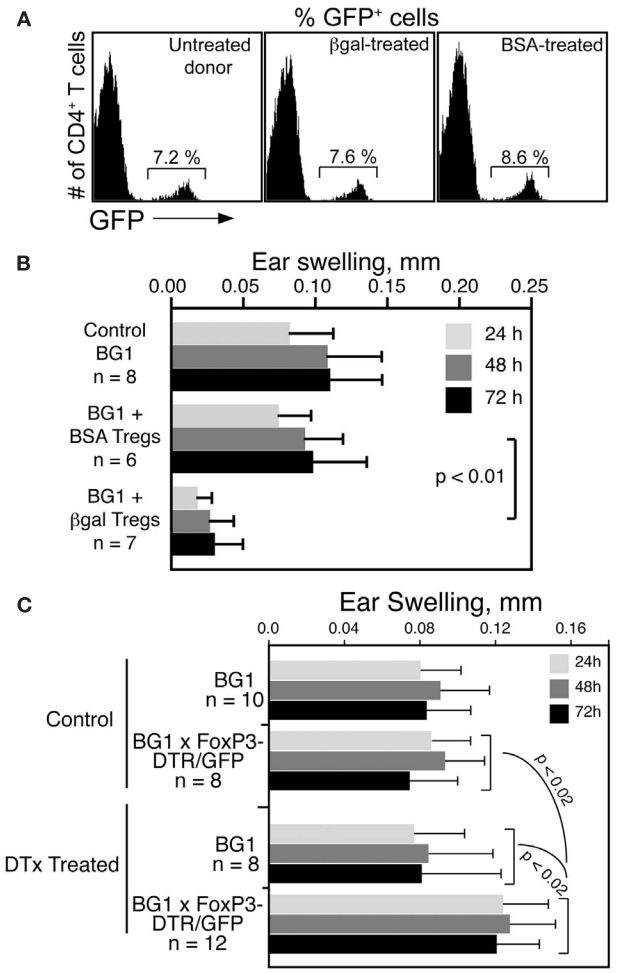

FIGURE 5 | βgal-specific Tregs modulate the DTH response of βgal-specific CD8$^+$ T cells. (A) Flow cytometry of Tregs (CD4$^+$GFP$^+$ T cells) in Foxp3-GFP mice following i.v. injection of 100 μg BSA or βgal. **(B)** Ear swelling assay in BG1 mice transferred with 5×10^5 total Tregs (GFP$^+$ cells) from the BSA or βgal-injected Foxp3-GFP mice shown in **(A)**. Ear pinna injections with βgal were done immediately after transfer of the GFP$^+$ Tregs. **(C)** Depletion of Tregs by systemic DTx treatment increases the DTH response of CD4$^+$ and CD8$^+$ T cells to βgal. DTx (250 ng/injection) was given i.p. on days 0, 3, and 6. βgal was injected into the ear pinna on day 7 to start the DTH response. Ear swelling was read on days 8–10. Results are given as the mean ± SD with *P* values determined by *t*-test.

treated control mice (**Figure 5C**). Even in the absence of retinal βgal expression as a transgene, there was still a sufficient number of Tregs to exert a detectable inhibition of the βgal DTH response, and these Tregs were depleted by DTx, leading to increased ear swelling.

TREGS SPECIFIC FOR RETINAL Ag ACT LOCALLY

We recently described a DC population in the retina of CD11c-DTR/GFP mice that could be eliminated from the retinal parenchyma by AC injection of as little as 1 ng of DTx (Lehmann et al., 2010). To ensure delivery of an effective, local Treg-depleting dose of DTx, administration of 25 ng of DTx into the AC of the eye was evaluated. The goal was to minimize systemic depletion of DTR$^+$ Tregs, but to ensure their depletion in retina. Tregs were depleted by i.p. delivery of DTx into Foxp3-DTR/GFP mice, but multiple injections of 25 ng of DTx into the AC had no effect on Treg numbers in blood (**Figure 6A**). Analysis of the DTH response to βgal showed no difference between right and left ears in mice that received right AC DTx injections, and that the response was not significantly different than in mice without DTx treatment (data not shown).

Since the number of Foxp3-GFP$^+$ Tregs in the retinal parenchyma of quiescent BG1 mice was too small to detect, functional measures of Treg activity were used, and based on susceptibility to EAU. The consequences of local Treg depletion was tested by adoptive transfer of activated BG1 × Foxp3-DTR/GFP T cells into Foxp3-DTR/GFP × arrβgal mice (**Figure 6B**). The recipients received unilateral AC injections of 25 ng DTx given three times per week to maintain local depletion of Foxp3$^+$ Tregs. This strategy would effectively deplete Tregs whether they were derived from the existing recipient pool, or from donor-derived Tregs, or

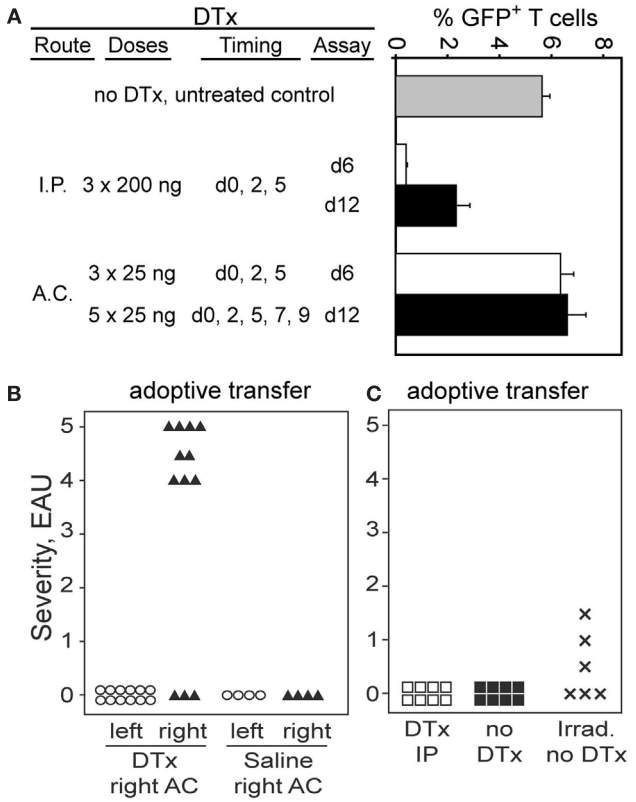

FIGURE 6 | Administration of DTx into the eye reveals that locally produced Tregs are responsible for protection of the retina from EAU. **(A)** Administration of DTx to Foxp3-DTR/GFP mice by the AC route does not deplete Tregs systemically. Graph shows % GFP$^+$ T cells in the fraction of CD3$^+$4$^+$ T cells in blood. **(B)** Transfer of activated BG1 × Foxp3-DTR/GFP T cells into Foxp3-DTR/GFP × arrβgal recipients, combined with local, unilateral depletion of Tregs leads to EAU in the depleted retina. Severe EAU was induced in retinas of eyes depleted of Tregs, but not in the contralateral retinas of the same animals. Control mice received an equal volume of saline in the AC. Mice were harvested 23 days post-transfer. Average EAU in all DTx treated eyes = 3.4 ± 2.1. **(C)** Systemic treatment with DTx did not promote EAU induction. Mice (Foxp3-DTR/GFP × arrβgal) were treated with 250 ng DTx on days 0, 3, and 6. T cells from BG1 mice lacking the Foxp3-DTR transgene were activated with Ag *in vitro*, and 7.5×10^6 T cells transferred on day 7. Control mice were not DTx treated, or were given radiation to induce lymphopenia, which promotes pathogenesis of EAU. DTx treatment given i.p. reduced the frequency of GFP$^+$ Tregs in blood from 3.6 to 0.4% 1 day after the last treatment.

were iTregs newly generated in the response to retinal βgal. Mice that received activated T cells, but no DTx injections, had no evidence of EAU in their retinas (**Figure 6B**). Mice that received multiple, unilateral (right eye) AC injections of DTx developed severe EAU in the ipsilateral right eyes (9/12 eyes, severity ≥ 4) but no EAU in the contralateral left eyes (**Figure 6B**). Unilateral injections of saline alone did not promote EAU induction (**Figure 6B**).

Controls for potential toxicity of DTx that was unrelated to expression of the DTR were done as follows. Since transfer of BG1 × Foxp3-DTR/GFP T cells gave a high incidence of severe EAU if the recipients were treated with DTx in the AC, the effect of the DTx on BG1 pathogenicity in the absence of the DTR was tested. If damage unrelated to Treg depletion by DTx was responsible for the EAU, then transfer with BG1 T cells lacking DTR should still give the severe EAU. Arrβgal mice lacking the DTR transgene were transferred with activated BG1 T cells also lacking the DTR transgene. Treatment of these mice with the same dose, frequency, and AC route of DTx injection used in **Figure 6B** gave no EAU in any of 6 mice (data not shown). Further, treatment of arrβgal × Foxp3-DTR/GFP mice with the same toxin regimen, but without BG1 T cell transfer also gave no EAU in any of 8 DTX treated mice (data not shown).

To test the possibility that the transferred βgal-specific T cells are the source of the Tregs, a set of recipient mice with DTx-sensitive Tregs (Foxp3-DTR/GFP × arrβgal) was given systemic treatments of DTx, followed by transfer of activated BG1 T cells that lacked the DTR transgene. Multiple systemic treatments with DTx did not increase susceptibility to EAU by transfer of activated BG1 T cells that did not carry the Foxp3-DTR transgene. These results indicated that the transferred T cells were the source of the EAU-protective Tregs, as those cells were not sensitive to DTx. As a control, the same preparation of T cells produced EAU in recipients in which radiation was used to induce lymphopenia (**Figure 6C**), showing that they possessed pathogenicity in lymphopenic recipients. Although the number of DTx-sensitive Tregs in quiescent retina of Foxp3-DTR/GFP mice is too small to detect their elimination, use of DTx in other tissue sites in the Foxp3-DTR/GFP mice has been reported to deplete them effectively (Kim et al., 2007, 2009).

The difference between Treg activity generated locally and the activity of circulating, preformed Tregs was further examined by experiments in BG1 × Foxp3-DTR/GFP × arrβgal mice. Our hypothesis proposes that Tregs generated locally and acting locally protect the retina, and was supported by the experiments above. Administering DTx systemically allows testing of the role of preformed circulating Tregs. In these mice, the FoxP3$^+$ Tregs are susceptible to DTx, and βgal is present in the retina, providing the combination of potentially pathogenic T cells and target Ag. No spontaneous EAU has been observed in a large number of these mice. Mice given four doses of 500 ng DTx/dose, at 4 days intervals failed to develop EAU, even though the systemic signs of autoimmunity associated with systemic depletion of Tregs appeared by the end of the 16-day course of the experiment (data not shown). Together, these results support the hypothesis that Tregs are generated in the retina, and inhibit EAU development.

PATHOGENESIS OF EAU BY AUTOREACTIVE CD8 T CELLS DOES NOT REQUIRE RECRUITMENT OF MHC CLASS I$^+$ CELLS TO THE RETINA

The role of retinal APC in the pathogenesis of EAU mediated by CD8 T cells was examined by preparation of radiation BM chimeras whose MHC class I expression was manipulated. Prior to adoptive transfer of activated BG1 T cells, the recipient mice were conditioned by sublethal irradiation. Control experiments in which class I$^+$ BM was grafted into class I$^+$ recipients, yielded mice that were susceptible to EAU induction by the subsequent transfer of activated BG1 T cells (Group A, **Figure 7**, Top). Conversely, no EAU was found in control mice made with class I$^{-/-}$ BM reconstitution of class I$^{-/-}$ recipients, and transferred with

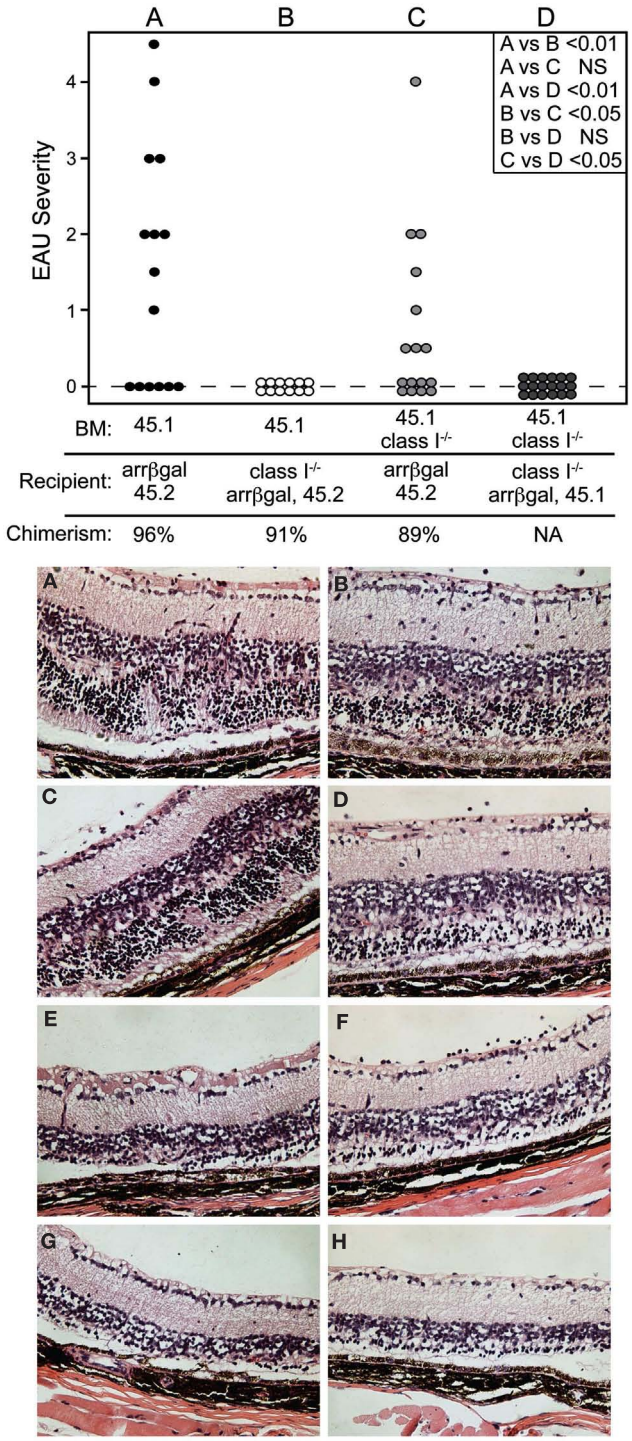

FIGURE 7 | Local expression of MHC class I is sufficient to support pathogenesis of CD8 T cell mediated EAU. TOP. Chimeric mice made in recipients that were MHC class I^+, but whose non-resident retinal immune cells were replaced by grafting class I^- BM (group C) were susceptible to EAU by adoptive transfer of activated BG1 T cells. Conversely, recipient mice that were class I^-, but given class I^+ BM (group B) were resistant to EAU. Control groups A and D were subjected to the chimerism procedures, but were fully class I^+ or class I^-, respectively. Recipients were sublethally *(Continued)*

FIGURE 7 | Continued
irradiated prior to transfer of the BG1 cells to induce the lymphopenia that promotes EAU induction. INSET. Comparisons of all eyes were made using non-parametric *P* values determined by Kruskal–Wallis test. Comparison of the severity in only the EAU-positive eyes (normally distributed) in groups A vs. C was also done by a *t*-test, and showed a *P* value of <0.05. BOTTOM. Histopathology of retinas from group A **(A–D)** and group C **(E–H)** showed that the presence or absence of class I^+ cells in the circulation had little effect on the cellularity of the inflammation and pathogenesis of CD8 T cell mediated EAU. All mice expressed retinal βgal.

the activated BG1 T cells (Group D, **Figure 7**, Top). No EAU was found in class $I^{-/-}$ recipients of wt BM transferred with activated BG1 T cells, suggesting that the βgal^+ photoreceptor cells do not express a sufficient level of MHC class I to support direct cytotoxic killing by the BG1 T cells (Group B, **Figure 7**, Top). It also suggests that the resident, class $I^{-/-}$ microglia in these retina, which turnover slowly in the absence of a stimulus, were not replaced by class I^+ donor cells during the 19-week period post-grafting to a level that supported pathogenesis based on class I expression by the donor-derived microglia. Class I^+ recipient mice receiving class $I^{-/-}$ BM developed were clearly susceptible to EAU (Group C, **Figure 7** Top), but the severity in the EAU-positive eyes only was less than found in the Group A controls. The outcomes in Groups A and C were consistent with local Ag presentation supporting EAU that could be augmented by class I^+ recruited cells, and strengthened our recent results demonstrating the significance of local Ag presentation in EAU (Heuss et al., 2012). The histopathology found in groups A and C was similar (**Figure 7**, Bottom). In all cases, there was minimal inflammatory infiltrate that was not significantly altered by the class I expression of the circulating cells available for recruitment.

DISCUSSION

With the exception of IRBP-induced CD4 T cell mediated EAU in the B10.R3 strain, mice are relatively resistant to retinal autoimmune inflammation (Caspi et al., 1992; Silver et al., 1995; Sun et al., 1997). A role for Tregs in that resistance has been described. For example, thymic expression of retinal IRBP resulting from *aire* gene activity, leads to thymic production of natural Tregs (nTregs) that suppress retinal autoimmunity (DeVoss et al., 2006). The presence or absence of thymic IRBP affected EAU susceptibility and systemic IRBP responses (Avichezer et al., 2003a,b). Expression of several immunopathogenic retinal autoAgs has been found in rodent and human thymus (Egwuagu et al., 1997; Gery and Egwuagu, 2002; Takase et al., 2005). Together, these findings support central tolerance as an important mechanism that contributes to retinal immune privilege. Our studies asked if Tregs are a factor in the resistance of murine retina to autoimmune disease directed to other retinal Ag, particularly in the context of pathogenic, autoreactive CD8 T cells.

Study of the control of CD8 T cell mediated autoimmunity by $Foxp3^+$ Tregs has received much less attention than autoimmune diseases mediated by CD4 T cells. Using viral CNS infection of mice with DTx-sensitive Tregs, the recruitment of CD8 T cells specific for known epitopes of the virus was unaffected by depletion of the Tregs (Cervantes-Barragan et al., 2012). In contrast,

the frequency of CD8 T cells specific for bystander epitopes was increased by the Treg depletion, leading to the hypothesis that Tregs in viral CNS inflammation largely serve to protect from self-reactive T cells, while allowing the anti-viral response to progress, protecting the CNS. In a model incorporating CD8 T cells specific for influenza hemagglutinin Ag, and lung expression of hemagglutinin, the evidence was consistent with Tregs exerting some control of self-reactive T cells, but the contribution of unknown mechanisms appeared to be dominant in preventing autoimmune inflammation (Tosiek et al., 2011).

Published and preliminary results from our lab show that retinal expression of the neo-self-Ag, βgal, promoted the generation of functionally significant βgal-specific Tregs from naive βgal-specific T cells transferred into $Rag^{-/-}$ mice expressing βgal in retina (Gregerson et al., 2008, 2009; McPherson et al., 2009; Heuss et al., 2012). iTregs are known to be generated in the periphery from mature T cells upon encounter with self and foreign Ag, under conditions that promote iTreg differentiation, including the presence of retinoic acid and TGFβ (Chen et al., 2003; Hall et al., 2011). In many cases the Treg-generating interactions take place in lymphoid tissues, especially LNs (del Rio et al., 2010). In light of these results, we proposed that the resistance of arrβgal mice to EAU is due to an efficient ongoing generation of iTregs from the mature, peripheral population of βgal-specific T cells, and sought evidence for their local activity in protecting from EAU.

We reported that quiescent retina of normal B6 mice contains a small number of α/β T cells, approximately 40–50 per retina, and that approximately 5% of these (2/retina) were $Foxp3^+$, based on their GFP expression in Foxp3-DTR/GFP mice (Heuss et al., 2012). The retina of the BG1 × Foxp3-DTR/GFP mice was similarly populated with T cells, including GFP^+ Tregs. Their specificity is unknown, but it seems improbable that this small number could block the challenge posed by transfer of several million activated CD8 T cells with specificity for a retinal antigen, as we have shown here. The experiments showing the role of retinal DC in the generation of $Foxp3^+$ Tregs (Heuss et al., 2012), and the data presented here showing that local depletion of Tregs allows development of EAU, points to the hypothesis that local Tregs provide the dynamic, effective resistance to CD8 T cell mediated EAU that has been found in the arrβgal mice. We suggest that this is a basic mechanism that is the foundation of the steady-state control of the susceptibility of the retina to autoimmune disease. Since this mechanism rapidly provides Tregs as needed, it lessens concerns for the lack of long-term stability in the iTreg population (Floess et al., 2007; Selvaraj and Geiger, 2007), or holes in the repertoire of specificities in the nTreg population. One could argue that the generation of the retina-protective Treg population we have described was an unusual phenomena limited to the high TGFβ and retinoic acid environment of the retina. However, retina, mucosal tissue, and brain share the characteristic of locales rich in TGFβ and retinoic acid (Chen et al., 2003; Coombes et al., 2007; Apostolou et al., 2008; Zhou et al., 2011). This feature may be found to be more widespread than previously thought.

The speed at which retinal protective Tregs were produced may be key to their effectiveness, as they appeared in the retina within 2 days of local antigen exposure in the retina (Heuss et al., 2012). Some studies using non-tissue-specific autoantigens identified LNs as a site of Treg generation and function. The Treg populations in those studies appear to require a sequential migratory pattern that includes transport through the vasculature to an inflamed tissue site, followed by emigration via the afferent lymphatic to draining LN where they acquire regulatory function (Zhang et al., 2009). From there, they are deployed in LN, where they inhibit recruitment and expansion of effector T cells, and also return to the site of inflammation where they provide local suppression (Suri-Payer et al., 1998; Huehn and Hamann, 2005). This rather lengthy process is unlikely to provide the rapid appearance of Tregs we found after 2 days in a site that is not inflamed, and lacks lymphatic drainage. Local regulatory activity has been found in the inflamed CNS using the EAE model (O'Connor et al., 2007). In unpublished experiments we have not found that DTx treatment of local LN impairs the Treg protection of retina.

We propose that the small number of Tregs present in quiescent retina were not a barrier to pathogenic T cells and EAU. The volume of the retina and the extensive vasculature would be a prohibitively large area for those few Tregs to peruse for invading T cells. However, local Treg depletion allowed the adoptive transfer model of EAU to produce a high incidence of severe EAU limited to the ipsilateral retina. Although LN that drain some ocular structures have been demonstrated (Camelo et al., 2006), the retina has not been demonstrated to be a tissue with lymphatic drainage. Tregs are not known to migrate anterograde from LN, through the lymphatics, to reach the retina or surrounding tissues. These results and considerations support our hypothesis that Tregs in the retina may be made locally, on demand. Their activity was clearly a dominant factor in retinal immune homeostasis, as their local depletion was permissive for destructive EAU. The appearance of Tregs in the retina upon local challenge is dependent on either the direct recruitment of Tregs from the circulation, or on their development from T cells recruited into the retina. If so, their rapid local production may be critical in providing regulation early in the response before pathogenesis begins, thus warding off disease onset at a time when the conditions are more manageable. The role of pre-existing, circulating Tregs has been more difficult to evaluate, since sustained, systemic depletion of Tregs in the Foxp3-DTR/GFP mice leads to progressive, lethal systemic autoimmunity (Kim et al., 2009). Several preliminary trials testing the effect of systemic depletion of Tregs in the Foxp3-DTR/GFP mice by DTx administration, with and without the βgal transgene in the retina or the BG1 TCR transgenes, failed to produce evidence of inflammation or damage to the retina. Although these trials were terminated at the first clear signs of stress, they did not produce results that would suggest that preformed Tregs were protecting the retina from autoimmunity. The existing nTregs may lack specificity for a local retinal Ag, or access to the retina, marginalizing their function in EAU. We interpret these results as supporting our hypothesis that the Tregs that protect the retina, as demonstrated in **Figure 6**, may be made locally and when needed.

Concerns for non-specific damage due to DTx-mediated injury of murine cells not targeted to express the primate DTR have

been raised. Although evidence for such damage has not been substantiated by experimentation in other experimental models (Kim et al., 2009), the possibility of toxicity was tested here. In our studies of the retina, multiple injections of 25 ng DTx into the AC of C57BL/6J control mice did not induce damage that could be distinguished from similar injections of saline alone. In conventional adoptive transfer experiments in which neither the activated BG1 T cells nor the arrβgal recipients expressed the DTR, no promotion of EAU was found. Control experiments for the injury due to repeated AC injections, separate from DTX toxicity, found that saline injections into the AC did not promote EAU induction in mice following adoptive transfer of activated BG1 T cells. Only local AC injection of DTx gave EAU in the ipsilateral retinas, confirming the local DTx dependency of the EAU.

The data presented here suggest several questions for future experiments. Is the function of the Tregs that protect retina proactive or reactive? Their numbers seem too sparse to be protective in a proactive manner, but if injury or other stimulus led to their rapid generation in the retina, where the target Ag was located, they might be able to alter the activity of the pathogenic T cells as they entered. If they are generated reactively, as part of the influx of pathogenic T cells, are they long-lived or do they act in the short term to protect only while the tissue is challenged with pathogenic T cells? The derivation of the Tregs is uncertain; although they appear to act locally, based on the depletion studies, where do they originate? Are they derived from naive cells, or from the infiltrating, activated T cells with specificity for a local, retinal antigen?

In the model we have described, the frequency of $CD8^+Foxp3^+$ T cells is only 0.1% of the CD8 T cells, whereas the $CD4^+Foxp3^+$ Tregs are approximately 9% of the CD4 T cells. The $CD8^+V\beta7^+$ T cells are the only cells in these mice with known specificity for the class I-restricted βgal peptide. Although a number of $CD8^+$ Treg subsets have been described (Tsai et al., 2011), only the $Foxp3^+$-DTR/GFP^+ subset can be the immediate precursors for the DTx-sensitive cells with Treg activity in our study. Further studies will be needed to determine the phenotype and specificity of the DTx-sensitive Tregs.

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REFERENCES

Apostolou, I., Verginis, P., Kretschmer, K., Polansky, J., Huhn, J., and Von Boehmer, H. (2008). Peripherally induced Treg: mode, stability, and role in specific tolerance. *J. Clin. Immunol.* 28, 619–624.

Avichezer, D., Grajewski, R. S., Chan, C. C., Mattapallil, M. J., Silver, P. B., Raber, J. A., Liou, G. I., Wiggert, B., Lewis, G. M., Donoso, L. A., and Caspi, R. R. (2003a). An immunologically privileged retinal antigen elicits tolerance: major role for central selection mechanisms. *J. Exp. Med.* 198, 1665–1676.

Avichezer, D., Liou, G. I., Chan, C. C., Lewis, G. M., Wiggert, B., Donoso, L. A., Nickerson, J. M., Crawford, M. A., and Caspi, R. R. (2003b). Interphotoreceptor retinoid-binding protein (IRBP)-deficient C57BL/6 mice have enhanced immunological and immunopathogenic responses to IRBP and an altered recognition of IRBP epitopes. *J. Autoimmun.* 21, 185–194.

Camelo, S., Kezic, J., Shanley, A., Rigby, P., and McMenamin, P. G. (2006). Antigen from the anterior chamber of the eye travels in a soluble form to secondary lymphoid organs via lymphatic and vascular routes. *Invest. Ophthalmol. Vis. Sci.* 47, 1039–1046.

Carson, M. J., Doose, J. M., Melchior, B., Schmid, C. D., and Ploix, C. C. (2006). CNS immune privilege: hiding in plain sight. *Immunol. Rev.* 213, 48–65.

Caspi, R. (2008). Autoimmunity in the immune privileged eye: pathogenic and regulatory T cells. *Immunol. Res.* 42, 41–50.

Caspi, R. R. (2006). Ocular autoimmunity: the price of privilege? *Immunol. Rev.* 213, 23–35.

Caspi, R. R., Grubbs, B. G., Chan, C. C., Chader, G. J., and Wiggert, B. (1992). Genetic control of susceptibility to experimental autoimmune uveoretinitis in the mouse model. Concomitant regulation by MHC and non-MHC genes. *J. Immunol.* 148, 2384–2389.

Cervantes-Barragan, L., Firner, S., Bechmann, I., Waisman, A., Lahl, K., Sparwasser, T., Thiel, V., and Ludewig, B. (2012). Regulatory T cells selectively preserve immune privilege of self-antigens during viral central nervous system infection. *J. Immunol.* 188, 3678–3685.

Chen, W., Jin, W., Hardegen, N., Lei, K. J., Li, L., Marinos, N., McGrady, G., and Wahl, S. M. (2003). Conversion of peripheral CD4+ CD25− naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J. Exp. Med.* 198, 1875–1886.

Coombes, J. L., Siddiqui, K. R., Arancibia-Carcamo, C. V., Hall, J., Sun, C. M., Belkaid, Y., and Powrie, F. (2007). A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J. Exp. Med.* 204, 1757–1764.

Cserr, H. F., Harling-Berg, C. J., and Knopf, P. M. (1992). Drainage of brain extracellular fluid into blood and deep cervical lymph and its immunological significance. *Brain Pathol.* 2, 269–276.

Curotto de Lafaille, M. A., Lino, A. C., Kutchukhidze, N., and Lafaille, J. J. (2004). CD25− T cells generate CD25+ Foxp3+ regulatory T cells by peripheral expansion. *J. Immunol.* 173, 7259–7268.

del Rio, M. L., Bernhardt, G., Rodriguez-Barbosa, J. I., and Forster, R. (2010). Development and functional specialization of CD103+ dendritic cells. *Immunol. Rev.* 234, 268–281.

DeVoss, J., Hou, Y., Johannes, K., Lu, W., Liou, G. I., Rinn, J., Chang, H., Caspi, R., Fong, L., and Anderson, M. S. (2006). Spontaneous autoimmunity prevented by thymic expression of a single self-antigen. *J. Exp. Med.* 203, 2727–2735.

DiPaolo, R. J., Brinster, C., Davidson, T. S., Andersson, J., Glass, D., and Shevach, E. M. (2007). Autoantigen-specific TGF beta-induced Foxp3+ regulatory T cells prevent autoimmunity by inhibiting dendritic cells from activating autoreactive T cells. *J. Immunol.* 179, 4685–4693.

Donohue, K. B., Grant, J. M., Tewalt, E. F., Palmer, D. C., Theoret, M. R., Restifo, N. P., and Norbury, C. C. (2006). Cross-priming utilizes antigen not available to the direct presentation pathway. *Immunology* 119, 63–73.

Egwuagu, C. E., Charukamnoetkanok, P., and Gery, I. (1997). Thymic expression of autoantigens correlates with resistance to autoimmune disease. *J. Immunol.* 159, 3109–3112.

Floess, S., Freyer, J., Siewert, C., Baron, U., Olek, S., Polansky, J., Schlawe, K., Chang, H. D., Bopp, T., Schmitt, E., Klein-Hessling, S., Serfling, E., Hamann, A., and Huehn, J. (2007). Epigenetic control of the foxp3 locus in regulatory T cells. *PLoS Biol.* 5, e38. doi:10.1371/journal.pbio.0050038

Fontenot, J. D., Rasmussen, J. P., Williams, L. M., Dooley, J. L., Farr, A. G., and Rudensky, A. Y. (2005). Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity* 22, 329–341.

Gery, I., and Egwuagu, C. E. (2002). Central tolerance mechanisms in control of susceptibility to autoimmune uveitic disease. *Int. Rev. Immunol.* 21, 89–100.

Grajewski, R. S., Silver, P. B., Agarwal, R. K., Su, S. B., Chan, C. C., Liou, G. I., and Caspi, R. R. (2006). Endogenous IRBP can be dispensable for generation of natural CD4+ CD25+ regulatory T cells that protect from IRBP-induced retinal autoimmunity. *J. Exp. Med.* 203, 851–856.

Gregerson, D. S. (1998). Immune privilege in the retina. *Ocul. Immunol. Inflamm.* 6, 257–267.

Gregerson, D. S. (2002). Peripheral expression of ocular antigens in regulation and therapy of ocular autoimmunity. *Int. Rev. Immunol.* 21, 101–121.

Gregerson, D. S., and Dou, C. (2002). Spontaneous induction of immunoregulation by an endogenous retinal antigen. *Invest. Ophthalmol. Vis. Sci.* 43, 2984–2991.

Gregerson, D. S., Heuss, N. D., Lehmann, U., and McPherson, S. W. (2008). Evidence for extrathymic generation of regulatory T cells specific for a retinal antigen. *Ophthalmic Res.* 40, 154–159.

Gregerson, D. S., Heuss, N. D., Lehmann, U., and Mcpherson, S. W. (2009). Peripheral induction of tolerance by retinal antigen expression. *J. Immunol.* 183, 814–822.

Gregerson, D. S., Obritsch, W. F., and Donoso, L. A. (1993). Oral tolerance in experimental autoimmune uveoretinitis. Distinct mechanisms of resistance are induced by low dose vs. high dose feeding protocols. *J. Immunol.* 151, 5751–5761.

Gregerson, D. S., Obritsch, W. F., Fling, S. P., and Cameron, J. D. (1986). S-antigen-specific rat T cell lines recognize peptide fragments of S-antigen and mediate experimental autoimmune uveoretinitis and pinealitis. *J. Immunol.* 136, 2875–2882.

Gregerson, D. S., Torseth, J. W., McPherson, S. W., Roberts, J. P., Shinohara, T., and Zack, D. J. (1999). Retinal expression of a neo-self antigen, beta-galactosidase, is not tolerogenic and creates a target for autoimmune uveoretinitis. *J. Immunol.* 163, 1073–1080.

Hall, J. A., Grainger, J. R., Spencer, S. P., and Belkaid, Y. (2011). The role of retinoic acid in tolerance and immunity. *Immunity* 35, 13–22.

Ham, D. I., Kim, S. J., Chen, J., Vistica, B. P., Fariss, R. N., Lee, R. S., Wawrousek, E. F., Takase, H., Yu, C. R., Egwuagu, C. E., Chan, C. C., and Gery, I. (2004). Central immunotolerance in transgenic mice expressing a foreign antigen under control of the rhodopsin promoter. *Invest. Ophthalmol. Vis. Sci.* 45, 857–862.

Heuss, N. D., Lehmann, U., Norbury, C. C., Mcpherson, S. W., and Gregerson, D. S. (2012). Local activation of dendritic cells alters the pathogenesis of autoimmune disease in the retina. *J. Immunol.* 188, 1191–1200.

Hori, J., Vega, J. L., and Masli, S. (2010). Review of ocular immune privilege in the year 2010: modifying the immune privilege of the eye. *Ocul. Immunol. Inflamm.* 18, 325–333.

Huehn, J., and Hamann, A. (2005). Homing to suppress: address codes for Treg migration. *Trends Immunol.* 26, 632–636.

Jutel, M., and Akdis, C. A. (2008). T-cell regulatory mechanisms in specific immunotherapy. *Chem. Immunol. Allergy* 94, 158–177.

Kim, J., Lahl, K., Hori, S., Loddenkemper, C., Chaudhry, A., Deroos, P., Rudensky, A., and Sparwasser, T. (2009). Cutting edge: depletion of Foxp3+ cells leads to induction of autoimmunity by specific ablation of regulatory T cells in genetically targeted mice. *J. Immunol.* 183, 7631–7634.

Kim, J. M., Rasmussen, J. P., and Rudensky, A. Y. (2007). Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. *Nat. Immunol.* 8, 191–197.

Lambe, T., Leung, J. C., Ferry, H., Bouriez-Jones, T., Makinen, K., Crockford, T. L., Jiang, H. R., Nickerson, J. M., Peltonen, L., Forrester, J. V., and Cornall, R. J. (2007). Limited peripheral T cell anergy predisposes to retinal autoimmunity. *J. Immunol.* 178, 4276–4283.

Lehmann, U., Heuss, N. D., McPherson, S. W., Roehrich, H., and Gregerson, D. S. (2010). Dendritic cells are early responders to retinal injury. *Neurobiol. Dis.* 40, 177–184.

Lohr, J., Knoechel, B., and Abbas, A. K. (2006). Regulatory T cells in the periphery. *Immunol. Rev.* 212, 149–162.

McPherson, S. W., and Gregerson, D. S. (1994). "Nucleotide sequences homologous to retinal S-antigen in the rat thymus lack a known EAU inducing epitope," in *Sixth International Symposium on the Immunology and Immunopathology of the Eye*, eds R. B. Nussenblatt, S. M. Whitcup, R. R. Caspi, and I. Gery (Amsterdam: Elsevier Sciences B.V.), 67–70.

McPherson, S. W., Heuss, N. D., and Gregerson, D. S. (2009). Lymphopenia-induced proliferation is a potent activator for CD4+ T cell-mediated autoimmune disease in the retina. *J. Immunol.* 182, 969–979.

McPherson, S. W., Heuss, N. D., Roehrich, H., and Gregerson, D. S. (2006). Bystander killing of neurons by cytotoxic T cells specific for a glial antigen. *Glia* 53, 457–466.

McPherson, S. W., Yang, J., Chan, C. C., Dou, C., and Gregerson, D. S. (2003). Resting CD8 T cells recognize beta-galactosidase expressed in the immune-privileged retina and mediate autoimmune disease when activated. *Immunology* 110, 386–396.

Niederkorn, J. Y. (2007). Regulatory T cells and the eye. *Chem. Immunol. Allergy* 92, 131–139.

Niederkorn, J. Y., and Wang, S. (2005). Immune privilege of the eye and fetus: parallel universes? *Transplantation* 80, 1139–1144.

O'Connor, R. A., Malpass, K. H., and Anderton, S. M. (2007). The inflamed central nervous system drives the activation and rapid proliferation of Foxp3+ regulatory T cells. *J. Immunol.* 179, 958–966.

Rizzo, L. V., Silver, P., Wiggert, B., Hakim, F., Gazzinelli, R. T., Chan, C.-C., and Caspi, R. R. (1996). Establishment and characterization of a murine CD4+ T cell line and clone that induce experimental autoimmune uveoretinitis in B10.A mice. *J. Immunol.* 156, 1654–1660.

Sakaguchi, S. (2011). Regulatory T cells: history and perspective. *Methods Mol. Biol.* 707, 3–17.

Selvaraj, R. K., and Geiger, T. L. (2007). A kinetic and dynamic analysis of Foxp3 induced in T cells by TGF-beta. *J. Immunol.* 178, 7667–7677.

Shevach, E. M., Dipaolo, R. A., Andersson, J., Zhao, D. M., Stephens, G. L., and Thornton, A. M. (2006). The lifestyle of naturally occurring CD4+ CD25+ Foxp3+ regulatory T cells. *Immunol. Rev.* 212, 60–73.

Silver, P. B., Agarwal, R. K., Su, S. B., Suffia, I., Grajewski, R. S., Luger, D., Chan, C. C., Mahdi, R. M., Nickerson, J. M., and Caspi, R. R. (2007). Hydrodynamic vaccination with DNA encoding an immunologically privileged retinal antigen protects from autoimmunity through induction of regulatory T cells. *J. Immunol.* 179, 5146–5158.

Silver, P. B., Rizzo, L. V., Chan, C. C., Donoso, L. A., Wiggert, B., and Caspi, R. R. (1995). Identification of a major pathogenic epitope in the human IRBP molecule recognized by mice of the H-2r haplotype. *Invest. Ophthalmol. Vis. Sci.* 36, 946–954.

Song, L., Le, J., Ye, F., Shao, H., Kaplan, H. J., and Sun, D. (2008). Sequence 168 to 177 of interphotoreceptor retinoid-binding protein (IRBP) is an antigenic epitope for autoreactive CD8 T cells in the B10RIII mouse. *J. Neuroimmunol.* 193, 68–76.

Stein-Streilein, J., and Taylor, A. W. (2007). An eye's view of T regulatory cells. *J. Leukoc. Biol.* 81, 593–598.

Streilein, J. W. (2003). Ocular immune privilege: the eye takes a dim but practical view of immunity and inflammation. *J. Leukoc. Biol.* 74, 179–185.

Su, S. B., Silver, P. B., Zhang, M., Chan, C. C., and Caspi, R. R. (2001). Pertussis toxin inhibits induction of tissue-specific autoimmune disease by disrupting G protein-coupled signals. *J. Immunol.* 167, 250–256.

Sun, B., Rizzo, L. V., Sun, S. H., Chan, C. C., Wiggert, B., Wilder, R. L., and Caspi, R. R. (1997). Genetic susceptibility to experimental autoimmune uveitis involves more than a predisposition to generate a T helper-1-like or a T helper-2-like response. *J. Immunol.* 159, 1004–1011.

Suri-Payer, E., Amar, A. Z., Thornton, A. M., and Shevach, E. M. (1998). CD4+CD25+ T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. *J. Immunol.* 160, 1212–1218.

Takase, H., Yu, C. R., Mahdi, R. M., Douek, D. C., Dirusso, G. B., Midgley, F. M., Dogra, R., Allende, G., Rosenkranz, E., Pugliese, A., Egwuagu, C. E., and Gery, I. (2005). Thymic expression of peripheral tissue antigens in humans: a remarkable variability among individuals. *Int. Immunol.* 17, 1131–1140.

Taylor, A. W. (2009). Ocular immune privilege. *Eye (Lond.)* 23, 1885–1889.

Tewalt, E. F., Grant, J. M., Granger, E. L., Palmer, D. C., Heuss, N. D., Gregerson, D. S., Restifo, N. P., and Norbury, C. C. (2009). Viral sequestration of antigen subverts cross presentation to CD8(+) T cells. *PLoS Pathog.* 5, e1000457. doi:10.1371/journal.ppat.1000457

Thorstenson, K. M., and Khoruts, A. (2001). Generation of anergic and potentially immunoregulatory CD25+ CD4 T cells in vivo after induction of peripheral tolerance with intravenous or oral antigen. *J. Immunol.* 167, 188–195.

Tosiek, M. J., Gruber, A. D., Bader, S. R., Mauel, S., Hoymann, H. G., Prettin, S., Tschernig, T., Buer, J., Gereke, M., and Bruder, D. (2011). CD4+CD25+Foxp3+ regulatory T cells are dispensable for controlling CD8+ T cell-mediated lung inflammation. *J. Immunol.* 186, 6106–6118.

Tsai, S., Clemente-Casares, X., and Santamaria, P. (2011). CD8(+) Tregs in autoimmunity: learning "self"-control from experience. *Cell. Mol. Life Sci.* 68, 3781–3795.

Wenkel, H., and Streilein, J. W. (1998). Analysis of immune deviation elicited by antigens injected into the subretinal space. *Invest. Ophthalmol. Vis. Sci.* 39, 1823–1834.

Yamada, S., Depasquale, M., Patlak, S. C., and Cserr, H. F. (1991). Albumin outflow into deep cervical lymph from different regions of rabbit brain. *Am. J. Physiol.* 261, H1197–H1204.

Zhang, N., Schroppel, B., Lal, G., Jakubzick, C., Mao, X., Chen, D., Yin, N., Jessberger, R., Ochando, J. C., Ding, Y., and Bromberg, J. S. (2009). Regulatory T cells sequentially migrate from inflamed tissues to draining lymph nodes to suppress the alloimmune response. *Immunity* 30, 458–469.

Zhang, X., Izikson, L., Liu, L., and Weiner, H. L. (2001). Activation of CD25(+)CD4(+) regulatory T cells by oral antigen administration. *J. Immunol.* 167, 4245–4253.

Zhou, R., Horai, R., Mattapallil, M. J., and Caspi, R. R. (2011). A new look at immune privilege of the eye: dual role for the vision-related molecule retinoic Acid. *J. Immunol.* 187, 4170–4177.

Zhou, R., Horai, R., Silver, P. B., Mattapallil, M. J., Zárate-Bladés, C. R., Chong, W. P., Chen, J., Rigden, R. C., Villasmil, R., and Caspi, R. R. (2012). The Living Eye "Disarms" Uncommitted Autoreactive T Cells by Converting Them to Foxp3+ Regulatory Cells following Local Antigen Recognition. *J. Immunol.* 188, 1742–1750.

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Influence of CD8+ T regulatory cells on intraocular tumor development

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The interior of the eye, or uvea, is a site of immune privilege where certain immune responses are attenuated or completely excluded to protect non-regenerating tissues essential for vision. One consequence of this immunoregulation is compromised immune mediated elimination of intraocular tumors. For example, certain murine tumor cell lines which are rejected by host immune responses when transplanted in the skin grow progressively when placed in the anterior chamber (a.c.) of the eye. Progressive ocular tumor growth occurs despite induction of tumor-specific CD8+ T cell responses capable of eliminating a subsequent tumor challenge in the skin or opposite eye. Why these CD8+ T effectors fail to eliminate established ocular tumors is not known. It is well appreciated that growth of tumors in the a.c. induces the generation of immunosuppressive CD8+ T regulatory (Treg) cells. However, the contribution of CD8+ Treg in ocular tumor progression remains unclear. Several studies indicate that these CD8+ Treg target responding CD4+ T cells to inhibit their induction of macrophage-dependent delayed type hypersensitivity (DTH) responses to tumor antigens (Ags). However, induction of tumor-specific CD4+ T cell responses does not assure intraocular tumor elimination. This review is focused on how CD8+ Treg could influence the tumoricidal activity of ocular tumor-specific CD8+ T effector cells.

Keywords: eye, tumor, CD8, Treg, CTL, immunosuppression, ACAID, immune evasion

INTRODUCTION

The concept of immune privilege was first advanced in the 1940s by the Nobel laureate Sir Peter Medawar. While studying tissue transplantation he observed that foreign skin grafts which were normally rejected when transplanted subcutaneously (s.c.), persisted sometimes indefinitely when transplanted into other sites that he termed "immune privileged" (Medawar, 1948). One immune privileged site was the anterior chamber (a.c.) of the eye, the aqueous humor (AqH) filled cavity located directly below the cornea and above the lens (**Figure 1**). As the a.c. is separated by a blood–AqH barrier and lacks demonstrable afferent lymphatic drainage, ocular immune privilege was originally explained by sequestration of ocular antigens (Ags) from the circulating immune system. However, seminal findings by Kaplan and Streilein in 1977 which demonstrated that antibody responses were generated to foreign Ags placed in the a.c. (Kaplan and Streilein, 1977) clearly showed that the immune system was not ignorant of ocular Ags. We now know that certain immune responses are attenuated or completely excluded from the eye to protect non-regenerating ocular tissues essential for vision. This ocular immune privilege is maintained by unique anatomical and biochemical features of the eye along with the generation of systemic tolerance to ocular Ags which is mediated by regulatory T cells (Treg). In this review we focus on how CD8+ Treg generated during intraocular tumor growth could influence the tumoricidal activity of tumor-specific CD8+ T effector cells.

MECHANISMS OF OCULAR IMMUNE PRIVILEGE

INFLUENCE OF OCULAR ANATOMY ON IMMUNE PRIVILEGE

The absence of afferent lymphatics (Bill, 1977), an avascular cornea (Patel and Dana, 2009), and tight junctions between vascular endothelial cells in the iris and retina (Crane and Liversidge, 2008) are barriers to the generation and expression of ocular immune responses. However, these barriers are not absolute as administration of soluble Ags into the a.c. has been shown to induce Ag-specific CD8+ (McKenna et al., 2002, 2005) and CD4+ (Egan et al., 1996; Perez et al., 2000) T cell expansion in the ipsilateral submandibular lymph nodes (LNs) and spleens of mice. In addition, activated T cells can enter even a non-inflamed retina to induce uveitis (Xu et al., 2003). Therefore, ocular anatomy may increase the threshold for the generation and expression of ocular immune responses but clearly does not prevent them.

Ags encountered within the a.c are thought to exit the eye via the normal drainage of AqH (Bill, 1977) (**Figure 1** inset). AqH is continually generated by the ciliary body, fills the a.c., and then primarily drains via the trabecular meshwork and Schlem's canal directly into the blood stream. A much smaller percentage of AqH travels by uveal-scleral flow into the cilary muscle and then traverses the choroid and sclera to be drained by conjunctival lymphatics (Bill, 1977). As we will discuss in subsequent sections, the spleen is essential for the generation of Treg which also contribute to maintaining ocular immune privilege. Therefore, preferential trafficking of ocular Ags via the bloodstream to the

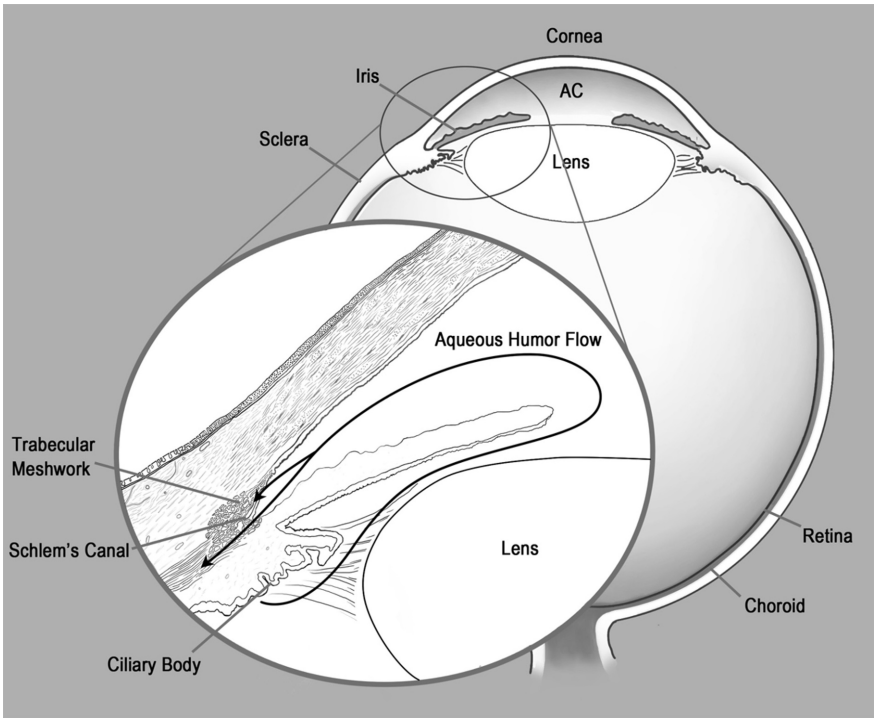

FIGURE 1 | Ocular anatomy and drainage. Anterior chamber (AC).

spleen may favor the induction of T cell tolerance. In addition, antigen presenting cells (APCs) from the iris and ciliary body have been shown to selectively traffic to the spleen (Wilbanks and Streilein, 1992).

BIOCHEMICAL BARRIERS TO OCULAR IMMUNE RESPONSES

T cells recognize processed peptides presented on major histocompatibility complex (MHC) molecules. In general, peptides presented by MHC Class I molecules are recognized by CD8+ T cells whereas CD4+ T cells recognize peptides complexed with MHC Class II molecules (**Figures 2A,B**). CD8+ T cells differentiate into cytotoxic T lymphocytes (CTL) which primarily eliminate infected or malignant cells by release of lytic granules although cytokines are also released (**Figure 2A**) whereas CD4+ Thelper cells primarily release cytokines to influence other immune cells. For example, during DTH responses, CD4+ T cells express IFNγ and/or IL-17 which recruits and activates macrophages and neutrophils to promote inflammation (**Figure 2B**). The response is delayed due to the requisite time for Ag-specific T cells to expand in draining LN and then migrate to the site of Ag exposure.

Although the majority of cells within the body express MHC Class I molecules and can be induced to express MHC Class II molecules by stimulation with interferon gamma (IFNγ), ocular tissues demonstrate atypical expression of MHC Class I and II. For example, corneal endothelial cells express very low levels of MHC Class I which protects these non-regenerating cells from lysis by CD8+ CTL (Abi-Hanna et al., 1988). Ocular melanocytes are impaired in expression of MHC Class II which may mitigate CD4+ T cell mediated inflammation (Radosevich et al., 2004, 2007).

AqH contains soluble immune suppressive molecules including cytokines, neuropeptides, and growth factors that have been shown to inhibit adaptive immune responses. Benezra and Sacks documented over forty years ago that AqH could inhibit proliferation of naïve T cells following PHA stimulation (Benezra and Sachs, 1974), and we now know that high concentrations of the cytokine TGFβ2 in AqH contributed to this suppression (Kaiser et al., 1989; Cousins et al., 1991). TGFβ2 is normally present in a latent form within the AqH and elegant work by Masli and coworkers demonstrated a critical role of thrombospondin-1 in activation of latent TGFβ2 to preserve ocular immune privilege (Masli et al., 2006).

Taylor and coworkers have clearly shown that AqH directs *in vitro* primed CD4+ T cells away from an IFNγ expressing phenotype and toward a TGFβ1 producing Treg type (Taylor et al., 1997). Factors within AqH including TGFβ2 and alpha-melanocyte stimulating hormone (α-MSH) can alone generate Treg (Nishida and Taylor, 1999). In combination, α-MSH increases the frequency of Treg by abrogating the anti-proliferative effects of TGFβ (Nishida and Taylor, 1999). Additional factors within AqH that favor Treg generation include somatostatin which induces α-MSH production in T cells (Taylor and Yee, 2003) and vasoactive intestinal peptide (VIP) which inhibits IFNγ production in effector T cells (Taylor et al., 1994).

AqH also contains molecules that inhibit innate immune responses. For example, high concentrations of ascorbic acid in AqH have been shown to inhibit myeloperoxidase activity of

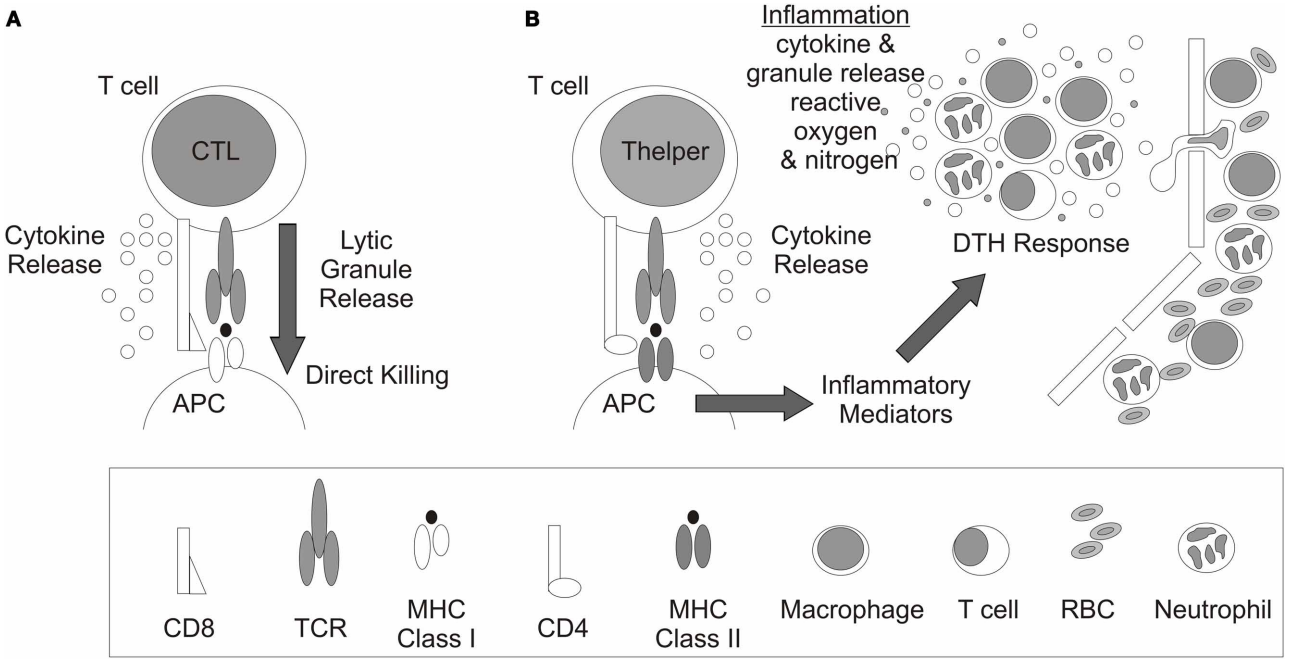

FIGURE 2 | T cell recognition and response. Antigen recognition and response by **(A)** CD8+ cytolytic T lymphocytes (CTL) and **(B)** CD4+ T helper cells. Antigen presenting cell (APC), cluster of differentiation (CD), delayed type hypersensitivity response (DTH), T cell receptor (TCR), major histocompatibility complex (MHC), and red blood cell (RBC).

neutrophils (Rosenbaum et al., 1985) and macrophage migration inhibitory factor (MIF) in AqH inhibits NK cell activity (Apte et al., 1998). AqH-mediated changes to the innate immune response also influence the adaptive immune response. For example, pretreatment of macrophages with TGFβ2 decreased their expression of CD40 and IL-12 and increased TGFβ1 expression (Takeuchi et al., 1998). Consequently, CD4+ T cells stimulated with TGFβ2 treated APC were deviated from IFNγ producing T cells to TGFβ1 producing Treg (Takeuchi et al., 1998; Keino et al., 2006b).

The interior of the eye is also lined by a continuous layer of pigmented epithelial (PE) cells of the iris, ciliary body, and retina along with the corneal endothelium. All of these ocular tissues have been shown to induce T cells to become immunosuppressive Treg *in vitro* (Sugita et al., 2006, 2008, 2009, 2011). Iris PE cells best convert CD8+ T cells into Treg via their expression of CD86 which engages CTLA-4 on activated CD8+ T cells (Sugita et al., 2008). In contrast, retinal PE cells and corneal endothelial cells express CTLA-2α which better converts CD4+ T cells into Tregs by decreasing cathepsin-L activity in T cells (Sugita et al., 2008, 2009). Expression of TGFβ1 by CD8+ (Sugita et al., 2008) and CD4+ Tregs (Sugita et al., 2006) contributes to their immunosuppressive activity. In addition, CD8+ Tregs generated by iris PE express CD86 (Sugita et al., 2006) and the immnosuppressive molecule programmed death 1 (PD-1) to suppress CTLA-4+ and PD-1 ligand + T cell effectors (Sugita et al., 2010).

Cells lining the interior of the eye also express death inducing molecules including CD95/FasL (Griffith et al., 1995a), and PD-1 ligand (Hori et al., 2006) which can induce apoptosis of effector T cells expressing CD95/Fas and PD-1 respectively. The significance of these death-inducing molecules is very apparent in corneal transplantation as corneal allografts deficient in CD95/FasL (Stuart et al., 1997; Yamagami et al., 1997) or PD-1 ligand (Hori et al., 2006) are rejected with increased frequency. Apoptotic CD4+ T cells were observed at the allograft junction of accepted corneas whereas CD4+ T cells accumulated in rejecting corneal allografts (Hori et al., 2006) which supported a death inducing mechanism for these molecules.

Taken together, the above data suggest a model (**Figure 3**) in which activated effector T cells are inactivated by their conversion into Treg as they extravasate from blood vessels and cross epithelial boundaries to enter the eye. Those effectors that escape this regulation may be converted into Treg by immunosuppressive factors within ocular fluids, like AqH, or induced to undergo apoptosis by death inducing molecules within the eye. Again, these barriers to ocular immune responses are not absolute as intravenous transfer of activated effector CD4+ T cells specific for ocular Ags can induce either anterior (Lai et al., 1999) or poster uveitis (Xu et al., 2003). Similarly, Zhou et al. (2012) recently showed that effector T cells specific for a retinal Ag did not become Treg when injected into the posterior chamber of the eye (Zhou et al., 2012). In addition, naïve mice are protected from an ocular tumor challenge if first infused intravenously with tumor-specific CD8+ T effectors (Niederkorn and Streilein, 1984). These data clearly indicate that activated effectors can overcome immunosuppressive mechanisms within the eye. Hence, these biochemical barriers must primarily raise the threshold of expression of ocular immune responses.

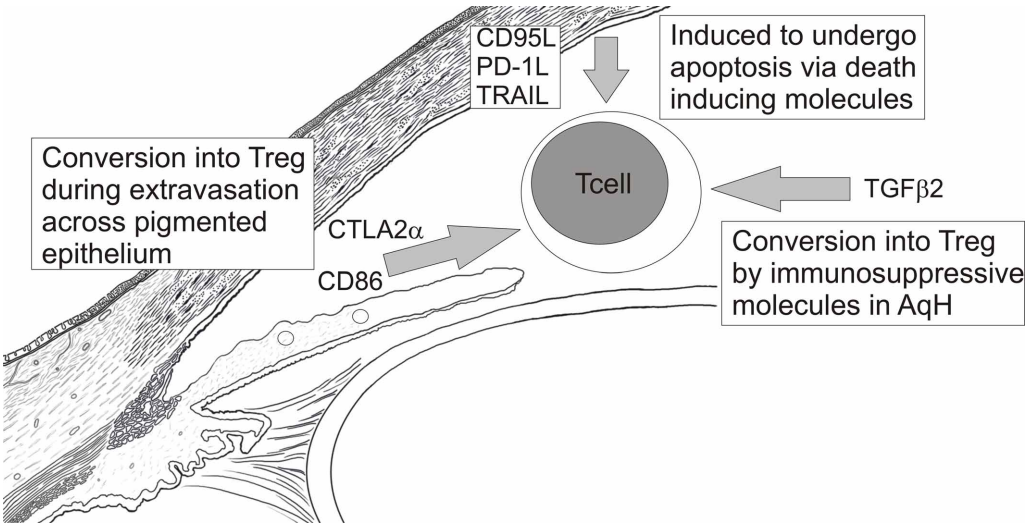

FIGURE 3 | Mechanisms of T cell inactivation within the anterior chamber.

Table 1 | Transplantable intraocular tumor models.

Tumor cell line	Tumor origin	Recipient mouse strain	Antigens on tumor	Intraocular tumor growth	Skin tumor tumor growth	References
P815	DBA/2	Balb/C	Minor MHC	Progressive	Rejection	Niederkorn et al., 1981
P815	DBA/2	C57Bl/6	Minor + Major MHC	Rejection	Rejection	Niederkorn et al., 1981
B16F10	C57Bl/6	A/J	Minor + Tumor Ag	Progressive	Rejection	Niederkorn and Streilein, 1983b
B16F10	C57Bl/6	C57Bl/6	Tumor Ag	Progressive	Progressive	Niederkorn, 1984
E.G7-OVA	C57Bl/6	C57Bl/6	Ovalbumin	Progressive	Rejection	McKenna and Kapp, 2006
Ad5E1	C57Bl/6	C57Bl/6	Adenovirus	Rejection	Rejection	Schurmans et al., 2001
UV-5C25	C57Bl/6	C57Bl/6	Tumor Ag	Rejection	Rejection	Knisely et al., 1987
P91	DBA/2	DBA/2	Tumor Ag	Rejection	Rejection	Knisely et al., 1987

ANTERIOR CHAMBER ASSOCIATED IMMUNE DEVIATION (ACAID)

One consequence of stringent control of ocular immune responses is compromised immune mediated elimination of intraocular tumors. For example, certain murine tumor cell lines (**Table 1**) that were rejected when transplanted in the skin grew progressively when placed in the a.c. of the eye. Progressive growth of these intraocular tumors was not due to a failure to prime immune responses. Rather, CD8+ CTL (Niederkorn and Streilein, 1983a; Ksander and Streilein, 1989) and cytotoxic antibody responses (Niederkorn and Streilein, 1982a) specific for tumor Ags were equivalent or greater than those observed in mice that rejected the same tumors in the skin. However, ocular tumor growth also generated immunosuppressive CD8+ Treg that inhibited CD4+ T cell mediated DTH responses to tumor Ags (Streilein and Niederkorn, 1985). This unique immune response which was deviated from the conventional immune response observed when tumors were injected in the skin was described by the general term a.c. associated immune deviation or ACAID.

ACAID has been primarily defined by suppression of DTH responses to Ags that were first encountered in the a.c. and has been demonstrated using tumors (Niederkorn et al., 1981), haptenated splenocytes (Waldrep and Kaplan, 1983), viruses (Ksander and Hendricks, 1987), and soluble Ags (Wilbanks and Streilein, 1990). The induction of ACAID is a complicated process which requires several tissues including the eye (Niederkorn and Streilein, 1982b), spleen (Streilein and Niederkorn, 1981), thymus (Wang et al., 1997), and sympathetic nervous system (Li et al., 2004; Vega et al., 2009). The current paradigm for the induction of ACAID (**Figure 4**) suggests that ocular Ags are processed by F4/80+ macrophages that were influenced by TGFβ2 in AqH (Wilbanks et al., 1991, 1992; Wilbanks and Streilein, 1991). These APCs travel from the eye via the bloodstream to the thymus and marginal zone of the spleen. Within the thymus CD4− CD8− NKT cells are generated (Wang et al., 2001) which migrate to the marginal zone of the spleen via a MIP-2 chemokine gradient created by F4/80+ macrophages from the eye (Faunce et al., 2001; Faunce and Stein-Streilein, 2002). These F4/80+ macrophages present Ags directly as well as release Ag which is internalized, processed and presented by B cells (D'Orazio et al., 2001). Coordinate interactions between F4/80+ macrophages (Lin et al., 2005), B cells (D'Orazio et al., 2001), NKT cells (Sonoda et al.,

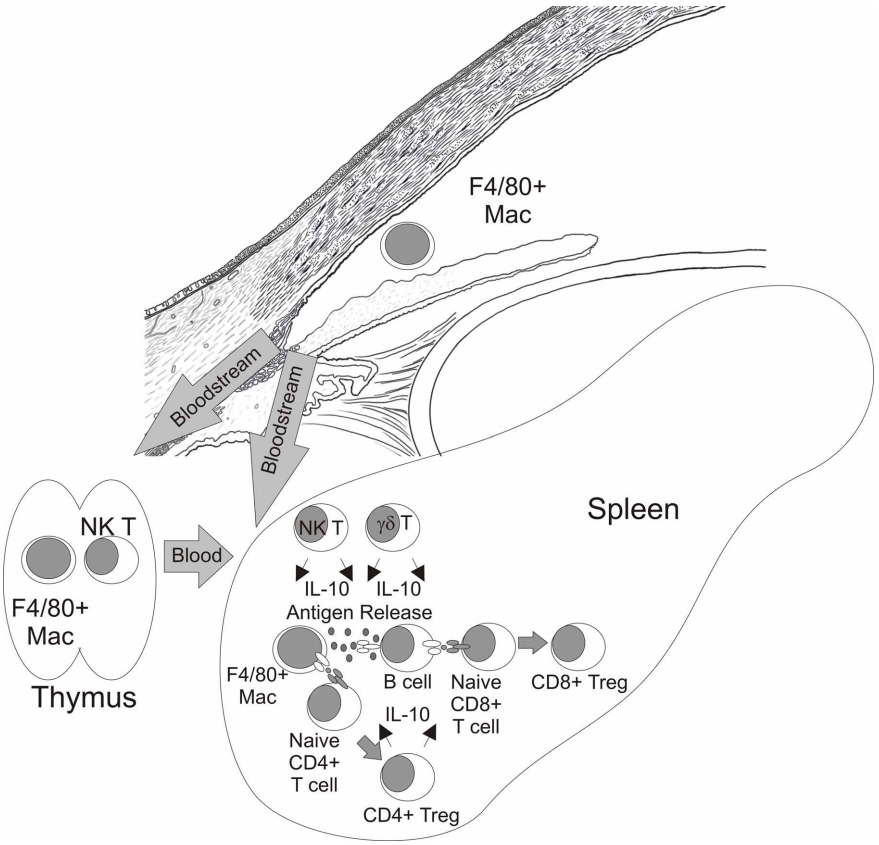

FIGURE 4 | Paradigm of CD8+ Treg generation in ACAID.

1999; Sonoda and Stein-Streilein, 2002), and γδ T cells (Skelsey et al., 2001; Xu and Kapp, 2002) along with production of IL-10 (D'Orazio and Niederkorn, 1998; Sonoda et al., 2001; Ashour and Niederkorn, 2006) culminates in the generation of CD4+ and CD8+ Treg which suppress the induction or expression of DTH responses respectively (Streilein and Niederkorn, 1985; Wilbanks and Streilein, 1990).

The significance of ACAID in ocular immune privilege is clearly demonstrated in splenectomized mice in which ACAID is terminated (Streilein and Niederkorn, 1981). Splenectomy prevents the generation of CD4+ and CD8+ Tregs, restores DTH responses to ocular Ags, and promotes spontaneous rejection of immunogenic tumors placed in the a.c. which would otherwise grow progressively (Streilein and Niederkorn, 1981). Corneal allografts are also rejected with greater frequency in splenectomized mice (Yamagami and Dana, 2001).

CD8+ Treg

T cells function to both eliminate pathogens and to resolve immune responses which, if left uncontrolled, would cause immunopathology and potentially autoimmunity. The seminal experiments by Sakaguchi in 1995 identified that immunosuppressive CD4+ CD25(IL-2Rα)+ T cells protected mice from autoimmunity mediated by CD4+ CD25– T cells (Sakaguchi et al., 1995). These CD4+ Treg represented a distinct lineage generated in the thymus which could be defined by expression of the transcription factor forkhead box P3 (FoxP3) (Fontenot et al., 2003). In addition to these "natural Treg," peripheral CD4+ T cells could also be induced to express FoxP3 and demonstrated regulatory activity although FoxP3 negative CD4+ T cells have also been shown to be immunosuppressive (Shevach, 2009).

It is important to note that immunosuppressive activity of T cells was first described twenty years earlier by Gershon and Kondo (Gershon and Kondo, 1971) who demonstrated that T cells could transfer Ag-specific tolerance to naïve mice. However, their "infectious tolerance" model was mediated by CD8+ T cells. Like CD4+ Treg, both natural and adaptive CD8+ Treg have been described. For example, transfer of natural CD122(common γ-chain receptor)+ CD8+ Treg prevented autoimmunity normally observed in CD122 deficient mice (Rifa'i et al., 2004). In addition, certain treatments (Glatiamer acetate and Fc-ILT3) which mitigate autoimmune conditions are associated with an expansion of induced CD8+ Treg cells (Tennakoon et al., 2006; Vlad et al., 2008). With the exception of FoxP3, there are no well defined markers that distinguish CD8+ T effector cells from CD8+ Treg cells.

IMMUNE RESPONSES TO INTRAOCULAR TUMORS

As mentioned previously, certain tumors transplanted in the a.c. of the eye grow progressively despite induction of tumor-specific

CTL (Niederkorn and Streilein, 1983a; Ksander and Streilein, 1989). A simple explanation for this phenomenon is that CD8+ CTL do not accumulate within the eye, either because they fail to infiltrate ocular tumors or because they undergo apoptosis within the eye due to ocular expression of death inducing molecules (Griffith et al., 1995a; Yamagami et al., 1997; Hori et al., 2006). However, this is clearly not the case as primary uveal melanomas are often infiltrated by CD8+ T cells (de la Cruz et al., 1990; Durie et al., 1990; Meecham et al., 1992; Ksander et al., 1998; McKenna et al., 2009). Similarly, CD8+ T cells accumulated within progressively growing tumors transplanted in the a.c. of mice (Ksander et al., 1991; Vicetti Miguel et al., 2010), and T cells isolated from spleens of mice primed to tumor Ags protected naïve mice from an ocular tumor challenge when transferred intravenously (Niederkorn and Streilein, 1984). As transferred T cells influenced tumor numbers within the a.c., these data also indicated that T cells could exert their tumoricidal effector function within the immune privileged eye at least in some circumstances. These data also argue against a conversion of CD8+ CTL into CD8+ Treg within the eye.

Progressive growth of tumors in the a.c. fails to generate CD4+ T cell dependent DTH responses to ocular tumor Ags (Niederkorn and Streilein, 1983a; Streilein and Niederkorn, 1985), and restoration of these DTH responses has been associated with rejection of intraocular tumors. For example, splenectomized Balb/C mice spontaneously eliminated P815 tumors placed in the a.c. and DTH responses to tumor Ags were restored (Streilein and Niederkorn, 1981). Similarly, P91 tumors (a P815 variant) induced strong DTH responses when transplanted in the a.c. of syngeneic DBA/2 mice and these intraocular tumors were also rejected (Niederkorn and Meunier, 1985). The immunopathological features of intraocular P91 tumor rejection resembled a DTH response as there was neutrophil infiltration, extensive damage to normal ocular tissues (including destruction of the microvasculature), and ischemic bulk necrosis (Knisely et al., 1987) resulting in ocular atrophy, termed phthsis. While these data suggested that DTH responses were critical for ocular tumor elimination, additional experiments showed that administration of anti-CD4 antibodies, which abrogated DTH responses measured in the footpad, did not prevent rejection of intraocular P91 tumors (Niederkorn et al., 1990). Similarly, we have observed that phthsical rejection of P815 tumors in splenectomized Balb/C mice was not influenced by CD4+ T cell depletion (our unpublished observations). In contrast, CD8 T cell depletion resulted in progressive growth of P815 (our unpublished observation and P91 tumors in the a.c. of DBA/2 mice (Niederkorn et al., 1990) although strong P91-specific DTH responses were observed in the footpad (Niederkorn et al., 1990). These data indicated that CD4+ T cell mediated DTH responses to tumor Ags were excluded from the eye. More importantly, CD8+ and not CD4+ T cells were most critical for elimination of intraocular P91, or P815 tumors by inducing expression of a "DTH like" immune response within the eye.

The immune suppressive mechanisms that normally exclude CD4+ T cell mediated DTH responses from the eye have not been defined. However, CD4+ T cell infiltration of ocular tumors does not appear to be compromised as Ad5E1 tumors are spontaneously rejected when placed in the a.c. by a process that requires CD4+ T cells, macrophages, and IFNγ (Schurmans et al., 2001; Wang et al., 2003; Boonman et al., 2006; Dace et al., 2008). Rejection of these ocular tumors is due to direct effects of IFNγ on tumors as Ad5E1 were also rejected in IFNγ receptor 1 (IFNγR1) deficient mice (Dace et al., 2007). As Ad5E1 tumors do not express MHC Class II these data suggest a model in which CD4+ T cells infiltrating ocular tumors express IFNγ only after engaging tumor Ags complexed with MHC Class II that are presented by ocular APC, most probably intratumoral macrophages. The direct effects of IFNγ on Ad5E1 tumors include inhibiting proliferation, and inducing expression of the death inducing molecule TRAIL-R2 and several anti-angiogenic molecules which in combination leads to non-phthsical ocular tumor elimination (Wang et al., 2003; Dace et al., 2007, 2008). Interestingly, Coursey and coworkers recently identified an Ad5E1 variant (Clone 2.1) that was rejected in a phthsical manner (Coursey et al., 2011). Destructive rejection of these tumors required CD4+ T cells and nitric oxide producing macrophages. TNFα expression was critical for inducing Ad5E1 tumor death in a manner which promoted differentiation of tumoricidal macrophages.

INFLUENCE OF Treg CELLS ON INTRAOCULAR TUMOR GROWTH

Administration of tumors into the a.c. of the eye induces CD8+ Tregs which transfer suppression of DTH responses to recipient mice (Streilein and Niederkorn, 1985). These CD8+ Treg are not generated in splenectomized mice that reject tumors transplanted in the a.c. so it is tempting to speculate that they contribute to progressive intraocular tumor growth. However, it is important to note that these Treg do not inhibit the generation of systemic CD8+ CTL or antibody responses directed against ocular tumors (Niederkorn and Streilein, 1983a; Ksander and Streilein, 1989). Therefore, their immunosuppressive effects could only be at the "efferent" stage of the immune response targeting effector immune cells within primary ocular tumors. This notion is somewhat complicated by the observation that mice bearing progressively growing tumors in one eye reject a subsequent tumor challenge in the opposite eye or skin− a phenomenon termed "intracamerally induced concomitant immunity" (Niederkorn and Streilein, 1983b; McKenna and Kapp, 2006). The induction of CD8+ Treg and CD8+ CTL occur with similar kinetics requiring at least 7–10 days to develop (Streilein and Niederkorn, 1981; McKenna and Kapp, 2006). Therefore as the second tumor challenge occurred at a time when CD8+ Treg would have already developed these data indicate that Treg do not influence the tumoricidal activity of immune responses within the microenvironment of the subsequent tumor challenge. However, the requirements for rejection of established primary ocular tumors could be different from those necessary to reject subsequent tumor challenges and thus more or less sensitive to immunoregulation. For example, although Ad5E1 tumors are spontaneously rejected in the a.c. of C57Bl/6 mice by a process that requires CD4+ T cells, macrophages and IFNγ (Schurmans et al., 2001; Wang et al., 2003; Boonman et al., 2006; Dace et al., 2007, 2008), IFNγ deficient mice reject Ad5E1 tumors placed s.c. in the skin and these mice also reject a subsequent Ad5E1 challenge in the a.c. (Dace et al., 2008). Hence, IFNγ is required for rejection of

established Ad5E1 ocular tumors but not for protection from a subsequent ocular tumor challenge.

Tumor burden is also significantly greater in established ocular tumors thereby requiring a stronger immune response to promote tumor elimination which should be more sensitive to immunoregulation. For example, serum or LN cells from mice immunized with P815 tumors s.c. protected naïve mice from an ocular tumor challenge if given 7 days before or at the same time as tumor administration in the a.c. but not if given four days after tumor inoculation (Niederkorn and Streilein, 1984). It is important to note that the kinetics and consequences of rejection of intraocular tumors by transferred sera or immune cells were different and significant. Ocular tumors never developed in mice given immune sera and the eye was preserved (Niederkorn and Streilein, 1984) suggesting that cytotoxic antibodies immediately eliminated tumors in the a.c. of the eye. In contrast, mice given immune LN cells developed established intraocular tumors which were then rejected by a process that caused phthsis (Niederkorn and Streilein, 1984). These data suggest that T cell and macrophage dependent DTH responses were involved in tumor elimination. Therefore, CD8+ Treg could target effector T cells or macrophages within ocular tumors to inhibit expression of DTH responses within the eye and as a result promote ocular tumor growth.

MECHANISMS OF SUPPRESSION BY CD8+ Treg IN ACAID

Characterization of the immune suppressive activity of CD8+ Treg in ACAID has relied entirely on assays that evaluate the inhibition of DTH responses. Specifically, splenocytes from mice given Ag in the a.c. have been shown to inhibit DTH responses when transferred to recipient mice previously immunized with the same Ag (Streilein and Niederkorn, 1985; Wilbanks and Streilein, 1990). In addition, splenocytes from mice given Ag in the a.c. were shown to suppress DTH responses when injected into the skin along with Ag and responder splenocytes from mice immunized with the same Ag in adjuvant. In this "local adoptive transfer assay" (LAT), responder cells alone induced DTH responses and these responses were not suppressed by naïve T cells, or splenocytes from mice given Ag s.c. but only by splenocytes from mice given Ag in the a.c. Further experimentation indicated that the splenic regulatory cell was a CD8+ T Cell (Wilbanks and Streilein, 1990) and this observation has been very reproducible in several different laboratories (Griffith et al., 1996; Nakamura et al., 2003; Cone et al., 2007; Paunicka et al., 2011).

CD8+ Treg isolated from mice given Ag in the a.c. suppress DTH responses in an exquisitely specific manner. For example, splenic CD8+ T cells from mice given bovine serum albumin (BSA) in the a.c. suppress BSA-specific responder cells but not OVA-specific responder cells even if both BSA and OVA Ag are co-injected in the LAT assay to activate BSA-specific CD8+ Treg (Wilbanks and Streilein, 1990). These data clearly argue against "linked suppression" in which CD8+ Treg target APC that express both Ags (Kapp et al., 2006, 2007).

The cellular target of CD8+ Tregs and their mechanism of immune suppression in the DTH response have not been fully elucidated. However, in a related system in which CD8+ Tregs were generated after intravenous injection of TGFβ2-treated Ag-pulsed macrophages, these CD8+ Treg inhibited DTH responses by inducing apoptosis of responder T cells through a Fas/FasL dependent mechanism (Kosiewicz et al., 2004). Similarly, Griffith and coworkers recently demonstrated that CD8+ Treg generated by the administration of HSV-1, or haptenated splenocytes (TNP-SPL) in the a.c. suppressed DTH responses by an apoptotic mechanism involving TRAIL/DR5 interactions (Griffith et al., 2011). In a third system in which CD8+ Treg were generated by injection of haptenated soluble Ag in the a.c., Cone and coworkers also showed that responding T cells were targeted by CD8+ Treg to suppress DTH responses (Cone et al., 2009b). Recognition of responding T cells by CD8+ Treg was restricted by Qa-1 and not MHC Class I (Cone et al., 2009a) which was consistent with the requirement for Qa-1 presentation of Ags by B cells in the generation of CD8+ Treg in ACAID (D'Orazio et al., 2001).

CD8+ Treg that recognize T cell receptor (TCR) peptides presented by Qa-1 have been shown to lyse Vβ3+ CD4+ T cells which normally expand in mice given the superantigen staphylococcal enterotoxin A (SEA) (Hu et al., 2004). These data suggest that under certain circumstances CD8+ Treg are primed to TCR determinants expressed by responding CD4+ T cells. Recognition of a TCR expressed by CD4+ T cells which expanded to Ags administered in the a.c. would nicely explain the specificity of CD8+ Treg in suppression of DTH responses in ACAID. As previously described, ACAID CD8+ Treg only suppress DTH responses to the same Ag as was originally delivered in the a.c. (Wilbanks and Streilein, 1990). Therefore, these data suggest a model in which CD8+ Treg eliminate responding T cell populations by recognizing a unique TCR determinant presented by Qa-1 on T cells (**Figure 5A**). In support of this model two independent laboratories have identified Ag-specific TCR proteins in serum after administration of Ag in the a.c. which transfer tolerance to naïve recipient mice (Ferguson et al., 1989; Griffith et al., 1995b; Hadjikouti et al., 1995). In addition, CD8+ Treg which suppress OVA-specific DTH responses can be generated by injection of the class II restricted peptide $OVA_{323-339}$ (Kosiewicz and Streilein, 1996) which supports that CD8+ Treg in ACAID are not restricted by MHC Class I and could indicate that CD8+ Treg recognized a TCR determinant expressed by responding $OVA_{323-339}$-specific CD4+ T cells. The inhibition of DTH responses by CD8+ Treg was also associated with absence of immune infiltrates at the site of the LAT assay in the skin which is consistent with elimination of responder T cells (Cone et al., 2009a).

A significant caveat to this model is that CD8+ Treg were also generated in CD4+ deficient MHC Class II−/− mice given soluble Ag in the a.c. (Nakamura et al., 2003). Therefore, CD8+ Treg with specificities other than TCR expressed by CD4+ T cells may also be generated. For example, CD8+ OT-I TCR transgenic T cells which recognize OVA peptide 257–264 complexed with Class I K^b were induced to become non-lytic CD103+ CD8+ Treg when stimulated with OVA-pulsed-TGFβ2 treated macrophages (Kezuka and Streilein, 2000; Keino et al., 2006a). These OT-I Treg were similar to those observed in ACAID in that they suppressed DTH responses in a LAT assay (Kezuka and Streilein,

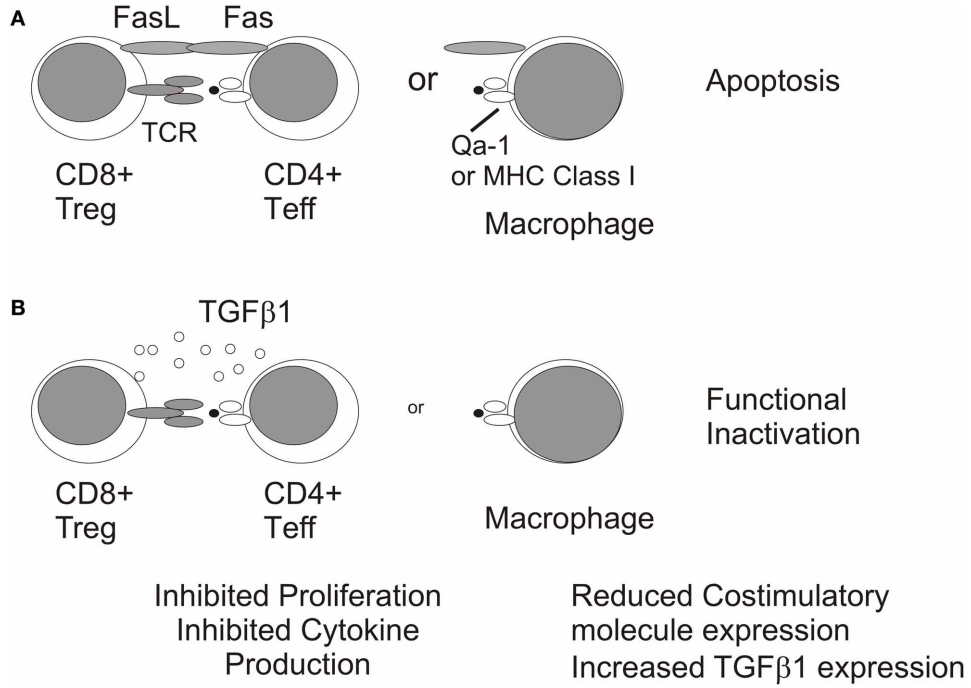

FIGURE 5 | Mechanisms of immunosuppression by CD8+ Treg. CD8+ Tregs may inhibit DTH responses by inducing apoptosis of CD4+ T cell effectors or macrophages **(A)** or by functionally inactivating these immune populations via TGFb1 expression **(B)**.

2000; Keino et al., 2006a). However, their cellular target may be APC (macrophages and/or dendritic cells) and not responder CD4+ T cells (**Figure 5B**) as Kapp and coworkers showed in a similar system that OT-I Treg reduced expression of the costimulatory molecule, CD86, on dendritic cells (Kapp et al., 2006). TGFβ has been shown to reduce costimulatory molecule expression on APC (Takeuchi et al., 1998) and is required for the suppressive activity of CD8+ Treg in ACAID (Cone et al., 2009b; Jiang et al., 2009) which could suggest additional non-lytic mechanism for inhibiting DTH responses. However, the suppression of immune responses by non-lytic OT-I CD8+ Treg does not require TGFβ (Kezuka and Streilein, 2000; Kapp et al., 2006) indicating that other immunosuppressive mediators may be involved. It is important to note that the potential contribution of CD8+ Treg in promoting ocular tumor growth would require their recognition of non-CD4+ T cells because CD4+ T cells do not contribute to elimination of intraocular P91 or P815 tumors (Niederkorn et al., 1990 and our unpublished observations). Therefore, CD8+ CTL effectors or intratumoral macrophages are logical targets of CD8+ Treg in the ocular tumor microenvironment (**Figure 6**).

INDIRECT TUMORICIDAL ACTIVITY OF CD8+ T CELLS

CD8+ T cells play a critical role in immunosurveillance of tumors by recognition of processed peptides presented by MHC Class I molecules on the tumor cell surface. Through release of cytotoxic granules containing granzyme B and perforin CD8+ CTL directly lyse tumor cells (Simon et al., 1997). In addition, CD8+ CTL express IFNγ which can induce apoptosis of certain tumors (Wall et al., 2003) and/or influence hematopoietic and non-hematopoietic cells within the tumor microenvironment to affect tumor growth (Blankenstein, 2005). For example, IFNγ dependent regression of B16-OVA melanomas transplanted in the skin by CD8+ OVA-specific OT-I T cell effectors required IFNγR1 expression by host cells (Schuler and Blankenstein, 2003). An inhibition of tumor angiogenesis preceded tumor regression in other models of IFNγ mediated rejection of tumors by CD8+ T cells suggesting that IFNγ targeted vascular endothelial cells, fibroblasts, and/or pro-angiogenic macrophages (Qin et al., 2003).

IFNγ may also promote tumoricidal activity in other immune cell populations within the tumor microenvironment. For example, regression of established E.G7-OVA skin tumors by CD8+ OT-I CTL effectors required IFNγ but not perforin expression by transferred T cells (Hollenbaugh et al., 2004; Hollenbaugh and Dutton, 2006). IFNγR1 and inducible nitric oxide synthase-2 (NOS2) expression in recipient mice were also required for tumor regression which indicated that CTL-expressed-IFNγ targeted host cells to eliminate skin tumors (Hollenbaugh et al., 2004; Hollenbaugh and Dutton, 2006). We recently demonstrated that transferred OT-I CTL induced F4/80+ macrophages within E.G7-OVA skin tumors to express tumoricidal concentrations of nitric oxide (NO) (Vicetti Miguel et al., 2010). Hence CD8+ T cell elimination of established E.G7-OVA skin tumors was indirect via their induction of tumoricidal activity in intratumoral macrophages. In contrast, established intraocular E.G7-OVA tumors were resistant to OT-I CTL transfer therapy although CTL infiltrated primary intraocular tumors and

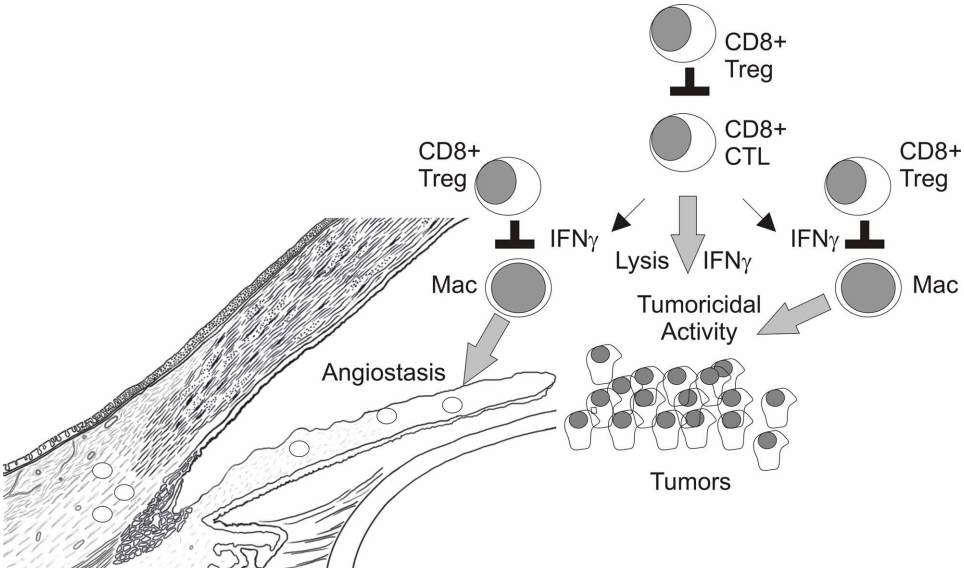

FIGURE 6 | Potential targets of CD8+ Treg in intraocular tumors.

expressed IFNγ which induced macrophages to express NOS2 protein; the enzyme responsible for NO production. However, ocular tumor associated macrophages did not produce appreciable amounts of NO and were not tumoricidal (Vicetti Miguel et al., 2010). Therefore, factors within the immune privileged eye normally inhibit NOS2 enzymatic activity in macrophages which contributes to ocular tumor progression. These data highlight that the interplay between CD8+ T cells and intratumoral macrophages is critical for elimination of intraocular tumors and represents a potential target of CD8+ Treg (**Figure 6**).

UVEAL MELANOMA

Immune suppressive mechanisms, which maintain ocular immune privilege, should theoretically decrease immunosurveillance of intraocular malignancies. However, the most common intraocular tumor, uveal melanoma (UM), is a rare malignancy. The frequency of UM is over 30 times lower than cutaneous melanoma (Singh and Topham, 2003; Garbe and Leiter, 2009). One explanation for this paradox is that the expression of death inducing molecules within the eye limits tumor outgrowth. For example, tumor necrosis factor (TNF) related apoptosis inducing ligand (TRAIL) is expressed by tissues lining the interior of the eye (Lee et al., 2002) and targets transformed cells for apoptosis (Wiley et al., 1995). P815 tumor cells transduced to express TRAIL receptor DR5 failed to develop into ocular tumors when injected into the a.c. of the eye of Balb/C mice (Lee et al., 2002) which supports this notion. Therefore, the lower frequency of ocular malignancies may be due to augmented expression of death inducing molecules that induce apoptosis in transformed cells. However, when transformed cells become resistant to apoptosis several observations suggest that ocular immune privilege favors tumor growth and persistence.

Uveal melanoma is unique in that primary tumors with an "inflammatory phenotype," characterized by significant infiltration with CD8+ T cells and CD68+ macrophages, are generally larger more vascularized tumors which express a genetic profile indicative of greater risk of liver metastasis (Maat et al., 2008). These data suggest that tumor-specific ocular immune responses are somehow converted within the ocular tumor microenvironment to favor tumor growth which is consistent with immunosuppression by ocular immune privilege.

CD8+ T cell function may be impaired within primary uveal melanomas as reduced CD3zeta chain expression, a marker of T cell dysfunction, was observed in T cells infiltrating ocular tumors that ultimately metastasized to the liver (Staibano et al., 2006). In addition, T cells isolated from primary uveal melanomas were generally non-responsive, proliferating poorly after stimulation (Ksander et al., 1998). In other malignancies reduced CD3zeta chain expression correlated with increased frequencies of activated CD11b+ CD15+ granulocytes in the blood (Schmielau and Finn, 2001; Zea et al., 2005) and we recently showed that CD11b+ CD15+ granulocytes were increased in a cohort of patients with primary UMs (McKenna et al., 2009) suggesting a possible mechanism for CD3zeta chain downmodulation. Although CD8+ T cells within primary UM appear to proliferate poorly, they still accumulate within primary uveal melanomas and indirect evidence suggests that they produce IFNγ. For example, primary UMs with increased numbers of CD8+ T cells are associated with increased HLA expression (de Waard-Siebinga et al., 1996) which is known to be influenced by IFNγ (de Waard-Siebinga et al., 1995). Moreover, in the E.G7-OVA/C57Bl/6 mouse model of intraocular tumor development we observed that CD8+ T cells which infiltrated intraocular tumors expressed IFNγ at levels which were equivalent to those observed in CD8+ T cells that infiltrated skin tumors (Vicetti Miguel et al., 2010).

IFNγ may actually promote immune evasion by ocular tumors as primary uveal melanoma lines treated with IFNγ expressed PD-L1 which inhibited T cell function *in vitro* (Yang et al., 2008). Similarly, IFNγ rendered uveal melanoma cell lines resistant to lysis by CD8+ T cells (Hallermalm et al., 2008). In addition, IFNγ is required for expression of the suppressive activity of CD8+ Treg in ACAID (Cone et al., 2007; Paunicka et al., 2011). Therefore, another explanation for primary uveal melanoma growth despite infiltration by CD8+ T cells is that these CD8+ T cells are not tumoricidal effectors but rather immune suppressive Treg.

As previously described the transcription factor FoxP3 faithfully identifies naturally occurring CD4+ Treg in mice and is also expressed in some induced CD4+ and CD8+ Tregs (Shevach, 2009). Two studies recently evaluated FoxP3 expression on T cells infiltrating primary uveal melanomas (Lagouros et al., 2009; Mougiakakos et al., 2010). FoxP3 expression was observed only in CD4+ T cells and their numbers were generally very low and correlated with the size of tumors and frequency of CD3+ T cells (Lagouros et al., 2009). As both tumor size and CD3+ T infiltration are negative prognostic indicators (Damato et al., 2011), it is difficult to discern the influence of CD4+ FOXP3+ Treg on tumor progression. However, the presence of FOXP3+ Treg in cycloxygenase-2 (COX-2) positive tumors did predict poor survival of uveal melanoma patients (Mougiakakos et al., 2010). It is important to note that these studies could not exclude that infiltrating CD8+ T cells, though FoxP3 negative, were immunosuppressive Treg.

Macrophages have also been shown to influence tumor growth (Mantovani et al., 2002). For example, macrophages stimulated with IFNγ and LPS, termed M1, demonstrate tumoricidal activity through expression of reactive oxygen and nitrogen production. In contrast, macrophages stimulated with IL-4 and IL-13, termed M2, are not tumoricidal and actually tumor promoting via expression of angiogenic factors including VEGF. Macrophages within primary uveal melanomas express CD163 which is a marker of M2 macrophages (Bronkhorst et al., 2010) and primary UMs heavily infiltrated with macrophages are more vascularized (Makitie et al., 2001). Taken together these data suggest that ocular tumor associated macrophages may promote tumor growth by inducing tumor angiogenesis. Therefore, it is possible that CD8+ Treg may contribute to ocular tumor growth by maintaining ocular tumor associated macrophages in an M2 phenotype.

CONCLUSIONS

It is very clear that progressive growth of intraocular tumors generates CD8+ Treg that inhibit CD4+ T cell dependent DTH responses to tumor Ags. However, as restoration of CD4+ T cell responses to tumor Ags does not assure ocular tumor elimination the contribution of CD8+ Treg in ocular tumor progression remains unclear. Interestingly, rejection of certain tumors transplanted into the a.c. of the eye involves a destructive process resembling a "DTH-like" response that requires CD8+ T cells and IFNγ. As several observations indicate that T cell production of IFNγ is not impaired within ocular tumors, CD8+ Treg may target macrophages in progressively growing ocular tumors to prevent their expression of inflammatory mediators that promote DTH responses which destroy the tumor vasculature, and/or directly kill tumor cells (**Figure 6**). One challenge to defining the role of CD8+ Treg in ocular tumor progression is a specific marker that discerns CD8+ CTL effectors from CD8+ Tregs. While the selective expression of CD103 on *in vitro* generated CD8+ Treg is encouraging (Keino et al., 2006a), *in vivo* expression of CD103 by ACAID CD8+ Treg has not been shown. Future experimentation which compares and contrasts tumor-specific immune responses in splenectomized mice that do not generate CD8+ Treg and eliminate tumors placed in the a.c. to mice with progressively growing intraocular tumors should help to define potential targets for CD8+ Tregs and may identify novel molecules expressed by CD8+ Treg.

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REFERENCES

Abi-Hanna, D., Wakefield, D., and Watkins, S. (1988). HLA antigens in ocular tissues. I. *In vivo* expression in human eyes. *Transplantation* 45, 610–613.

Apte, R. S., Sinha, D., Mayhew, E., Wistow, G. J., and Niederkorn, J. Y. (1998). Cutting edge: role of macrophage migration inhibitory factor in inhibiting NK cell activity and preserving immune privilege. *J. Immunol.* 160, 5693–5696.

Ashour, H. M., and Niederkorn, J. Y. (2006). Gammadelta T cells promote anterior chamber-associated immune deviation and immune privilege through their production of IL-10. *J. Immunol.* 177, 8331–8337.

Benezra, D., and Sachs, U. (1974). Growth factors in aqueous humor of normal and inflamed eyes of rabbits. *Invest. Ophthalmol.* 13, 868–870.

Bill, A. (1977). Basic physiology of the drainage of aqueous humor. *Exp.Eye Res.* 25(Suppl.) 291–304.

Blankenstein, T. (2005). The role of tumor stroma in the interaction between tumor and immune system. *Curr. Opin. Immunol.* 17, 180–186.

Boonman, Z. F., Schurmans, L. R., Van, R. N., Melief, C. J., Toes, R. E., and Jager, M. J. (2006). Macrophages are vital in spontaneous intraocular tumor eradication. *Invest. Ophthalmol. Vis. Sci.* 47, 2959–2965.

Bronkhorst, I. H., Ly, L. V., Jordanova, E. S., Vrolijk, H., Versluis, M., Luyten, G. P., and Jager, M. J. (2010). Detection of M2 macrophages in uveal melanoma and relation with survival. *Invest. Ophthalmol. Vis. Sci.* 52, 643–650.

Cone, R. E., Chattopadhyay, S., Sharafieh, R., Lemire, Y., and O'Rourke, J. (2009a). The suppression of hypersensitivity by ocular-induced CD8(+) T cells requires compatibility in the Qa-1 haplotype. *Immunol. Cell Biol.* 87, 241–248.

Cone, R. E., Chattopadhyay, S., Sharafieh, R., Lemire, Y., O'Rourke, J., Flavell, R. A., and Clark, R. B. (2009b). T cell sensitivity to TGF-beta is required for the effector function but not the generation of splenic CD8+ regulatory T cells induced via the injection of antigen into the anterior chamber. *Int. Immunol.* 21, 567–574.

Cone, R. E., Li, X., Sharafieh, R., O'Rourke, J., and Vella, A. T. (2007). The suppression of delayed-type hypersensitivity by CD8+ regulatory T cells requires interferon-gamma. *Immunology* 120, 112–119.

Coursey, T. G., Chen, P. W., and Niederkorn, J. Y. (2011). Abrogating TNF-alpha expression prevents bystander destruction of normal tissues during iNOS-mediated elimination of intraocular tumors. *Cancer Res.* 71, 2445–2454.

Cousins, S. W., McCabe, M. M., Danielpour, D., and Streilein, J. W. (1991). Identification of transforming growth factor-beta as an immunosuppressive factor in aqueous humor. *Invest. Ophthalmol. Vis. Sci.* 32, 2201–2211.

Crane, I. J., and Liversidge, J. (2008). Mechanisms of leukocyte migration across the blood-retina barrier. *Semin. Immunopathol.* 30, 165–177.

Dace, D. S., Chen, P. W., Alizadeh, H., and Niederkorn, J. Y. (2007). Ocular immune privilege is circumvented by CD4+ T cells, leading to the rejection of intraocular tumors in an IFN-{gamma}-dependent manner. *J. Leukoc. Biol.* 81, 421–429.

Dace, D. S., Chen, P. W., and Niederkorn, J. Y. (2008). CD4+ T-cell-dependent tumour rejection in an immune-privileged environment requires macrophages. *Immunology* 123, 367–377.

Damato, B., Eleuteri, A., Taktak, A. F., and Coupland, S. E. (2011). Estimating prognosis for survival after treatment of choroidal melanoma. *Prog. Retin. Eye Res.* 30, 285–295.

de la Cruz, P. O. Jr., Specht, C. S., and McLean, I. W. (1990). Lymphocytic infiltration in uveal malignant melanoma. *Cancer* 65, 112–115.

de Waard-Siebinga, I., Creyghton, W. M., Kool, J., and Jager, M. J. (1995). Effects of interferon alfa and gamma on human uveal melanoma cells *in vitro*. *Br. J. Ophthalmol.* 79, 847–855.

de Waard-Siebinga, I., Hilders, C. G., Hansen, B. E., van Delft, J. L., and Jager, M. J. (1996). HLA expression and tumor-infiltrating immune cells in uveal melanoma. *Graefes Arch. Clin. Exp. Ophthalmol.* 234, 34–42.

D'Orazio, T. J., Mayhew, E., and Niederkorn, J. Y. (2001). Ocular immune privilege promoted by the presentation of peptide on tolerogenic B cells in the spleen. II. Evidence for presentation by Qa-1. *J. Immunol.* 166, 26–32.

D'Orazio, T. J., and Niederkorn, J. Y. (1998). A novel role for TGF-beta and IL-10 in the induction of immune privilege. *J. Immunol.* 160, 2089–2098.

Durie, F. H., Campbell, A. M., Lee, W. R., and Damato, B. (1990). Analysis of lymphocytic infiltration in uveal melanoma. *Invest. Ophthalmol. Vis. Sci.* 31, 2106–2110.

Egan, R. M., Yorkey, C., Black, R., Loh, W. K., Stevens, J. L., and Woodward, J. G. (1996). Peptide-specific T cell clonal expansion *in vivo* following immunization in the eye, an immune-privileged site. *J. Immunol.* 157, 2262–2271.

Faunce, D. E., Sonoda, K. H., and Stein-Streilein, J. (2001). MIP-2 recruits NKT cells to the spleen during tolerance induction. *J. Immunol.* 166, 313–321.

Faunce, D. E., and Stein-Streilein, J. (2002). NKT cell-derived RANTES recruits APCs and CD8+ T cells to the spleen during the generation of regulatory T cells in tolerance. *J. Immunol.* 169, 31–38.

Ferguson, T. A., Hayashi, J. D., and Kaplan, H. J. (1989). The immune response and the eye. III. Anterior chamber-associated immune deviation can be adoptively transferred by serum. *J. Immunol.* 143, 821–826.

Fontenot, J. D., Gavin, M. A., and Rudensky, A. Y. (2003). Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol.* 4, 330–336.

Garbe, C., and Leiter, U. (2009). Melanoma epidemiology and trends. *Clin. Dermatol.* 27, 3–9.

Gershon, R. K., and Kondo, K. (1971). Infectious immunological tolerance. *Immunology* 21, 903–914.

Griffith, T. S., Brincks, E. L., Gurung, P., Kucaba, T. A., and Ferguson, T. A. (2011). Systemic immunological tolerance to ocular antigens is mediated by TRAIL-expressing CD8+ T cells. *J. Immunol.* 186, 791–798.

Griffith, T. S., Brunner, T., Fletcher, S. M., Green, D. R., and Ferguson, T. A. (1995a). Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science* 270, 1189–1192.

Griffith, T. S., Herndon, J. M., Lima, J., Kahn, M., and Ferguson, T. A. (1995b). The immune response and the eye. TCR alpha-chain related molecules regulate the systemic immunity to antigen presented in the eye. *Int. Immunol.* 7, 1617–1625.

Griffith, T. S., Yu, X., Herndon, J. M., Green, D. R., and Ferguson, T. A. (1996). CD95-induced apoptosis of lymphocytes in an immune privileged site induces immunological tolerance. *Immunity* 5, 7–16.

Hadjikouti, C. A., Wang, Y., O'Rourke, J., and Cone, R. E. (1995). Intracameral injection of antigen potentiates the production of antigen-specific T cell proteins in serum after the induction of delayed-type hypersensitivity. *Invest. Ophthalmol. Vis. Sci.* 36, 1470–1476.

Hallermalm, K., Seki, K., De, G. A., Motyka, B., Bleackley, R. C., Jager, M. J., Froelich, C. J., Kiessling, R., Levitsky, V., and Levitskaya, J. (2008). Modulation of the tumor cell phenotype by IFN-gamma results in resistance of uveal melanoma cells to granule-mediated lysis by cytotoxic lymphocytes. *J. Immunol.* 180, 3766–3774.

Hollenbaugh, J. A., and Dutton, R. W. (2006). IFN-gamma regulates donor CD8 T cell expansion, migration, and leads to apoptosis of cells of a solid tumor. *J. Immunol.* 177, 3004–3011.

Hollenbaugh, J. A., Reome, J., Dobrzanski, M., and Dutton, R. W. (2004). The rate of the CD8-dependent initial reduction in tumor volume is not limited by contact-dependent perforin, Fas ligand, or TNF-mediated cytolysis. *J. Immunol.* 173, 1738–1743.

Hori, J., Wang, M., Miyashita, M., Tanemoto, K., Takahashi, H., Takemori, T., Okumura, K., Yagita, H., and Azuma, M. (2006). B7-H1-induced apoptosis as a mechanism of immune privilege of corneal allografts. *J. Immunol.* 177, 5928–5935.

Hu, D., Ikizawa, K., Lu, L., Sanchirico, M. E., Shinohara, M. L., and Cantor, H. (2004). Analysis of regulatory CD8 T cells in Qa-1-deficient mice. *Nat. Immunol.* 5, 516–523.

Jiang, L., He, H., Yang, P., Lin, X., Zhou, H., Huang, X., and Kijlstra, A. (2009). Splenic CD8+ T cells secrete TGF-beta1 to exert suppression in mice with anterior chamber-associated immune deviation. *Graefes Arch. Clin. Exp. Ophthalmol.* 247, 87–92.

Kaiser, C. J., Ksander, B. R., and Streilein, J. W. (1989). Inhibition of lymphocyte proliferation by aqueous humor. *Reg. Immunol.* 2, 42–49.

Kaplan, H. J., and Streilein, J. W. (1977). Immune response to immunization via the anterior chamber of the eye. I. F1-lymphocyte induced immune deviation. *J. Immunol.* 118, 809–814.

Kapp, J. A., Honjo, K., Kapp, L. M., Goldsmith, K., and Bucy, R. P. (2007). Antigen, in the presence of TGF-beta, induces up-regulation of FoxP3gfp+ in CD4+ TCR transgenic T cells that mediate linked suppression of CD8+ T cell responses. *J. Immunol.* 179, 2105–2114.

Kapp, J. A., Honjo, K., Kapp, L. M., Xu, X., Cozier, A., and Bucy, R. P. (2006). TCR transgenic CD8+ T cells activated in the presence of TGFbeta express FoxP3 and mediate linked suppression of primary immune responses and cardiac allograft rejection. *Int. Immunol.* 18, 1549–1562.

Keino, H., Masli, S., Sasaki, S., Streilein, J. W., and Stein-Streilein, J. (2006a). CD8+ T regulatory cells use a novel genetic program that includes CD103 to suppress Th1 immunity in eye-derived tolerance. *Invest. Ophthalmol. Vis. Sci.* 47, 1533–1542.

Keino, H., Takeuchi, M., Kezuka, T., Hattori, T., Usui, M., Taguchi, O., Streilein, J. W., and Stein-Streilein, J. (2006b). Induction of eye-derived tolerance does not depend on naturally occurring CD4+CD25+ T regulatory cells. *Invest. Ophthalmol. Vis. Sci.* 47, 1047–1055.

Kezuka, T., and Streilein, J. W. (2000). *In vitro* generation of regulatory CD8+ T cells similar to those found in mice with anterior chamber-associated immune deviation. *Invest. Ophthalmol. Vis. Sci.* 41, 1803–1811.

Knisely, T. L., Luckenbach, M. W., Fischer, B. J., and Niederkorn, J. Y. (1987). Destructive and non-destructive patterns of immune rejection of syngeneic intraocular tumors. *J. Immunol.* 138, 4515–4523.

Kosiewicz, M. M., Alard, P., Liang, S., and Clark, S. L. (2004). Mechanisms of tolerance induced by transforming growth factor-beta-treated antigen-presenting cells: CD8 regulatory T cells inhibit the effector phase of the immune response in primed mice through a mechanism involving Fas ligand. *Int. Immunol.* 16, 697–706.

Kosiewicz, M. M., and Streilein, J. W. (1996). Intraocular injection of class II-restricted peptide induces an unexpected population of CD8 regulatory cells. *J. Immunol.* 157, 1905–1912.

Ksander, B. R., Bando, Y., Acevedo, J., and Streilein, J. W. (1991). Infiltration and accumulation of precursor cytotoxic T-cells increase with time in progressively growing ocular tumors. *Cancer Res.* 51, 3153–3158.

Ksander, B. R., Geer, D. C., Chen, P. W., Salgaller, M. L., Rubsamen, P., and Murray, T. G. (1998). Uveal melanomas contain antigenically specific and non-specific infiltrating lymphocytes. *Curr. Eye Res.* 17, 165–173.

Ksander, B. R., and Hendricks, R. L. (1987). Cell-mediated immune

tolerance to HSV-1 antigens associated with reduced susceptibility to HSV-1 corneal lesions. *Invest. Ophthalmol. Vis. Sci.* 28, 1986–1993.

Ksander, B. R., and Streilein, J. W. (1989). Analysis of cytotoxic T cell responses to intracameral allogeneic tumors. *Invest. Ophthalmol. Vis. Sci.* 30, 323–329.

Lagouros, E., Salomao, D., Thorland, E., Hodge, D. O., Vile, R., and Pulido, J. S. (2009). Infiltrative T regulatory cells in enucleated uveal melanomas. *Trans. Am. Ophthalmol. Soc.* 107, 223–228.

Lai, J. C., Lobanoff, M. C., Fukushima, A., Wawrousek, E. F., Chan, C. C., Whitcup, S. M., and Gery, I. (1999). Uveitis induced by lymphocytes sensitized against a transgenically expressed lens protein. *Invest. Ophthalmol. Vis. Sci.* 40, 2735–2739.

Lee, H. O., Herndon, J. M., Barreiro, R., Griffith, T. S., and Ferguson, T. A. (2002). TRAIL: a mechanism of tumor surveillance in an immune privileged site. *J. Immunol.* 169, 4739–4744.

Li, X., Taylor, S., Zegarelli, B., Shen, S., O'Rourke, J., and Cone, R. E. (2004). The induction of splenic suppressor T cells through an immune-privileged site requires an intact sympathetic nervous system. *J. Neuroimmunol.* 153, 40–49.

Lin, H. H., Faunce, D. E., Stacey, M., Terajewicz, A., Nakamura, T., Zhang-Hoover, J., Kerley, M., Mucenski, M. L., Gordon, S., and Stein-Streilein, J. (2005). The macrophage F4/80 receptor is required for the induction of antigen-specific efferent regulatory T cells in peripheral tolerance. *J. Exp. Med.* 201, 1615–1625.

Maat, W., Ly, L. V., Jordanova, E. S., de Wolff-Rouendaal, D., Schalij-Delfos, N. E., and Jager, M. J. (2008). Monosomy of chromosome 3 and an inflammatory phenotype occur together in uveal melanoma. *Invest. Ophthalmol. Vis. Sci.* 49, 505–510.

Makitie, T., Summanen, P., Tarkkanen, A., and Kivela, T. (2001). Tumor-infiltrating macrophages (CD68(+) cells) and prognosis in malignant uveal melanoma. *Invest. Ophthalmol. Vis. Sci.* 42, 1414–1421.

Mantovani, A., Sozzani, S., Locati, M., Allavena, P., and Sica, A. (2002). Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol.* 23, 549–555.

Masli, S., Turpie, B., and Streilein, J. W. (2006). Thrombospondin orchestrates the tolerance-promoting properties of TGFbeta-treated antigen-presenting cells. *Int. Immunol.* 18, 689–699.

McKenna, K. C., Anderson, K. M., and Kapp, J. A. (2005). CD8+ T-cell tolerance induced by delivery of antigen to the anterior chamber is not the result of de facto intravenous or mucosal administration of antigen. *Ocul. Immunol. Inflamm.* 13, 149–157.

McKenna, K. C., Beatty, K. M., Bilonick, R. A., Schoenfield, L., Lathrop, K. L., and Singh, A. D. (2009). Activated CD11b+ CD15+ granulocytes increase in blood of uveal melanoma patients. *Invest. Ophthalmol. Vis. Sci.* 50, 4295–4303.

McKenna, K. C., and Kapp, J. A. (2006). Accumulation of immunosuppressive CD11b+ myeloid cells correlates with the failure to prevent tumor growth in the anterior chamber of the eye. *J. Immunol.* 177, 1599–1608.

McKenna, K. C., Xu, Y., and Kapp, J. A. (2002). Injection of soluble antigen into the anterior chamber of the eye induces expansion and functional unresponsiveness of antigen-specific CD8+ T cells. *J. Immunol.* 169, 5630–5637.

Medawar, P. (1948). Immunity to homologous grafted skin: III. The fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. *Br. J. Exp. Pathol.* 29, 58–69.

Meecham, W. J., Char, D. H., and Kaleta-Michaels, S. (1992). Infiltrating lymphocytes and antigen expression in uveal melanoma. *Ophthalmic Res.* 24, 20–26.

Mougiakakos, D., Johansson, C. C., Trocme, E., ll-Ericsson, C., Economou, M. A., Larsson, O., Seregard, S., and Kiessling, R. (2010). Intratumoral forkhead box P3-positive regulatory T cells predict poor survival in cyclooxygenase-2-positive uveal melanoma. *Cancer* 116, 2224–2233.

Nakamura, T., Sonoda, K. H., Faunce, D. E., Gumperz, J., Yamamura, T., Miyake, S., and Stein-Streilein, J. (2003). CD4(+) NKT cells, but not conventional CD4(+) T cells, are required to generate efferent CD8(+) T regulatory cells following antigen inoculation in an immune-privileged site. *J. Immunol.* 171, 1266–1271.

Niederkorn, J. Y. (1984). Suppressed cellular immunity in mice harboring intraocular melanomas. *Invest. Ophthalmol. Vis. Sci.* 25, 447–454.

Niederkorn, J. Y., Benson, J. L., and Mayhew, E. (1990). Efferent blockade of delayed-type hypersensitivity responses in the anterior chamber of the eye. *Reg. Immunol.* 3, 349–354.

Niederkorn, J. Y., and Meunier, P. C. (1985). Spontaneous immune rejection of intraocular tumors in mice. *Invest. Ophthalmol. Vis. Sci.* 26, 877–884.

Niederkorn, J. Y., Shadduck, J. A., and Streilein, J. W. (1981). Immunogenetic basis for immunologic privilege in the anterior chamber of the eye. *Immunogenetics* 13, 227–236.

Niederkorn, J. Y., and Streilein, J. W. (1982a). Analysis of antibody production induced by allogeneic tumor cells inoculated into the anterior chamber of the eye. *Transplantation* 33, 573–577.

Niederkorn, J. Y., and Streilein, J. W. (1982b). Induction of anterior chamber-associated immune deviation (ACAID) by allogeneic intraocular tumors does not require splenic metastases. *J. Immunol.* 128, 2470–2474.

Niederkorn, J. Y., and Streilein, J. W. (1983a). Alloantigens placed into the anterior chamber of the eye induce specific suppression of delayed-type hypersensitivity but normal cytotoxic T lymphocyte and helper T lymphocyte responses. *J. Immunol.* 131, 2670–2674.

Niederkorn, J. Y., and Streilein, J. W. (1983b). Intracamerally induced concomitant immunity: mice harboring progressively growing intraocular tumors are immune to spontaneous metastases and secondary tumor challenge. *J. Immunol.* 131, 2587–2594.

Niederkorn, J. Y., and Streilein, J. W. (1984). Adoptive transfer of immunity to intraocular tumors in mice. *Invest. Ophthalmol. Vis. Sci.* 25, 336–342.

Nishida, T., and Taylor, A. W. (1999). Specific aqueous humor factors induce activation of regulatory T cells. *Invest. Ophthalmol. Vis. Sci.* 40, 2268–2274.

Patel, S. P., and Dana, R. (2009). Corneal lymphangiogenesis: implications in immunity. *Semin. Ophthalmol.* 24, 135–138.

Paunicka, K., Chen, P. W., and Niederkorn, J. Y. (2011). Role of IFN-gamma in the establishment of anterior chamber-associated immune deviation (ACAID)-induced CD8+ T regulatory cells. *J. Leukoc. Biol.* 91, 475–483.

Perez, V. L., Biuckians, A., and Streilein, J. W. (2000). *In-vivo* impaired T helper 1 cell development in submandibular lymph nodes due to Il-12 deficiency following antigen injection into the anterior chamber of the eye. *Ocul. Immunol. Inflamm.* 8, 9–24.

Qin, Z., Schwartzkopff, J., Pradera, F., Kammertoens, T., Seliger, B., Pircher, H., and Blankenstein, T. (2003). A critical requirement of interferon gamma-mediated angiostasis for tumor rejection by CD8+ T cells. *Cancer Res.* 63, 4095–4100.

Radosevich, M., Jager, M., and Ono, S. J. (2007). Inhibition of MHC class II gene expression in uveal melanoma cells is due to methylation of the CIITA gene or an upstream activator. *Exp. Mol. Pathol.* 82, 68–76.

Radosevich, M., Song, Z., Gorga, J. C., Ksander, B., and Ono, S. J. (2004). Epigenetic silencing of the CIITA gene and posttranscriptional regulation of class II MHC genes in ocular melanoma cells. *Invest. Ophthalmol. Vis. Sci.* 45, 3185–3195.

Rifa'i, M., Kawamoto, Y., Nakashima, I., and Suzuki, H. (2004). Essential roles of CD8+CD122+ regulatory T cells in the maintenance of T cell homeostasis. *J. Exp. Med.* 200, 1123–1134.

Rosenbaum, J. T., Howes, E. L. Jr., and English, D. (1985). Ascorbate in aqueous humor protects against myeloperoxidase-induced oxidation. *Am. J. Pathol.* 120, 244–247.

Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., and Toda, M. (1995). Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* 155, 1151–1164.

Schmielau, J., and Finn, O. J. (2001). Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of t-cell function in advanced cancer patients. *Cancer Res.* 61, 4756–4760.

Schuler, T., and Blankenstein, T. (2003). Cutting edge: CD8+ effector T cells reject tumors by direct antigen recognition but indirect action on host cells. *J. Immunol.* 170, 4427–4431.

Schurmans, L. R., Diehl, L., den Boer, A. T., Sutmuller, R. P., Boonman, Z. F., Medema, J. P., van der Voort, E. I., Laman, J., Melief, C. J., Jager, M. J., and Toes, R. E. (2001). Rejection of intraocular tumors by CD4(+) T cells without induction of phthisis. *J. Immunol.* 167, 5832–5837.

Shevach, E. M. (2009). Mechanisms of foxp3+ T regulatory cell-mediated suppression. *Immunity* 30, 636–645.

Simon, M. M., Hausmann, M., Tran, T., Ebnet, K., Tschopp, J., ThaHla, R., and Mullbacher, A. (1997). *In vitro*- and *ex vivo*-derived cytolytic leukocytes from granzyme A x B double knockout mice are defective in granule-mediated apoptosis but not lysis of targeT cells. *J. Exp. Med.* 186, 1781–1786.

Singh, A. D., and Topham, A. (2003). Incidence of uveal melanoma in the United States: 1973–1997. *Ophthalmology* 110, 956–961.

Skelsey, M. E., Mellon, J., and Niederkorn, J. Y. (2001). Gamma delta T cells are needed for ocular immune privilege and corneal graft survival. *J. Immunol.* 166, 4327–4333.

Sonoda, K. H., Exley, M., Snapper, S., Balk, S. P., and Stein-Streilein, J. (1999). CD1-reactive natural killer T cells are required for development of systemic tolerance through an immune-privileged site. *J. Exp. Med.* 190, 1215–1226.

Sonoda, K. H., Faunce, D. E., Taniguchi, M., Exley, M., Balk, S., and Stein-Streilein, J. (2001). NK T cell-derived IL-10 is essential for the differentiation of antigen-specific T regulatory cells in systemic tolerance. *J. Immunol.* 166, 42–50.

Sonoda, K. H., and Stein-Streilein, J. (2002). CD1d on antigen-transporting APC and splenic marginal zone B cells promotes NKT cell-dependent tolerance. *Eur. J. Immunol.* 32, 848–857.

Staibano, S., Mascolo, M., Tranfa, F., Salvatore, G., Mignogna, C., Bufo, P., Nugnes, L., Bonavolonta, G., and De, R. G. (2006). Tumor infiltrating lymphocytes in uveal melanoma: a link with clinical behavior? *Int. J. Immunopathol. Pharmacol.* 19, 171–179.

Streilein, J. W., and Niederkorn, J. Y. (1981). Induction of anterior chamber-associated immune deviation requires an intact, functional spleen. *J. Exp. Med.* 153, 1058–1067.

Streilein, J. W., and Niederkorn, J. Y. (1985). Characterization of the suppressor cell(s) responsible for anterior chamber-associated immune deviation (ACAID) induced in BALB/c mice by P815 cells. *J. Immunol.* 134, 1381–1387.

Stuart, P. M., Griffith, T. S., Usui, N., Pepose, J., Yu, X., and Ferguson, T. A. (1997). CD95 ligand (FasL)-induced apoptosis is necessary for corneal allograft survival. *J. Clin. Invest.* 99, 396–402.

Sugita, S., Horie, S., Nakamura, O., Futagami, Y., Takase, H., Keino, H., Aburatani, H., Katunuma, N., Ishidoh, K., Yamamoto, Y., and Mochizuki, M. (2008). Retinal pigment epithelium-derived CTLA-2alpha induces TGFbeta-producing T regulatory cells. *J. Immunol.* 181, 7525–7536.

Sugita, S., Horie, S., Nakamura, O., Maruyama, K., Takase, H., Usui, Y., Takeuchi, M., Ishidoh, K., Koike, M., Uchiyama, Y., Peters, C., Yamamoto, Y., and Mochizuki, M. (2009). Acquisition of T regulatory function in cathepsin L-inhibited T cells by eye-derived CTLA-2alpha during inflammatory conditions. *J. Immunol.* 183, 5013–5022.

Sugita, S., Horie, S., Yamada, Y., Keino, H., Usui, Y., Takeuchi, M., and Mochizuki, M. (2010). Suppression of bystander T helper 1 cells by iris pigment epithelium-inducing regulatory T cells via negative costimulatory signals. *Invest. Ophthalmol. Vis. Sci.* 51, 2529–2536.

Sugita, S., Ng, T. F., Lucas, P. J., Gress, R. E., and Streilein, J. W. (2006). B7+ iris pigment epithelium induce CD8+ T regulatory cells; both suppress CTLA-4+ T cells. *J. Immunol.* 176, 118–127.

Sugita, S., Yamada, Y., Horie, S., Nakamura, O., Ishidoh, K., Yamamoto, Y., Yamagami, S., and Mochizuki, M. (2011). Induction of T regulatory cells by cytotoxic T-lymphocyte antigen-2alpha on corneal endothelial cells. *Invest. Ophthalmol. Vis. Sci.* 52, 2598–2605.

Takeuchi, M., Alard, P., and Streilein, J. W. (1998). TGF-b promotes immune deviation by altering accessory signals of antigen-presenting cells. *J. Immunol.* 160, 1589–1597.

Taylor, A. W., Alard, P., Yee, D. G., and Streilein, J. W. (1997). Aqueous humor induces transforming growth factor-beta (TGF-beta)-producing regulatory T-cells. *Curr. Eye Res.* 16, 900–908.

Taylor, A. W., Streilein, J. W., and Cousins, S. W. (1994). Immunoreactive vasoactive intestinal peptide contributes to the immunosuppressive activity of normal aqueous humor. *J. Immunol.* 153, 1080–1086.

Taylor, A. W., and Yee, D. G. (2003). Somatostatin is an immunosuppressive factor in aqueous humor. *Invest. Ophthalmol. Vis. Sci.* 44, 2644–2649.

Tennakoon, D. K., Mehta, R. S., Ortega, S. B., Bhoj, V., Racke, M. K., and Karandikar, N. J. (2006). Therapeutic induction of regulatory, cytotoxic CD8+ T cells in multiple sclerosis. *J. Immunol.* 176, 7119–7129.

Vega, J. L., Keino, H., and Masli, S. (2009). Surgical denervation of ocular sympathetic afferents decreases local transforming growth factor-beta and abolishes immune privilege. *Am. J. Pathol.* 175, 1218–1225.

Vicetti Miguel, R. D., Cherpes, T. L., Watson, L. J., and McKenna, K. C. (2010). CTL induction of tumoricidal nitric oxide production by intratumoral macrophages is critical for tumor elimination. *J. Immunol.* 185, 6706–6718.

Vlad, G., D'Agati, V. D., Zhang, Q. Y., Liu, Z., Ho, E. K., Mohanakumar, T., Hardy, M. A., Cortesini, R., and Suciu-Foca, N. (2008). Immunoglobulin-like transcript 3-Fc suppresses T-cell responses to allogeneic human islet transplants in hu-NOD/SCID mice. *Diabetes* 57, 1878–1886.

Waldrep, J. C., and Kaplan, H. J. (1983). Anterior chamber associated immune deviation induced by TNP-splenocytes (TNP-ACAID). I. Systemic tolerance mediated by suppressor T-cells. *Invest. Ophthalmol. Vis. Sci.* 24, 1086–1092.

Wall, L., Burke, F., Barton, C., Smyth, J., and Balkwill, F. (2003). IFN-gamma induces apoptosis in ovarian cancer cells *in vivo* and *in vitro*. *Clin. Cancer Res.* 9, 2487–2496.

Wang, S., Boonman, Z. F., Li, H. C., He, Y., Jager, M. J., Toes, R. E., and Niederkorn, J. Y. (2003). Role of TRAIL and IFN-gamma in CD4+ T cell-dependent tumor rejection in the anterior chamber of the eye. *J. Immunol.* 171, 2789–2796.

Wang, Y., Goldschneider, I., Foss, D., Wu, D. Y., O'Rourke, J., and Cone, R. E. (1997). Direct thymic involvement in anterior chamber-associated immune deviation: evidence for a nondeletional mechanism of centrally induced tolerance to extrathymic antigens in adult mice. *J. Immunol.* 158, 2150–2155.

Wang, Y., Goldschneider, I., O'Rourke, J., and Cone, R. E. (2001). Blood mononuclear cells induce regulatory NK T thymocytes in anterior chamber-associated immune deviation. *J. Leukoc. Biol.* 69, 741–746.

Wilbanks, G. A., Mammolenti, M., and Streilein, J. W. (1991). Studies on the induction of anterior chamber-associated immune deviation (ACAID). II. Eye-derived cells participate in generating blood-borne signals that induce ACAID. *J. Immunol.* 146, 3018–3024.

Wilbanks, G. A., Mammolenti, M., and Streilein, J. W. (1992). Studies on the induction of anterior chamber-associated immune deviation (ACAID). III. Induction of ACAID depends upon intraocular transforming growth factor-b. *Eur. J. Immunol.* 22, 165–173.

Wilbanks, G. A., and Streilein, J. W. (1991). Studies on the induction of anterior chamber-associated immune deviation (ACAID). 1. Evidence that an antigen-specific, ACAID-inducing, cell-associated signal exists in the peripheral blood. *J. Immunol.* 146, 2610–2617.

Wilbanks, G. A., and Streilein, J. W. (1992). Macrophages capable of inducing anterior chamber associated immune deviation demonstrate spleen-seeking migratory properties. *Reg. Immunol.* 4, 130–137.

Wilbanks, G. A., and Streilein, J. W. (1990). Characterization of suppressor cells in anterior chamber-associated immune deviation (ACAID) induced by soluble antigen. Evidence of two functionally and phenotypically distinct T-suppressor cell populations. *Immunology* 71, 383–389.

Wiley, S. R., Schooley, K., Smolak, P. J., Din, W. S., Huang, C. P., Nicholl, J. K., Sutherland, G. R., Smith, T. D., Rauch, C., and Smith, C. A. (1995). Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* 3, 673–682.

Xu, H., Manivannan, A., Liversidge, J., Sharp, P. F., Forrester, J. V., and Crane, I. J. (2003). Requirements for passage of T lymphocytes across non-inflamed retinal microvessels. *J. Neuroimmunol.* 142, 47–57.

Xu, Y., and Kapp, J. A. (2002). Gamma delta T cells in anterior chamber-induced tolerance in CD8(+) CTL responses. *Invest. Ophthalmol. Vis. Sci.* 43, 3473–3479.

Yamagami, S., and Dana, M. R. (2001). The critical role of lymph nodes in corneal alloimmunization and graft rejection. *Invest. Ophthalmol. Vis. Sci.* 42, 1293–1298.

Yamagami, S., Kawashima, H., Tsuru, T., Yamagami, H., Kayagaki, N., Yagita, H., Okumura, K., and Gregerson, D. S. (1997). Role of Fas-Fas ligand interactions in the immunorejection of allogeneic mouse corneal transplants. *Transplantation* 64, 1107–1111.

Yang, W., Chen, P. W., Li, H., Alizadeh, H., and Niederkorn, J. Y. (2008). PD-L1, PD-1 interaction contributes to the functional suppression of T-cell responses to human uveal melanoma cells *in vitro*. *Invest. Ophthalmol. Vis. Sci.* 49, 2518–2525.

Zea, A. H., Rodriguez, P. C., Atkins, M. B., Hernandez, C., Signoretti,

S., Zabaleta, J., McDermott, D., Quiceno, D., Youmans, A., O'Neill, A., Mier, J., and Ochoa, A. C. (2005). Arginase-producing myeloid suppressor cells in renal cell carcinoma patients: a mechanism of tumor evasion. *Cancer Res.* 65, 3044–3048.

Zhou, R., Horai, R., Silver, P. B., Mattapallil, M. J., Zarate-Blades, C. R., Chong, W. P., Chen, J., Rigden, R. C., Villasmil, R., and Caspi, R. R. (2012). The living eye "disarms" uncommitted autoreactive T cells by converting them to Foxp3(+) regulatory cells following local antigen recognition. *J. Immunol.* 188, 1742–1750.

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The privileged immunity of immune privileged organs: the case of the eye

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Understanding of ocular diseases and the search for their cure have been based on the common assumption that the eye is an immune privileged site, and the consequent conclusion that entry of immune cells to this organ is forbidden. Accordingly, it was assumed that when immune cell entry does occur, this reflects an undesired outcome of breached barriers. However, studies spanning more than a decade have demonstrated that acute insults to the retina, or chronic conditions resulting in retinal ganglion cell loss, such as in glaucoma, result in an inferior outcome in immunocompromised mice; likewise, steroidal treatment was found to be detrimental under these conditions. Moreover, even conditions that are associated with inflammation, such as age-related macular degeneration, are not currently believed to require immune suppression for treatment, but rather, are thought to benefit from immune modulation. Here, we propose that the immune privilege of the eye is its ability to enable, upon need, the entry of selected immune cells for its repair and healing, rather than to altogether prevent immune cell entry. The implications for acute and chronic degenerative diseases, as well as for infection and inflammatory diseases, are discussed.

Keywords: immune privilege, visual system, immunomodulation, neuroprotection and neuronal repair, inflammation

INTRODUCTION

Over the past decades, the mammalian central nervous system (CNS), including the eye, brain, and spinal cord, were believed to be sealed from the circulation. Thus, immune activity at these sites was considered forbidden, and was collectively assumed to be consistently detrimental. As a consequence, the inflammatory response in the eye or the brain was assessed solely based on counting the number of immune cells, without regard to their phenotype or function. Thus, the poor ability of the optic nerve to regenerate following injury, as well as the poor recovery following acute injury to any other parts of the CNS, were assumed to be an outcome of local detrimental immune activity seen at the lesion site (Fitch et al., 1999; Popovich et al., 1999; Ghirnikar et al., 2001). Such a view was almost universally accepted from the early 1980s and supported the use of anti-inflammatory drugs to treat victims of CNS injuries (Constantini and Young, 1994; Carlson et al., 1998).

With time and the advance of technologies, there was an increase in the understanding of the heterogeneity of innate and adaptive immunity in general, and in the CNS in particular, with respect to both functional cell subsets (Korn et al., 2007; Gee et al., 2008; O'Shea et al., 2008; Auffray et al., 2009; Prinz et al., 2011; Zhu et al., 2011) and origin (Geissmann et al., 2010; Ginhoux et al., 2010; Prinz et al., 2011). As a corollary, it became clear that some of the blanket assumptions regarding the eye and the brain were not accurate, and, accordingly, that some experimental findings had not been properly interpreted. Thus, it became evident that the response to CNS injury, similar to that in other tissues in the body, is a multi-step process that requires a set sequence, and synchrony of events in time and space; many of the steps that take place in the healing process following "sterile" injuries are similar if not identical to processes occurring outside the CNS with respect to the immune response (Dusart and Schwab, 1994; Frank and Wolburg, 1996; Arnold et al., 2007; Nahrendorf et al., 2007; Rolls et al., 2009; Shechter et al., 2009; Stirling et al., 2009; London et al., 2011). The early innate immune response involves cells that are needed for cleaning the lesion site, yet the activity of these cells must be followed by immune cells that terminate this initial response and subsequently contribute to the repair. Both stages involve innate immune cells of distinct phenotypes; the cells that contribute to the termination of the local early response are largely monocyte-derived macrophages that acquire and exert a local anti-inflammatory function (Kigerl et al., 2009; Shechter et al., 2009; London et al., 2011; Zhu et al., 2011). The obvious question is how such a response can be reconciled with the traditional view of the eye as an immune privileged site; do these findings change our understanding of the privilege, or do they require breaking of privilege under severe conditions? Here, focusing on the eye, we will discuss a different view of the physiological meaning of the CNS as an immune privileged site, and its manifestations under pathological conditions.

THE EYE AS AN IMMUNE PRIVILEGED ORGAN

Immune privileged organs were operationally defined as sites in the body where foreign tissue grafts can survive for extended, often indefinite periods of time, whereas similar grafts placed at regular sites in the body are acutely rejected (Medawar, 1948). These organs include the eye and the brain, as well as the pregnant uterus, testis, and several others (Streilein, 2003b; Niederkorn, 2006). Such immune privilege is thought to be an evolutionary adaptation to

protect tissues that are indispensable, yet have limited regeneration capacity, like the brain and the eye, from the potentially damaging effects of an uncontrolled inflammatory immune response. Thus, immune privileged organs were considered as ones to which immune cell entry is forbidden; leukocytes were believed to be excluded from these vital organs by the presence of specialized physical barriers, the blood–tissue barriers.

In contrast to the previous view that immune privilege is maintained by immune cell exclusion, it is now increasingly accepted that the privileged status is preserved by local active mechanisms that suppress responses to antigens within the privileged tissues (Niederkorn and Stein-Streilein, 2010). In the eye, one such mechanism is anterior chamber-associated immune deviation (ACAID), referring to a phenomenon in which antigenic material introduced into the anterior chamber of the eye elicits a systemic immune response that results in immune deviation, characterized by the suppression of T cell-mediated immunity, while enabling the production of non-complement-fixing antibodies (Kaplan et al., 1975; Streilein, 2003b; Niederkorn, 2006). ACAID involves the migration of specialized antigen presenting cells from the eye to the thymus and spleen, and is associated with an elevation in regulatory, γδ, and natural killer T cells (Streilein, 2003b; Niederkorn, 2006). Other mechanisms aimed at maintaining the immune privileged state of the eye include the reduced expression of MHC molecules on ocular cells, and the existence of an intraocular anti-inflammatory environment, mediated by resident cells, and various molecules, both surface-bound and soluble, all of which serve to modulate the activity of infiltrating immune cells, *in situ* (Streilein, 2003b; Schewitz-Bowers et al., 2010; Zhou et al., 2012). These well-orchestrated, multifaceted mechanisms, known to involve numerous pathways, were long thought to be designed to ensure limited infiltration of circulating immune cells to the eye, leaving behind a tissue that was considered autonomous in terms of repair. It is puzzling, however, why a fragile and precious organ such as the eye would evolve such complex tolerance mechanisms, if their sole purpose were to guarantee immune ignorance. Moreover, several studies have shown that immunocompromised mice exhibit worse recovery from optic nerve and retinal insult than do their immunocompetent counterparts (Kipnis et al., 2001; Schori et al., 2001; Yoles et al., 2001), similar to the case in peripheral nerve injury (Serpe et al., 1999). Similarly, recent studies have demonstrated that well-regulated immune responses in the CNS, rather than immune ignorance, are optimal for the recovery of the tissue after insult, whether sterile or immune-induced (Kerr et al., 2008; Shechter et al., 2009; Caspi et al., 2011; London et al., 2011; London et al., under revision). Thus, it is becoming increasingly clear that immune privilege is not aimed at entirely suppressing immune responses in the target organ, but rather at maintaining a specialized, tightly regulated immunological niche to preserve the integrity of especially vulnerable organs, such as the brain and the eye (Streilein, 2003b; Niederkorn, 2006).

REGULATED IMMUNE RESPONSES ARE BENEFICIAL IN MITIGATING EYE PATHOLOGIES

Inflammation is the body's adaptive response to any insult, be it mechanical, biochemical, or immune-mediated. However, inflammation is beneficial only on the condition that it ends in active resolution (Gronert, 2010). Studies on wound healing outside the CNS have characterized distinct subsets of macrophages that infiltrate the site of injury and display different functions corresponding to the changing needs of the tissue along the course of healing; these include the clearing of dead cells and tissue debris at the first stage, and the secretion of anti-inflammatory cytokines and growth factors at the later stage, to aid tissue regrowth and restoration of immune homeostasis (Arnold et al., 2007; Nahrendorf et al., 2007). Recently, our team demonstrated that a subset of monocyte-derived macrophages, which manifests an immune-resolving phenotype, is essential for the resolution of inflammation after sterile insults, in models of spinal cord injury and retinal glutamate intoxication (Shechter et al., 2009; London et al., 2011). In both of these cases, such macrophages were found to be crucial for recovery, as was measured by a functional motor scale after spinal cord injury, and directly in terms of cell survival in the retina. Thus, despite the classification of these organs as immune privileged, they nevertheless derive benefit from the controlled recruitment of innate immune cells from the circulation, to assist in their healing. Notably, while the CNS contains its own population of immune cells, the resident microglia, we have shown that infiltrating blood-derived macrophages are nonetheless crucial for neuroprotective and anti-inflammatory activities at the injury site; we have therefore proposed that the infiltrating cells fulfill specialized functions in the recovery process, which the resident immune cells either fail to display, or at least do not manifest at the right time or at sufficient levels (Shechter et al., 2009). In animal models of optic nerve injury, it was found that macrophages can modify the non-permissive nature of the optic nerve for regeneration *in vitro* (David et al., 1990), and that transplantation of activated macrophages into the injured optic nerve can facilitate regrowth *in vivo* (Lazarov-Spiegler et al., 1996). In line with these observations, the important contribution of a macrophage-derived molecule, oncomodulin, to the regeneration of the optic nerve, was identified by Benowitz and colleagues (Yin et al., 2006, 2009; Cui et al., 2009), who coined the term "inflammation-induced regeneration." Collectively, these results attribute to innate immunity an important role in eye repair, and reveal the ability of macrophages to orchestrate neuroprotection and axonal regeneration.

The beneficial role of adaptive immunity in neuroprotection was initially observed in animal models simulating different aspects of glaucoma, where it was found that the extent of retinal ganglion cell loss is increased in immunocompromised animals relative to immunocompetent ones (Kipnis et al., 2001; Schori et al., 2001; Yoles et al., 2001; Bakalash et al., 2002). Moreover, T cell-based vaccinations, both passive and active, promote neuroprotection after optic nerve crush (Moalem et al., 1999; Fisher et al., 2001). Importantly, the potential benefit derived from T cells in these systems relies, at least in part, on a delicate balance between effector and regulatory subsets of these cells (Kipnis et al., 2002, 2004). More recently, results obtained in different models of CNS insult suggested that the beneficial effects of T cells might be mediated in part by controlling the recruitment of monocyte-derived macrophages from the circulation (Butovsky et al., 2007; Schwartz

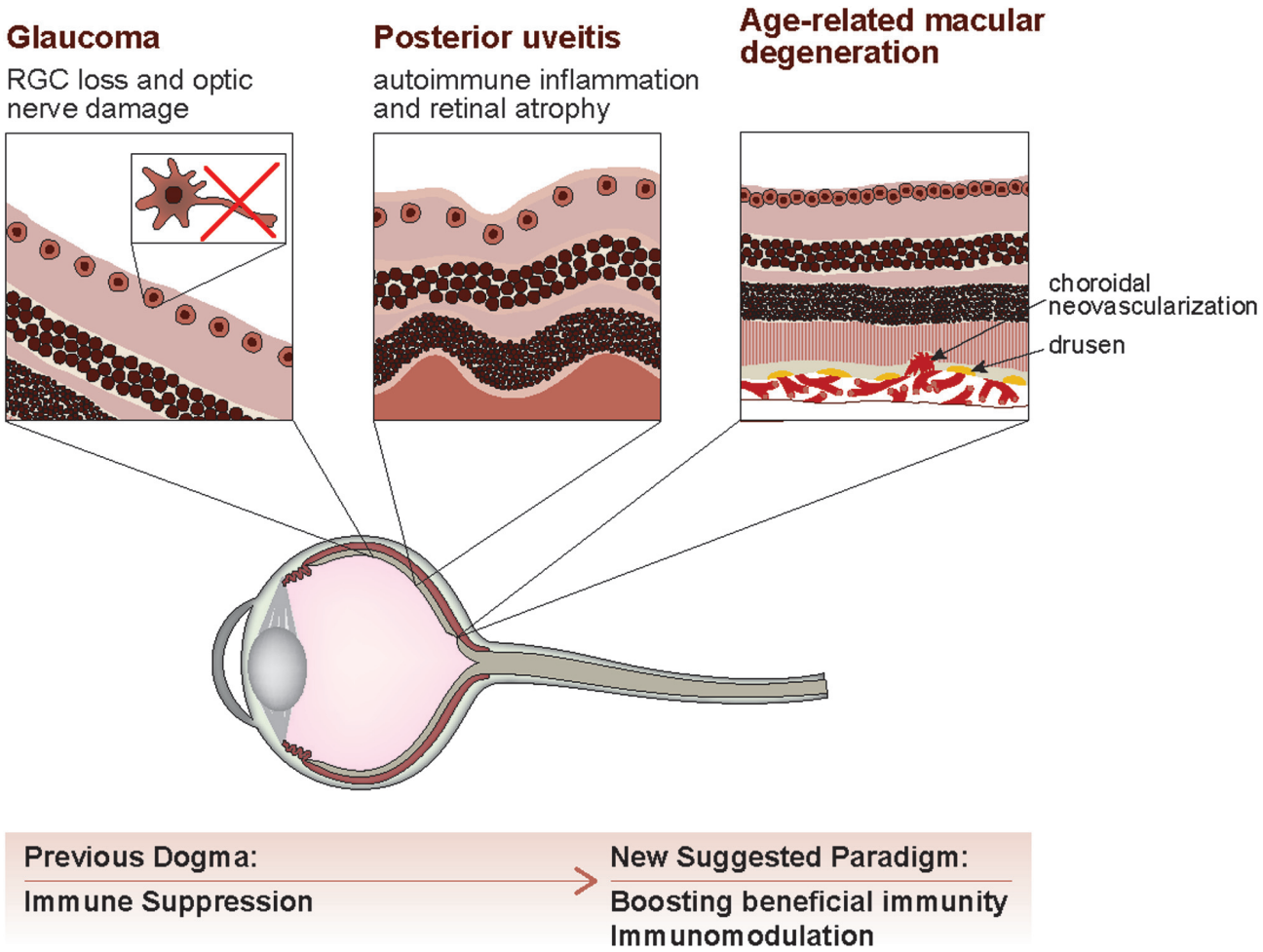

FIGURE 1 | An evolving view of immune involvement in the eye. Ocular pathologies are initiated by multiple factors, and take on various manifestations. Glaucoma, a slowly progressing neurodegenerative disease, is characterized by loss of retinal ganglion cells and damage to the optic nerve. In posterior uveitis, retinal atrophy, and neuronal death are commonly induced by autoimmune inflammation, and age-related macular degeneration presents with drusen ("dry" AMD) and choroidal neovascularization ("wet" AMD). While the traditional dogma stated that immune privilege implies the exclusion of immune activity from the eye under any circumstances, our evolving understanding of immune privilege proposes that boosting beneficial immunity in the eye, in a well-regulated manner, rather than general immune suppression, is most favorable for coping with ocular pathologies, regardless of their initiating factors.

et al., 2009; Shechter et al., 2009). Thus, the well-orchestrated collaboration between the innate and adaptive arms of the immune system appears to be optimal for achieving neuroprotection.

The beneficial involvement of immune cells in the eye is also observed in diseases that are immune-induced, such as autoimmune posterior uveitis, a potentially blinding inflammatory condition affecting the retina and the choroid of the eye. Studies in experimental autoimmune uveitis (EAU), an animal model of human posterior uveitis, demonstrate the heterogeneity of immune cells along this disease. Beside the well-characterized pro-inflammatory cells known to initiate EAU, the uveitic eye is also endowed with regulatory immune populations (Robertson et al., 2002; Kerr et al., 2008; Caspi et al., 2011; London et al., under revision). These cells, including subsets of macrophages and T cells, act to limit inflammation, presumably bringing the disease to a state of equilibrium and remission.

An additional pathology in which the immune system has been shown to fulfill various, perhaps opposing functions, is age-related macular degeneration (AMD), the leading cause of blindness in the elderly. Naturally, the etiology of AMD is very diverse; the disease is associated with numerous immune-related factors. Here too, the role of macrophages has been a matter of debate; on the one hand, it was found that aging is accompanied by a pathological shift to M2 macrophages, which are known to promote angiogenesis, and would therefore seem likely candidates for promoting choroidal neovascularization (CNV), the process by which abnormal blood vessels develop beneath the retina (Espinosa-Heidmann et al., 2003; Sakurai et al., 2003; Cao et al., 2011). On the other hand, studies have also shown that prevention of macrophage entry into the eye promotes CNV, whereas injection of macrophages inhibits it (Apte et al., 2006). Patel and Chan (2008) reviewed the seemingly contradictory

functions of macrophages in AMD, and proposed that these conflicting findings reflect a dual role of macrophages in this pathology, where the uncontrolled pro-inflammatory M1 macrophages induce tissue damage, and the M2 macrophages, which are recruited to terminate the M1 response and to clear drusen and other age-related deposits, could also adversely affect disease progression by displaying pro-angiogenic activity. Among the additional factors associated with AMD pathogenesis and progression, a pivotal role has been attributed over the past several years to the complement system and its dysregulation (Klein et al., 2005; Patel and Chan, 2008; Anderson et al., 2010). These findings emphasize the need for a regulated immune response, in terms of timing, duration, and phenotype, and further support the argument that there are no "good" or "bad" immune cells; it is all a matter of their control and coordination. Moreover, the accumulating evidence on beneficial immune involvement in AMD and in the other ocular pathologies mentioned above give further reinforcement to the current contention that although the eye is an immune privileged site, it can enjoy the benefits of immune support, and thus immune regulation, rather than immune suppression, is the key to disease resolution, as in other parts of the body (**Figure 1**).

A DIFFERENT VIEW OF IMMUNE PRIVILEGE

Immune privilege is an evolutionary adaptation aimed at protecting especially vulnerable organs from overwhelming inflammation that could abolish their functions and jeopardize the well-being of the individual. As vision is crucial for survival, it is understandable why the eye would be particularly protected from these risks (Streilein, 2003a). However, we propose that the immune privileged designation of the eye means that it has the privilege to enable selective immune responses most suitable and effective for its proper function in health and pathology. We contend that this is true for all other parts of the CNS, as well.

As many CNS pathologies are associated with local inflammation, they are generally treated with anti-inflammatory and immunosuppressive drugs. However, this treatment approach has shown limited success in animal models of ocular pathologies and other neurodegenerative disorders, as well as in the clinic, and in some cases was even found to exacerbate disease (Levin et al., 1999; Solberg et al., 1999; Bakalash et al., 2003; Ohlsson et al., 2004; Dimitriu et al., 2008; Schwartz and Shechter, 2010). The benefits of those drugs, if any, are often temporary, as they help relieve some of the symptoms but do not address the underlying pathological processes (Gronert, 2010). Bearing in mind the heterogeneity of immune cells and their changing functions along the course of disease, together with the delicate balance of counter-regulatory signals required for effective resolution of inflammation (Gronert, 2010), we suggest that a more efficient approach to treating such disorders would be to manipulate specific immune subsets in a timely manner, rather than to globally inhibit the immune response (**Figure 1**).

Finally, our interpretation of the privilege of immunity in immune privileged sites does not negate the possibility that under certain conditions, immune privilege is breached in order to preserve the life of the individual, at the expense of local loss of function; this is the case in certain microbial infections, or in the presence of highly immunogenic tumors (Morrison et al., 1989; Niederkorn, 1991; Li and Niederkorn, 1997; Streilein et al., 1997; Saint Andre et al., 2002; Niederkorn and Stein-Streilein, 2010), in which a powerful immune response is essential, and the risk of blindness is accepted for the sake of survival (Niederkorn and Stein-Streilein, 2010).

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REFERENCES

Anderson, D. H., Radeke, M. J., Gallo, N. B., Chapin, E. A., Johnson, P. T., Curletti, C. R., Hancox, L. S., Hu, J., Ebright, J. N., Malek, G., Hauser, M. A., Rickman, C. B., Bok, D., Hageman, G. S., and Johnson, L. V. (2010). The pivotal role of the complement system in aging and age-related macular degeneration: hypothesis revisited. *Prog. Retin. Eye Res.* 29, 95–112.

Apte, R. S., Richter, J., Herndon, J., and Ferguson, T. A. (2006). Macrophages inhibit neovascularization in a murine model of age-related macular degeneration. *PLoS Med.* 3, e310. doi: 10.1371/journal.pmed.0030310

Arnold, L., Henry, A., Poron, F., Baba-Amer, Y., Van Rooijen, N., Plonquet, A., Gherardi, R. K., and Chazaud, B. (2007). Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. *J. Exp. Med.* 204, 1057–1069.

Auffray, C., Sieweke, M. H., and Geissmann, F. (2009). Blood monocytes: development, heterogeneity, and relationship with dendritic cells. *Annu. Rev. Immunol.* 27, 669–692.

Bakalash, S., Kessler, A., Mizrahi, T., Nussenblatt, R., and Schwartz, M. (2003). Antigenic specificity of immunoprotective therapeutic vaccination for glaucoma. *Invest. Ophthalmol. Vis. Sci.* 44, 3374–3381.

Bakalash, S., Kipnis, J., Yoles, E., and Schwartz, M. (2002). Resistance of retinal ganglion cells to an increase in intraocular pressure is immune-dependent. *Invest. Ophthalmol. Vis. Sci.* 43, 2648–2653.

Butovsky, O., Kunis, G., Koronyo-Hamaoui, M., and Schwartz, M. (2007). Selective ablation of bone marrow-derived dendritic cells increases amyloid plaques in a mouse Alzheimer's disease model. *Eur. J. Neurosci.* 26, 413–416.

Cao, X., Shen, D., Patel, M. M., Tuo, J., Johnson, T. M., Olsen, T. W., and Chan, C. C. (2011). Macrophage polarization in the maculae of age-related macular degeneration: a pilot study. *Pathol. Int.* 61, 528–535.

Carlson, S. L., Parrish, M. E., Springer, J. E., Doty, K., and Dossett, L. (1998). Acute inflammatory response in spinal cord following impact injury. *Exp. Neurol.* 151, 77–88.

Caspi, R. R., Horai, R., Chen, J., Hansen, A., Mcmanigle, W., Villasmil, R., and Silver, P. (2011). Retina-specific T regulatory cells accumulate in the eye during autoimmune uveitis and act to control inflammation. *J. Immunol.* 186, 115.22.

Constantini, S., and Young, W. (1994). The effects of methylprednisolone and the ganglioside GM1 on acute spinal cord injury in rats. *J. Neurosurg.* 80, 97–111.

Cui, Q., Yin, Y., and Benowitz, L. I. (2009). The role of macrophages in optic nerve regeneration. *Neuroscience* 158, 1039–1048.

David, S., Bouchard, C., Tsatas, O., and Giftochristos, N. (1990). Macrophages can modify the non-permissive nature of the adult mammalian central nervous system. *Neuron* 5, 463–469.

Dimitriu, C., Bach, M., Lagreze, W. A., and Jehle, T. (2008). Methylprednisolone fails to preserve retinal ganglion cells and visual function after ocular ischemia in rats. *Invest. Ophthalmol. Vis. Sci.* 49, 5003–5007.

Dusart, I., and Schwab, M. E. (1994). Secondary cell death and the inflammatory reaction after dorsal hemisection of the rat spinal cord. *Eur. J. Neurosci.* 6, 712–724.

Espinosa-Heidmann, D. G., Suner, I. J., Hernandez, E. P., Monroy, D., Csaky, K. G., and Cousins, S. W. (2003). Macrophage depletion

diminishes lesion size and severity in experimental choroidal neovascularization. *Invest. Ophthalmol. Vis. Sci.* 44, 3586–3592.

Fisher, J., Levkovitch-Verbin, H., Schori, H., Yoles, E., Butovsky, O., Kaye, J. F., Ben-Nun, A., and Schwartz, M. (2001). Vaccination for neuroprotection in the mouse optic nerve: implications for optic neuropathies. *J. Neurosci.* 21, 136–142.

Fitch, M. T., Doller, C., Combs, C. K., Landreth, G. E., and Silver, J. (1999). Cellular and molecular mechanisms of glial scarring and progressive cavitation: in vivo and in vitro analysis of inflammation-induced secondary injury after CNS trauma. *J. Neurosci.* 19, 8182–8198.

Frank, M., and Wolburg, H. (1996). Cellular reactions at the lesion site after crushing of the rat optic nerve. *Glia* 16, 227–240.

Gee, J. M., Kalil, A., Thullbery, M., and Becker, K. J. (2008). Induction of immunologic tolerance to myelin basic protein prevents central nervous system autoimmunity and improves outcome after stroke. *Stroke* 39, 1575–1582.

Geissmann, F., Manz, M. G., Jung, S., Sieweke, M. H., Merad, M., and Ley, K. (2010). Development of monocytes, macrophages, and dendritic cells. *Science* 327, 656–661.

Ghirnikar, R. S., Lee, Y. L., and Eng, L. F. (2001). Chemokine antagonist infusion promotes axonal sparing after spinal cord contusion injury in rat. *J. Neurosci. Res.* 64, 582–589.

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., Mehler, M. F., Conway, S. J., Ng, L. G., Stanley, E. R., Samokhvalov, I. M., and Merad, M. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330, 841–845.

Gronert, K. (2010). Resolution, the grail for healthy ocular inflammation. *Exp. Eye Res.* 91, 478–485.

Kaplan, H. J., Streilein, J. W., and Stevens, T. R. (1975). Transplantation immunology of the anterior chamber of the eye. II. Immune response to allogeneic cells. *J. Immunol.* 115, 805–810.

Kerr, E. C., Raveney, B. J., Copland, D. A., Dick, A. D., and Nicholson, L. B. (2008). Analysis of retinal cellular infiltrate in experimental autoimmune uveoretinitis reveals multiple regulatory cell populations. *J. Autoimmun.* 31, 354–361.

Kigerl, K. A., Gensel, J. C., Ankeny, D. P., Alexander, J. K., Donnelly, D. J., and Popovich, P. G. (2009). Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. *J. Neurosci.* 29, 13435–13444.

Kipnis, J., Cardon, M., Avidan, H., Lewitus, G. M., Mordechay, S., Rolls, A., Shani, Y., and Schwartz, M. (2004). Dopamine, through the extracellular signal-regulated kinase pathway, downregulates CD4+CD25+ regulatory T-cell activity: implications for neurodegeneration. *J. Neurosci.* 24, 6133–6143.

Kipnis, J., Mizrahi, T., Hauben, E., Shaked, I., Shevach, E., and Schwartz, M. (2002). Neuroprotective autoimmunity: naturally occurring CD4+CD25+ regulatory T cells suppress the ability to withstand injury to the central nervous system. *Proc. Natl. Acad. Sci. U.S.A.* 99, 15620–15625.

Kipnis, J., Yoles, E., Schori, H., Hauben, E., Shaked, I., and Schwartz, M. (2001). Neuronal survival after CNS insult is determined by a genetically encoded autoimmune response. *J. Neurosci.* 21, 4564–4571.

Klein, R. J., Zeiss, C., Chew, E. Y., Tsai, J. Y., Sackler, R. S., Haynes, C., Henning, A. K., Sangiovanni, J. P., Mane, S. M., Mayne, S. T., Bracken, M. B., Ferris, F. L., Ott, J., Barnstable, C., and Hoh, J. (2005). Complement factor H polymorphism in age-related macular degeneration. *Science* 308, 385–389.

Korn, T., Reddy, J., Gao, W., Bettelli, E., Awasthi, A., Petersen, T. R., Backstrom, B. T., Sobel, R. A., Wucherpfennig, K. W., Strom, T. B., Oukka, M., and Kuchroo, V. K. (2007). Myelin-specific regulatory T cells accumulate in the CNS but fail to control autoimmune inflammation. *Nat. Med.* 13, 423–431.

Lazarov-Spiegler, O., Solomon, A. S., Zeev-Brann, A. B., Hirschberg, D. L., Lavie, V., and Schwartz, M. (1996). Transplantation of activated macrophages overcomes central nervous system regrowth failure. *FASEB J.* 10, 1296–1302.

Levin, L. A., Beck, R. W., Joseph, M. P., Seiff, S., and Kraker, R. (1999). The treatment of traumatic optic neuropathy: the International Optic Nerve Trauma Study. *Ophthalmology* 106, 1268–1277.

Li, X. Y., and Niederkorn, J. Y. (1997). Immune privilege in the anterior chamber of the eye is not extended to intraocular *Listeria monocytogenes*. *Ocul. Immunol. Inflamm.* 5, 245–257.

London, A., Itskovich, E., Benhar, I., Kalchenko, V., Mack, M., Jung, S., and Schwartz, M. (2011). Neuroprotection and progenitor cell renewal in the injured adult murine retina requires healing monocyte-derived macrophages. *J. Exp. Med.* 208, 23–39.

Medawar, P. B. (1948). Immunity to homologous grafted skin; the fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. *Br. J. Exp. Pathol.* 29, 58–69.

Moalem, G., Leibowitz-Amit, R., Yoles, E., Mor, F., Cohen, I. R., and Schwartz, M. (1999). Autoimmune T cells protect neurons from secondary degeneration after central nervous system axotomy. *Nat. Med.* 5, 49–55.

Morrison, R. P., Lyng, K., and Caldwell, H. D. (1989). Chlamydial disease pathogenesis. Ocular hypersensitivity elicited by a genus-specific 57-kD protein. *J. Exp. Med.* 169, 663–675.

Nahrendorf, M., Swirski, F. K., Aikawa, E., Stangenberg, L., Wurdinger, T., Figueiredo, J. L., Libby, P., Weissleder, R., and Pittet, M. J. (2007). The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. *J. Exp. Med.* 204, 3037–3047.

Niederkorn, J. Y. (1991). The immunopathology of intraocular tumour rejection. *Eye (Lond.)* 5(Pt 2), 186–192.

Niederkorn, J. Y. (2006). See no evil, hear no evil, do no evil: the lessons of immune privilege. *Nat. Immunol.* 7, 354–359.

Niederkorn, J. Y., and Stein-Streilein, J. (2010). History and physiology of immune privilege. *Ocul. Immunol. Inflamm.* 18, 19–23.

Ohlsson, M., Westerlund, U., Langmoen, I. A., and Svensson, M. (2004). Methylprednisolone treatment does not influence axonal regeneration or degeneration following optic nerve injury in the adult rat. *J. Neuroophthalmol.* 24, 11–18.

O'Shea, J. J., Hunter, C. A., and Germain, R. N. (2008). T cell heterogeneity: firmly fixed, predominantly plastic or merely malleable? *Nat. Immunol.* 9, 450–453.

Patel, M., and Chan, C. C. (2008). Immunopathological aspects of age-related macular degeneration. *Semin. Immunopathol.* 30, 97–110.

Popovich, P. G., Guan, Z., Wei, P., Huitinga, I., Van Rooijen, N., and Stokes, B. T. (1999). Depletion of hematogenous macrophages promotes partial hindlimb recovery and neuroanatomical repair after experimental spinal cord injury. *Exp. Neurol.* 158, 351–365.

Prinz, M., Priller, J., Sisodia, S. S., and Ransohoff, R. M. (2011). Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. *Nat. Neurosci.* 14, 1227–1235.

Robertson, M. J., Erwig, L. P., Liversidge, J., Forrester, J. V., Rees, A. J., and Dick, A. D. (2002). Retinal microenvironment controls resident and infiltrating macrophage function during uveoretinitis. *Invest. Ophthalmol. Vis. Sci.* 43, 2250–2257.

Rolls, A., Shechter, R., and Schwartz, M. (2009). The bright side of the glial scar in CNS repair. *Nat. Rev. Neurosci.* 10, 235–241.

Saint Andre, A., Blackwell, N. M., Hall, L. R., Hoerauf, A., Brattig, N. W., Volkmann, L., Taylor, M. J., Ford, L., Hise, A. G., Lass, J. H., Diaconu, E., and Pearlman, E. (2002). The role of endosymbiotic *Wolbachia* bacteria in the pathogenesis of river blindness. *Science* 295, 1892–1895.

Sakurai, E., Anand, A., Ambati, B. K., Van Rooijen, N., and Ambati, J. (2003). Macrophage depletion inhibits experimental choroidal neovascularization. *Invest. Ophthalmol. Vis. Sci.* 44, 3578–3585.

Schewitz-Bowers, L. P., Lee, R. W. J., and Dick, A. D. (2010). Immune mechanisms of intraocular inflammation. *Expert Rev. Ophthalmol.* 5, 43–58.

Schori, H., Yoles, E., and Schwartz, M. (2001). T-cell-based immunity counteracts the potential toxicity of glutamate in the central nervous system. *J. Neuroimmunol.* 119, 199–204.

Schwartz, M., London, A., and Shechter, R. (2009). Boosting T-cell immunity as a therapeutic approach for neurodegenerative conditions: the role of innate immunity. *Neuroscience* 158, 1133–1142.

Schwartz, M., and Shechter, R. (2010). Systemic inflammatory cells fight off neurodegenerative disease. *Nat. Rev. Neurol.* 6, 405–410.

Serpe, C. J., Kohm, A. P., Huppenbauer, C. B., Sanders, V. M., and Jones, K. J. (1999). Exacerbation of facial motoneuron loss after facial nerve transection in severe combined immunodeficient (scid) mice. *J. Neurosci.* 19, RC7.

Shechter, R., London, A., Varol, C., Raposo, C., Cusimano, M., Yovel, G., Rolls, A., Mack, M., Pluchino, S., Martino, G., Jung, S., and Schwartz, M. (2009). Infiltrating blood-derived macrophages are vital cells playing an anti-inflammatory role in recovery from spinal cord injury in mice. *PLoS Med.* 6, e1000113. doi: 10.1371/journal.pmed.1000113

Solberg, Y., Dubinski, G., Tchirkov, M., Belkin, M., and Rosner, M. (1999). Methylprednisolone therapy for retinal laser injury. *Surv. Ophthalmol.* 44(Suppl. 1), S85–S92.

Stirling, D. P., Liu, S., Kubes, P., and Yong, V. W. (2009). Depletion of Ly6G/Gr-1 leukocytes after spinal cord injury in mice alters wound healing and worsens neurological outcome. *J. Neurosci.* 29, 753–764.

Streilein, J. W. (2003a). Ocular immune privilege: the eye takes a dim but practical view of immunity and inflammation. *J. Leukoc. Biol.* 74, 179–185.

Streilein, J. W. (2003b). Ocular immune privilege: therapeutic opportunities from an experiment of nature. *Nat. Rev. Immunol.* 3, 879–889.

Streilein, J. W., Dana, M. R., and Ksander, B. R. (1997). Immunity causing blindness: five different paths to herpes stromal keratitis. *Immunol. Today* 18, 443–449.

Yin, Y., Cui, Q., Gilbert, H. Y., Yang, Y., Yang, Z., Berlinicke, C., Li, Z., Zaverucha-Do-Valle, C., He, H., Petkova, V., Zack, D. J., and Benowitz, L. I. (2009). Oncomodulin links inflammation to optic nerve regeneration. *Proc. Natl. Acad. Sci. U.S.A.* 106, 19587–19592.

Yin, Y., Henzl, M. T., Lorber, B., Nakazawa, T., Thomas, T. T., Jiang, F., Langer, R., and Benowitz, L. I. (2006). Oncomodulin is a macrophage-derived signal for axon regeneration in retinal ganglion cells. *Nat. Neurosci.* 9, 843–852.

Yoles, E., Hauben, E., Palgi, O., Agranov, E., Gothilf, A., Cohen, A., Kuchroo, V., Cohen, I. R., Weiner, H., and Schwartz, M. (2001). Protective autoimmunity is a physiological response to CNS trauma. *J. Neurosci.* 21, 3740–3748.

Zhou, R., Horai, R., Silver, P. B., Mattapallil, M. J., Zarate-Blades, C. R., Chong, W. P., Chen, J., Rigden, R. C., Villasmil, R., and Caspi, R. R. (2012). The living eye "disarms" uncommitted autoreactive T cells by converting them to Foxp3(+) regulatory cells following local antigen recognition. *J. Immunol.* 188, 1742–1750.

Zhu, B., Kennedy, J. K., Wang, Y., Sandoval-Garcia, C., Cao, L., Xiao, S., Wu, C., Elyaman, W., and Khoury, S. J. (2011). Plasticity of Ly-6C(hi) myeloid cells in T cell regulation. *J. Immunol.* 187, 2418–2432.

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Manifestations of immune tolerance in the human female reproductive tract

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Like other mucosal surfaces (e.g., the gastrointestinal tract, the respiratory tract), the human female reproductive tract acts as an initial barrier to foreign antigens. In this role, the epithelial surface and subepithelial immune cells must balance protection against pathogenic insults against harmful inflammatory reactions and acceptance of particular foreign antigens. Two common examples of these acceptable foreign antigens are the fetal allograft and human semen/sperm. Both are purposely deposited into the female genital tract and appropriate immunologic response to these non-self antigens is essential to the survival of the species. In light of the weight of this task, it is not surprising that multiple, redundant and overlapping mechanisms are involved. For instance, cells at the immunologic interface between self (female reproductive tract epithelium) and non-self (placental trophoblast cells or human sperm) express glycosylation patterns that mimic those on many metastatic cancer cells and successful pathogens. The cytokine/chemokine milieu at this interface is altered through endocrine and immunologic mechanisms to favor tolerance of non-self. The "foreign" cells themselves also play an integral role in their own immunologic acceptance, since sperm and placental trophoblast cells are unusual and unique in their antigen presenting molecule expression patterns. Here, we will discuss these and other mechanisms that allow the human female reproductive tract to perform this delicate and indispensible balancing act.

Keywords: cervix, semen, trophoblast, immune privilege, human, vagina

INTRODUCTION

There are specific locations in human tissues and organs where alloantigens and autoantigens are tolerated by the immune system. This tolerance can exist indefinitely or for defined periods of time like pregnancy. This uncoupling of the adaptive immune response confers a physiological state known as immune privilege (Streilein, 1995). Evidence suggesting the existence of immune privilege was first obtained by van Dooremal, who documented the extended survival of murine skin xenografts in the anterior chamber of the dog eye in 1873 (van Dooremaal, 1873). Other well-established immune privileged tissues and organs include the uteroplacental unit (Medawar, 1953; Beer and Billingham, 1971), brain (Muldoon et al., 2013), testes (Li et al., 2012), and the prostate (Neaves and Billingham, 1979; Leibovitz et al., 2004).

Peter Medawar initially recognized that the mammalian fetus is an allograft due to the contribution of its foreign paternal alloantigens (Medawar, 1953). Unrelated surrogate mothers readily accommodate a completely foreign fetus as well as their own offspring, confirming that maternal histocompatibility is unnecessary. These observations indicate that the female reproductive tract is immune privileged during pregnancy. However, a sexually active woman's immune components must respond robustly to pathogens at all times, yet remain selectively tolerant to male-associated antigens present in human seminal plasma and sperm. The goal of this review is to consider the current evidence describing how immune privilege is manifested in the female reproductive tract during pregnancy and semen exposure, both of which should otherwise provoke potent immune responses.

IMMUNE PRIVILEGE DURING PREGNANCY

THE HUMAN ZONA PELLUCIDA (ZP)

The mammalian egg is surrounded by the zona pellucida (ZP), which acts as a specialized extracellular matrix for binding sperm. For fertilization to occur, sperm must first bind to this matrix, transit through this barrier and fuse with the egg cell to form a zygote (Clark, 2010). The zygote is an equal combination of both the maternal and foreign paternal genomes. It is unknown at which stage the human pre-embryo begins to express paternal major histocompatibility (MHC) molecules. However, in the mouse, MHC expression is readily detected at the eight cell stage (Ewoldsen et al., 1987). Cytotoxic T lymphocyte cells (CTL) sensitized to paternal MHC antigens are unable to kill mouse pre-embryos surrounded by an intact ZP, but pre-embryos denuded of this matrix are immediately destroyed (Ewoldsen et al., 1987).

The ZP has been suggested to simply act as a physical barrier against the rejection of the human pre-embryo (Ewoldsen et al., 1987). However, many types of immune cells transit through similar types of physical barriers during the rejection of a foreign organ transplant (Krensky et al., 1990). Human ZP glycoproteins express N-glycans terminated with multivalent sialyl-Lewis^x sequences (SLEX) that mediate sperm binding (**Figure 1**) (Pang et al., 2011). SLEX is also the universal ligand for the selectins,

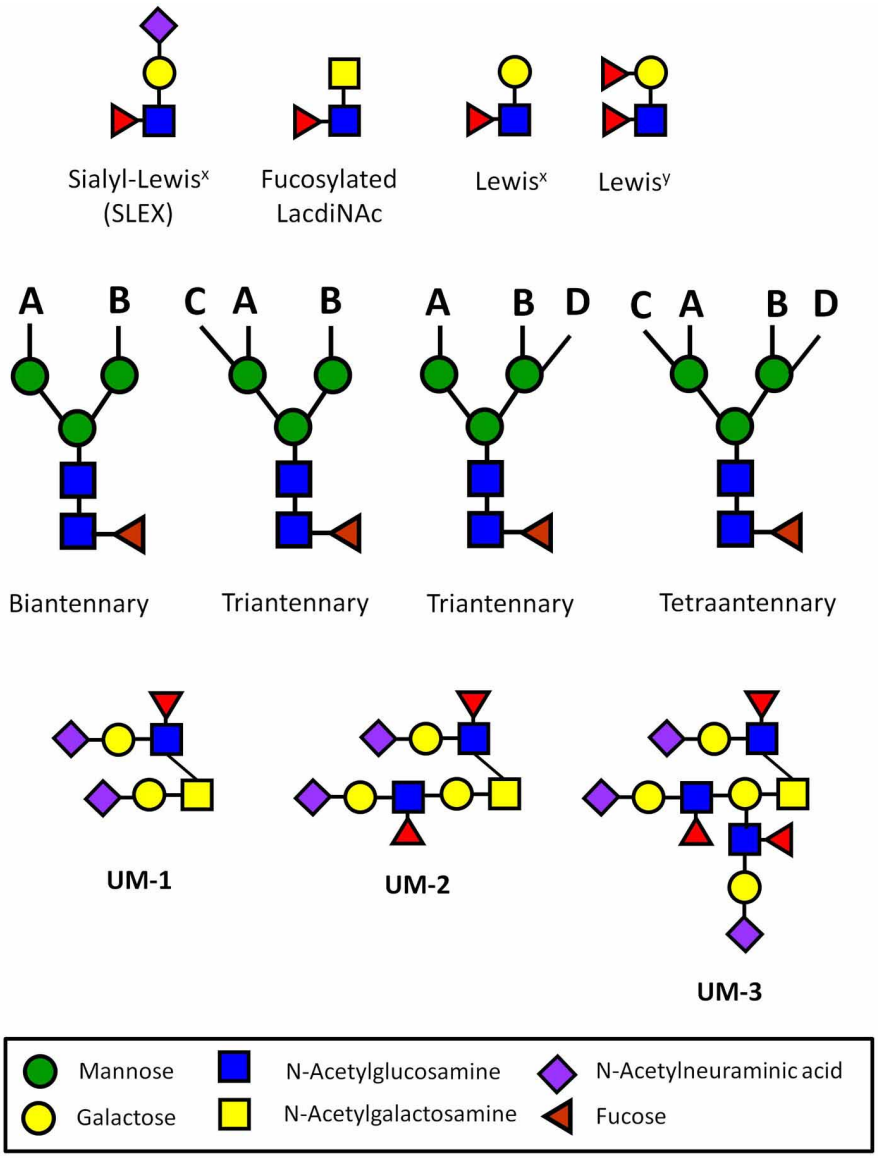

FIGURE 1 | Carbohydrate sequences involved in immune privilege in the human reproductive system. N-glycans usually have two (biantennary), three (triantennary), or four (tetraantennary) antennae linked at up to four positions (designated A–D). On the human ZP, there are biantennary and triantennary N-glycans terminated on every antenna with the SLEX sequences on every antenna (Pang et al., 2011). Tetraantennary N-glycans bearing three SLEX antenna are also present. Human sperm and seminal plasma express bi-, tri-, and tetra-antennary N-glycans terminated exclusively with Lewis^x or exclusively with Lewis^y sequences on their antennae, though many carry a mixture of both of these sequences (Pang et al., 2007, 2009). Glycodelin-A bears the fucosylated lacdiNAc sequence on 60% of its total N-glycans (Dell et al., 1995). Uromodulin expresses one (UM-1), two (UM-2), or three SLEX sequences on a single O-glycan (Easton et al., 2000). These types of presentations have not been found in other normal cells or tissues outside of the human reproductive system.

cell adhesion molecules that mediate both the binding of immune cells to inflamed vascular endothelium and lymphocyte homing (Foxall et al., 1992; Fukuda et al., 1999). SLEX is also a ligand for siglec-9, an immunoglobulin-like lectin that carries an immunoreceptor tyrosine-based inhibitory motif (ITIM) that generates an inhibitory signal in several immune cell populations (Angata and Varki, 2000; Avril et al., 2004). The possibility has been raised that the carbohydrate sequences expressed on the ZP act as functional groups to protect the early pre-embryo before blastocyst hatching (Clark et al., 1996, 1997). In short, the human egg itself could be an immune privileged cell type both before and after fertilization.

GLYCODELIN-A (Gda) AND CA125

When the histoincompatible human pre-embryo hatches out of the blastocyst, it faces the daunting task of invading the maternal endometrial lining where four major immune cell populations are present: uterine NK cells (uNK), macrophages, T cells, and

dendritic cells (DC) (King et al., 1998; Tirado-Gonzalez et al., 2012). During the early stages of implantation, trophoblast cells secrete the chemokine MIP-1α that induces uNK cells and monocytes to migrate to the implantation site, resulting in the formation of a dense infiltrate of these cells in the decidua basalis (Drake et al., 2001). It is apparent that the implanting human embryo is challenged at an early stage by these immune cells. One major question becomes apparent: how does the encircled human embryo resist challenge by these immune cells at this early stage?

Glycodelin-A (GdA) (PP14) is a luteal phase endometrial glycoprotein that is secreted beginning 2 days after ovulation (Dalton et al., 1995). This 27 kDa glycoprotein manifests many different immune deviating effects when present at physiological concentrations *in vitro* (**Table 1**). If implantation succeeds, the synthesis of GdA is massively induced, becoming 4–16% of the total protein content expressed in early stage decidua (7–11 weeks of pregnancy) (Julkunen et al., 1985). GdA is also taken up by the placenta and concentrated in this organ. This glycoprotein is also present in physiologically relevant concentrations in amniotic fluid, reaching levels averaging 46 μg/ml between 12 and 20 weeks (Julkunen et al., 1985). However, the level of GdA decreases dramatically after 20 weeks, becoming a minor component of decidual proteins and the amniotic fluid at term.

Glycodelin has also been isolated from seminal plasma and has been designated GdS. Its protein backbone is identical to GdA, but GdS does not cause the diverse immunomodulatory effects associated with GdA. Instead, *in vitro* studies indicate that it blocks the capacitation of human sperm (Chiu et al., 2005). Biophysical analyses of GdA and GdS confirm major differences in their N-glycosylation patterns. GdA expresses very unusual fucosylated lacdiNAc and Sd^a sequences on the antennae of its N-glycans; these are completely lacking in GdS (Morris et al., 1996; Lee et al., 2009). The fucosylated lacdiNAc sequence is a carbohydrate ligand for both selectins and DC-SIGN, two immune lectins that have been implicated in leukocyte/lymphocyte binding and the modulation of the adaptive immune response, respectively (Grinnell et al., 1994; van Liempt et al., 2006). The carbohydrate sequences linked to GdA have been implicated as functional groups that enable this glycoprotein to mediate its immunomodulatory effects (Clark et al., 1996, 1997).

Table 1 | Immunomodulatory activities of Glycodelin-A.

Effect	References
Inhibits T cell proliferation by PHA and other activators	Pockley et al., 1988
Decreases production of IL-2 following T cell activation	Pockley and Bolton, 1989
Induces apoptosis of activated T cells	Mukhopadhyay et al., 2001
Binds CD45 on T cells via a potential lectin-like activity	Ish-Shalom et al., 2006
Inhibits lysis of K562 target cells by large granular lymphocytes	Okamoto et al., 1991
Diminishes IgM secretion and MHC class II expression in B cells	Yaniv et al., 2003
Blocks chemoattractant induced migration of monocytes	Mukhopadhyay et al., 2001
Inhibitor of E-selectin-mediated cell adhesion	Jeschke et al., 2003
Stimulates IL-6 secretion by monocytes/ macrophages via interaction with L-selectin and the extracellular signal regulated kinase pathway	Lee et al., 2012

CA125 (MUC 16) is the largest mucinous glycoprotein in the human genome, coding for ∼24,000 amino acids (Yin et al., 2002). It is best known for its role as a specific marker for epithelial ovarian cancer (Bast et al., 1981, 1983). CA125 isolated from the human ovarian cancer cell line, OVCAR-3, is heavily N- and O-glycosylated, and its constituent glycans have been sequenced (Kui Wong et al., 2003). CA125 is secreted by endometrial epithelial cells during the same temporal window of the menstrual cycle as GdA (Kui Wong et al., 2003). Like GdA, this mucinous glycoprotein also becomes a major secreted product during the first trimester of human pregnancy. CA125 derived from OVCAR-3 cells suppresses cytotoxicity mediated by NK and lymphokine activated killer cells (LAK) *in vitro* when present at the physiological concentrations seen in the endometrium and decidua during the first trimester of pregnancy (Patankar et al., 2005). CA125 manifests this specific effect by blocking NK cell synapse formation, which results in the direct inhibition of NK cell mediated cytotoxicity (Gubbels et al., 2010).

In summary, GdA and CA125 likely participate in suppressing the maternal immune responses before implantation and continue to do so until mid-trimester. Defective expression of these glycoproteins during this stage of pregnancy would likely result in implantation failure or early pregnancy loss. However, whether defective expression of these modulators sets the stage for other pathological processes that are manifested after midtrimester is currently unknown.

DIFFERENTIAL EXPRESSION OF HUMAN MAJOR HISTOCOMPATIBILITY (MHC) ANTIGENS

The human leukocyte antigen (HLA) region of human chromosome 6 encodes many immune system genes, including the MHC complex class I and II molecules, which can be found on the surface of almost all nucleated cell types. HLA expression is tightly regulated at the feto-maternal interface, perhaps because direct engagement of foreign paternal or maternal antigens could trigger fetal rejection. When the human embryo first makes contact with the maternal endometrial epithelium, placental trophoblast cells at the adhesion site fuse and form a syncytium of multinucleated cells called syncytiotrophoblast (SynT) cells. Unlike normal cells and tissues, SynT and the underlying villous cytotrophoblasts (CytoT) do not express HLA class I and class II molecules (Hutter et al., 1996). This unusual characteristic ensures that paternal HLA antigens that could trigger histocompatibility based immune rejection are not expressed on these invasive trophoblast populations.

This lack of HLA expression, however, is somewhat problematic from an immunological perspective. About 70% of the immune cells at the implantation site during the early stages of pregnancy are uNK cells (King et al., 1996). Though not as potent

in this activity as peripheral NK cells, uNK cells lyse HLA negative cells based on the "missing self" hypothesis (King et al., 1989; Karre, 1991). Currently, there is no explanation for how SynT resist these NK cell-mediated responses, since they are somewhat sensitive to lymphokine activated killing (King and Loke, 1990). However, there are large amounts of GdA and CA125 in the uterus during implantation that could substantially affect uNK cell mediated cytotoxicity.

Further complexity in this system is added by the expression of uncommon HLA class I molecules on specific, highly-invasive trophoblast cell subpopulations. The placental villi are a complex series of branching structures that contain a core of fetal vessels surrounded by stroma (**Figure 2**). Separating the stroma and fetal vessels from the maternal blood present in the spaces between villous structures are an inner, non-continuous layer of villous cytotrophoblast cells (CytoT) and an outer, continuous layer of fused, multinuclear SynT cells (Georgiades et al., 2002). Some placental villae float freely in the intervillous space, while others traverse the space to attach or anchor themselves to the maternal decidualized endometrium (decidua). The third trophoblast cell type, extravillous cytotrophoblast (EVTB) cells arise at the tips of anchoring villae and invade deeply into the maternal decidua (Benirschke, 1994). Invasion in humans typically extends into the inner third of the uterine muscle (myometrium). EVTB also invade the maternal uterine vessels, called spiral arteries due to their anatomic appearance. EVTB plug maternal spiral arteries until approximately 10 weeks of pregnancy (Burton and Jauniaux, 2004) and remodel the vessel walls to convert them from highly vasoactive structures into relatively unresponsive conduits for blood flow from mother to baby. After 10 weeks of gestation, EVTB vascular plugs disappear to allow unobstructed flow through these conduits (Meekins et al., 1997).

EVTB are unlike all other human cells in their MHC class I expression. HLA-G and HLA-E are MHC class Ib molecules with restricted polymorphism that can be detected on the surface of EVTB (Juch et al., 2012). uNK cells bear inhibitory killer immunoglobulin-like receptors (KIR) that specifically recognize these molecules. HLA-E binds to CD94/NKG2A (King et al., 2000), whereas HLA-G binds to KIR2DL4 (Rajagopalan and Long, 1999). EVTB also express HLA-C, which is a polymorphic MHC class I molecule like HLA-A and HLA-B and could—but does not appear to—strongly stimulate harmful antipaternal adaptive immune responses (Chazara et al., 2011). HLA-C molecules also specifically bind to NK cell KIRs.

OTHER IMMUNOLOGICAL ACTIVITIES OF HLA-G

Currently, seven different isoforms of HLA-G, designated G1–G7, have been identified. HLA-G1, -G2, -G3 and -G4 are membrane-associated forms whereas HLA-G5, -G6 and -G7 (**Figure 3**) are soluble forms (Favier et al., 2007b). Both soluble and membrane associated forms of HLA-G can induce many immunomodulatory effects *in vitro* (Hunt, 2006; Favier et al., 2007b). These include: (1) inhibition of NK cell-mediated responses by either soluble or cell surface associated forms; (2) abrogation of the lytic activity mediated by CTLs via either soluble or membrane bound isoforms; (3) suppression of the IFN-γ mediated upregulation of CD8α mRNA by recombinant soluble forms of HLA-G1 and HLA-G2; (4) inhibition of the alloproliferative responses of $CD4^+$ T cells as a membrane-bound molecule expressed by third party inert cells or by cells presenting a stimulating antigen;

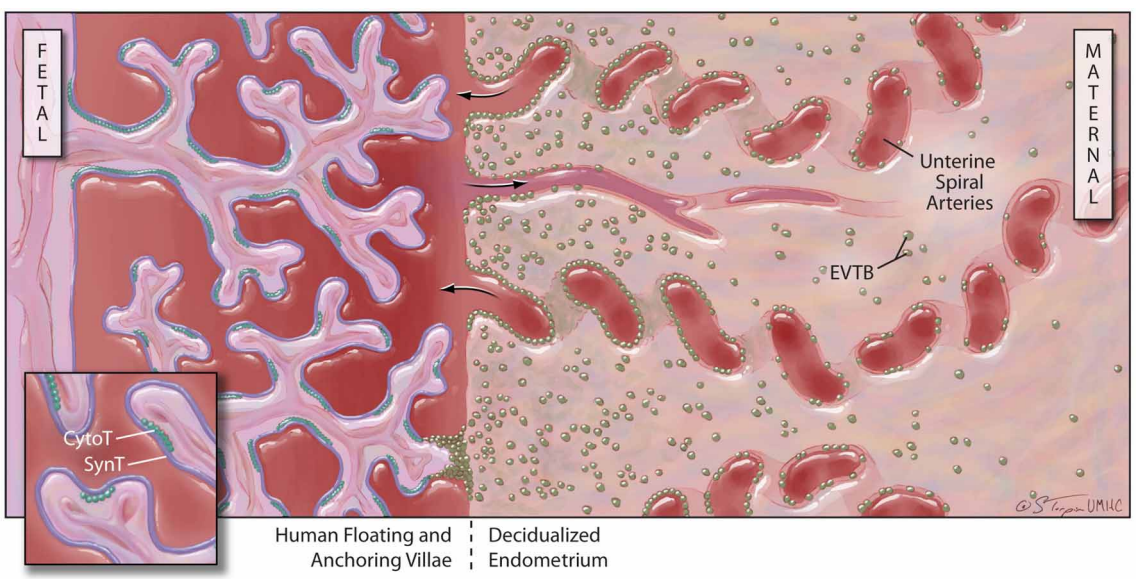

FIGURE 2 | Human placental structure (after 12 weeks of gestation): the human placenta has a fetal and a maternal side. The fetal side consists of a mass of tree-like villous structures that are bathed in maternal blood. Unlike floating villae, anchoring villae traverse the blood-filled intervillous space and attach to the maternal decidualized endometrium. The maternal decidua is populated by stromal and immune cells and is crossed by spiral arteries that dump blood into the intervillous space. Floating and anchoring placental villae are coated by an inner layer of individual, fetally-derived cytotrophoblast (Cyto-T) cells and an outer layer of fused syncytiotrophoblast (Syn-T) cells. A third population of fetally-derived trophoblast cells arises from Cyto-T at the tips of anchoring villae. These extravilous cytotrophoblast (EVTB) cells invade deeply into the maternal tissues and remodel maternal spiral arteries.

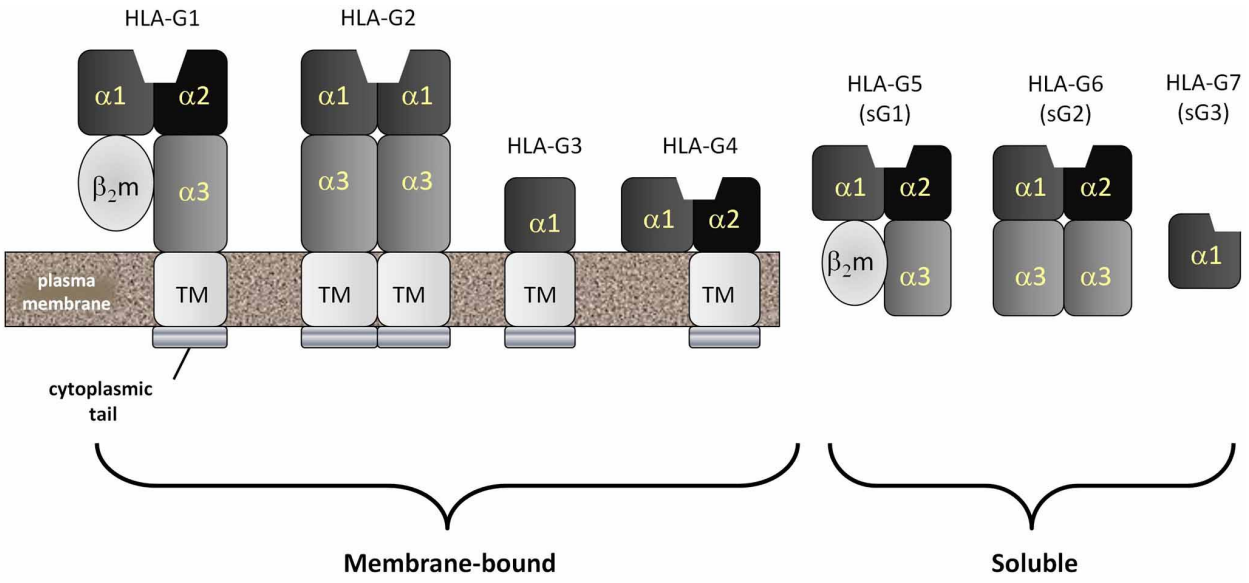

FIGURE 3 | HLA-G: the most common form of the non-classical MHC class Ib molecule, HLA-G, mimics HLA-A and -B in structure and is called HLA-G1. HLA-A, -B and -G1 are all homodimers of an MHC class I heavy chain comprised of five domains and a stabilizing second molecule, beta-2 microglobulin (β_2m). The MHC class I heavy chain consists of an α1 and α2 domain (forming the antigenic peptide-binding groove), an α3 domain, a transmembrane domain and a cytopalasmic tail. Unlike classical MHC class I molecules, the cytoplasmic tail of HLA-G is very short, containing only six amino acids. Also unlike classical MHC class Ia molecules, HLA-G can be detected as several spliced variants. The most common of these are the membrane-bound HLA-G1, -G2, -G3 and -G4 and the soluble HLA-G5, -G6 and -G7. Soluble forms have lost their transmembrane segments and cytoplasmic tails during splicing.

(5) induction of the differentiation of Tregs by stimulating antigen-producing cells; and (6) disruption of DC maturation. These effects are mediated through direct binding to inhibitory receptors designated immunoglobulin-like transcript-2 and -4 (ILT2, ILT4) and the KIR, KIR2DL4 (Favier et al., 2007a). ILT2 is expressed by both myeloid and lymphoid cells. ILT4 is exclusively expressed by myeloid cells and KIR2DL4 is expressed by NK and some T cells bearing CD81.

The effects of HLA-G *in vitro* are quite varied and affect many different types of potential immune responses in the pregnant uterus. However, the exact protein levels of the soluble and cell surface-associated isoforms of HLA-G in the fetoplacental unit and in the maternal decidua and periphery have yet to be determined. There also remain questions about HLA-G expression in different physiological states, including the proposal that the soluble forms of HLA-G (HLA-G5, -G6, and -G7) are not present in the pregnant uterus at all (Blaschitz et al., 2005; Sargent, 2005). In fact, the functions of HLA-G during the early human implantation have been difficult to definitively establish. Specifically, decidual NK (dNK) cells do not reproducibly express KIR2DL4 and the interaction between this KIR and HLA-G has not been consistently demonstrated (Apps et al., 2008). In addition, Moffet et al. have recently shown that HLA-G has no effect on freshly isolated human dNK cells in several functional tests that are known to greatly affect peripheral blood NK cells (Apps et al., 2011).

REGULATORY T CELLS (TREGS)

Tregs are essential for the development of immune privilege in the uterus during early but not late mouse pregnancy (Aluvihare et al., 2004; Shima et al., 2010). In humans, these cells have a specific phenotype ($CD4^+CD25^+FoxP3^+$) and accumulate in the endometrium during the follicular phase of the menstrual cycle (Arruvito et al., 2007). These numbers are maintained if implantation proceeds, but otherwise decrease dramatically in the luteal phase. A decrease in the number of decidual Tregs during early pregnancy in women is correlated with spontaneous abortions (Sasaki et al., 2004). Tregs decrease the cytolytic activity of NK cells (Ghiringhelli et al., 2006), impede the development of DCs (Bluestone and Tang, 2005) and negatively impact the proliferation of and cytokine release by $CD3^+$ T cells (Earle et al., 2005).

GALECTINS

Galectins are a family of small lectin molecules that generally have a universal affinity for N-acetyllactosamine (galactose in β1-4 linkage to N-acetylglucosamine; Galβ1-4GlcNAc) but which can, in some cases, bind to other carbohydrate sequences (Barondes et al., 1994). For example, several galectins, including galectin-3, also bind to the Thomsen–Friedenreich (T–F) antigen (Galβ1-3GalNAc) (Bian et al., 2011). In 1983, placental protein 13 was the first galectin isolated from the female reproductive tract (Bohn et al., 1983). Galectin-1 is another major galectin that is found in villous CytoT. This galectin has been reported to induce T cell apoptosis (Perillo et al., 1995), although there is some debate about whether this effect was due to non-physiological incubation conditions (Stowell et al., 2007). Nonetheless, interest in the expression of human placental galectins increased substantially when galectin-1 deficient mice in a stress-induced model

of pregnancy failure were reported to display higher rates of fetal loss than control mice (Blois et al., 2007). These investigators also demonstrated that this effect could be completely reversed by the administration of recombinant galectin-1. Galectin-1 has been proposed to mediate its effects via a variety of different mechanisms including: (1) differentially regulating the survival of Th cell subsets (Toscano et al., 2007; Motran et al., 2008); (2) controlling T cell trafficking (He and Baum, 2006; Norling et al., 2008); and (3) promoting the differentiation of tolerogenic DCs (Ilarregui et al., 2009; Kuo et al., 2011). In one recent study, compelling evidence was provided that suggests the overall serum level of galectin-1 is decreased dramatically (eight-fold) in spontaneous abortion patients when compared to normal pregnant controls (Tirado-Gonzalez et al., 2013).

INDOLEAMINE 2,3-DIOXYGENASE (IDO)

Indoleamine 2,3-dioxygenase (IDO) is an enzyme that can be induced in specific macrophages following their stimulation with IFN-γ and other mediators (Munn and Armstrong, 1993). Activation of this enzyme inhibits T cell-mediated responses by catabolizing the tryptophan that is essential for normal T cell proliferation. IDO is also synthesized by human Syn-T isolated from fresh placenta (Kamimura et al., 1991). Based on these effects, this enzyme was proposed to be a major mediator of immune tolerance during pregnancy. To test this hypothesis, Munn et al. exposed pregnant mice carrying either syngeneic or allogeneic fetuses to 1-methyl-tryptophan, a pharmacologic agent that inhibits IDO activity (Munn et al., 1998). Pregnant mice treated with this agent accommodated syngeneic but not allogeneic pups, suggesting IDO mediated tolerance. However, matings of male and female IDO-deficient mice generate litters of normal size, suggesting that the expression of IDO is not absolutely obligatory for mouse reproduction (Baban et al., 2004). Nonetheless, IDO likely plays a complementary role to other pregnancy-specific factors that insure the suppression of responses against the semiallogeneic fetus.

UROMODULIN

Tamm–Horsfall glycoprotein (THP) is the major protein/glycoprotein component in human urine (Tamm and Horsfall, 1950). Uromodulin is a differentially glycosylated form of THP that is present in the urine of pregnant human females, but not human males or non-pregnant females (Easton et al., 2000). It is 13 times more potent in the inhibition of antigen-induced T cell proliferation *in vitro* than THP derived from non-pregnant females and males (Hession et al., 1987). Rigorous analysis of uromodulin-derived oligosaccharides revealed the presence of unusual core 2 type O-glycans terminated with up to three SLEX terminals (Easton et al., 2000). By comparison, THP from non-pregnant females and males expresses very simple mono- and di-sialylated derivatives of Core 1 O-glycans, indicating a dramatic remodeling of THP glycosylation. Analysis of THP O-glycans isolated from females 2 months postpartum revealed a near total loss of SLEX modifications and a reversion back to forms that characterize the non-pregnant state. This observation suggests that uromodulin expression relies on human pregnancy hormones (e.g., estrogens, progesterone) or other related factors. How uromodulin contributes to the maintenance of immune privilege during pregnancy remains an enigma.

IMMUNE PRIVILEGE FOR SEMEN AND SPERM IN THE FEMALE REPRODUCTIVE TRACT

The immune system in the human female reproductive tract is also challenged by both the cellular and soluble components of human semen. The vagina and the cervix represent a relatively hostile environment for human sperm (Drobnis and Overstreet, 1992). Soon after sexual intercourse, maternal neutrophils, monocytes, and lymphocytes are released from the cervical epithelium during a cellular response known as *the leukocyte reaction* (Pandya and Cohen, 1985; Thompson et al., 1992). Human sperm do not express paternal MHC class I or II molecules on their surface and should not trigger histocompatibility-based responses (Hutter and Dohr, 1998). However, immature germ cells, epithelial cells, and leukocytes are present in semen, although they usually comprise less than 15% of the total cellular fraction (Fedder, 1996). Unlike sperm, these cells express paternal MHC molecules that could stimulate an MHC-restricted response at the surface of the cervico-vaginal epithelium (Zinkernagel and Doherty, 1974).

Sperm arise from testicular germ cells after the commencement of puberty and long after the period of thymic education (Fijak and Meinhardt, 2006). Proteins that are unique to human sperm are therefore foreign to the immune system and could be classified as either autoantigens or neoantigens. Evidence favoring this categorization includes the known induction of autoimmune orchitis following the autologous injection of testicular homogenates at sites distal to the testes (Tung et al., 1981). However, allografts and xenografts resist rejection following transplantation into the testis itself (Head and Billingham, 1985). This immune privileged state was initially thought to be due to the presence of a blood-testis barrier that protected germ cells from immune effector cells and antibodies (Setchell, 1967; Dym, 1973). Arguing against this paradigm is the fact that germ cell neoantigens on spermatogonia and early spermatocytes can be found in the basal compartments of the testis, which lack the blood-testis barrier (Yule et al., 1988; Saari et al., 1996). Further, neoantigens can also be detected in seminal plasma, which contains components that are produced by several glands in the male urogenital tract (Gupta et al., 1988).

Many of the known sperm and seminal plasma neoantigens are produced in response to androgen stimulation. While these antigens should provoke a potent immune response in the female reproductive system, the incidence of women with antisperm antibodies is only 2–3%. When antibodies are produced, however, subfertility or infertility often follows (Rumke and Hellinga, 1959; Lombardo et al., 2001). Allergic reactions to the fluid components of seminal plasma are also rare (Sublett and Bernstein, 2011). These results suggest that powerful immune-deviating effects are in play within the female genital tract.

Based on the excellent outcomes of IVF and artificial insemination procedures, which separate germ cells from seminal plasma, it is apparent that seminal plasma components are not required for successful fertilization. On the other hand, exposure to seminal plasma may be crucial to reductions in certain disease

states in humans. Preeclampsia is a common but incompletely understood complication of pregnancy with pleomorphic pathological effects (Pennington et al., 2012). Interestingly, women who have had prolonged exposure to semen via unprotected oral or vaginal sex exhibit a considerably lower risk of developing preeclampsia than women who have had a much more limited duration of semen exposure (Basso et al., 2001; Kho et al., 2009). These results lead to the conclusion that seminal plasma suppresses specific immune responses in the female reproductive tract and, in several instances, tolerizes the female to paternal immune challenges. The net effect would be to protect the female from other immune-related disease states of pregnancy. Several factors in seminal plasma have been proposed to be responsible for this immune deviation.

PROSTAGLANDINS

Human seminal plasma contains very high concentrations of prostaglandins when compared to other bodily secretions. These bioactive compounds were initially independently identified in this fluid by von Euler and Goldblatt in 1935 (Goldblatt, 1935; von Euler, 1935, 1936). Prostaglandin E_1, E_2, E_3, $F_{1\alpha}$, and $F_{2\alpha}$ have been detected in human seminal plasma (Samuelsson, 1963). It is now apparent that PGE_2, 19-hydroxyprostaglandin E_1 and 19-hydroxyprostaglandin E_2 are the three major prostaglandins in human seminal plasma, each being present in millimolar concentrations (Taylor and Kelly, 1974; Kelly et al., 1976; Templeton et al., 1978). Since these lipid mediators often manifest their effects in the μM to nM concentration range, virtually all pathways that are affected by these lipids are operating under saturating conditions in human semen.

PGE_2 is a potent modulator of immune function. The effects of PGE_2 have been the subject of intense investigation for over 20 years because of its association with cancer and other pathological states. This prostaglandin can simultaneously manifest both proinflammatory and immunosuppressive effects. These effects are summarized with references in **Table 2**.

The overall effects of PGE_2 include: (1) inhibition of responses mediated by phagocytic cells (neutrophils, macrophages); (2) suppression of NK, CTL, and T helper type 1 responses; (3) activation of DCs but limitation of their ability to attract naïve, memory, and effector T cells; and (4) stimulation of the production of regulatory T cells and myeloid-derived suppressor cells. In summary, the effects of PGE_2 are consistent with a role in the inhibition of antigen-driven Th1 responses and in the promotion of Th2 responses. This overall response is essentially that which would be necessary to suppress responses directed against neoantigens while simultaneously maintaining the effectiveness of select beneficial immune responses.

CYTOKINE EXPRESSION

Human seminal plasma contains substantial amounts of a potent immunoregulatory cytokine known as transforming growth factor-β (TGF-β) (Nocera and Chu, 1993), although only ∼7% of TGF-β in seminal plasma is in the active form (Nocera and Chu, 1995). Latent TGF-β can be activated by acidic conditions (transient acidification to pH 3.2) (Wakefield et al., 1987). Still, even though the healthy vaginal environment is acidic at baseline, it is unlikely that a significant amount of TGF-β is activated here after deposition in the vagina because the buffering capacity of the relatively large volume of basic human seminal plasma causes the pH of the vaginal environment to increase from 4.3 to 7.2 within 8 s after ejaculation (Fox et al., 1973).

The levels of other cytokines in human seminal plasma have also been studied (Maegawa et al., 2002). The levels of IL-1α, IL-2, IL-4, IL-6, IL-8, TNF-α, interferon-γ, granulocyte colony-stimulating factor [G-CSF] and macrophage CFS [M-CSF] have been analyzed. The pro-inflammatory cytokines (IL-1α, TNF-α), chemokine (IL-8) and G-CSF are present, but at low levels. The remainder are undetectable. In short, the levels of inflammatory cytokines in the lower female genital tract are very low under normal physiological conditions.

Very elegant, but difficult to perform investigations have been conducted to assess the effects of seminal plasma on cytokine expression in the human cervix. Twelve hours after unprotected vaginal intercourse with ejaculation, the mRNA levels for colony stimulating factor 2, IL-6. IL-8 and IL-1α in human cervical biopsies are enhanced when compared to controls (abstention or condom-protected controls) (Sharkey et al., 2012b). Seminal fluid not only induces the expression of pro-inflammatory cytokines and chemokines in the cervix, but also causes a major influx of macrophages, DCs, and memory T cells (Sharkey et al., 2012b). Still, TGF-β has been the component of seminal plasma most directly implicated in this response (Sharkey et al., 2012a).

UNUSUAL GLYCOSYLATION OF HUMAN SPERM AND SEMINAL PLASMA GLYCOPROTEINS

The role of glycosylation in inducing immune privilege, particularly in the reproductive tract, has been understudied. Historically, carbohydrate ligands and their complementary lectin-like immune receptors have been difficult to isolate and characterize. However, the development of ultrasensitive mass spectrometric (MS) techniques for sequencing oligosaccharides, when combined with the use of glycan arrays to define carbohydrate binding specificities, have recently changed the research landscape in this area (Blixt et al., 2004; North et al., 2009). The molecular bases underlying the ability of specific carbohydrate sequences to act as functional groups that suppress immune function are now being revealed and previous predictions about these relationships are being validated (Clark et al., 1996, 1997).

Ultrasensitive MS profiling of the N-glycans associated with human sperm and seminal plasma has uncovered the expression of unusual glycans (Pang et al., 2007, 2009). A distinguishing feature of these glycans is the presence of $Lewis^x$ and $Lewis^y$ sequences that are rarely found on the oligosaccharides present on the surface of other normal cell and tissue types outside of the male reproductive system. These sequences are displayed in multivalent presentations on the terminal ends of biantennary, triantennary, and tetraantennary N-glycans (**Figure 1**). The N-glycans linked to the human ZP are similar, except that they are terminated with multivalent SLEX rather than $Lewis^x$ or $Lewis^y$ sequences (Pang et al., 2011).

The endogenous glycoprotein ligands for immune type lectins have been proposed to be the true mediators of immune homeostasis (Garcia-Vallejo and van Kooyk, 2009). There are four

Table 2 | Effects of PGE_2 on immune function.

Immunological effects	References
Inhibits granulocyte functions	Smith, 1977
Limits the phagocytic activity of alveolar macrophages and their pathogen killing function	Hubbard et al., 2010
Promotes the tissue influx of neutrophils, macrophages, and mast cells	Yu and Chadee, 1998; Nakayama et al., 2006; Weller et al., 2007
Converts DCs to myeloid derived suppressor cells	Obermajer et al., 2011
Suppresses NK cell mediated cytotoxicity	Bankhurst, 1982; Goto et al., 1983
Inhibits NK cell responses to IL-12, IL-15, and IL-2	Joshi et al., 2001; Walker and Rotondo, 2004
Blocks NK cell production of IFN-γ, inhibiting NK cell helper function	Mailliard et al., 2005
Disrupts early stages of differentiation of dendritic cells (DCs)	Kaliński et al., 1997
Promotes the induction of mast cells and their local attraction and degranulation	Hu et al., 1995; Gomi et al., 2000
Directly inhibits T cell production of IL-2 and IL-2 responsiveness	Walker et al., 1983
Enhances the production of Th2-attracting chemokines	McIlroy et al., 2006
Supports the induction of fully mature DCs	Jonuleit et al., 1997
Accelerates DC maturation and elevates their costimululatory molecules when present in combination with IL-1β and TNF-α	Rieser et al., 1997; Kaliński et al., 1998
Promotes the expression of CCR7, the receptor for chemokines L19 and L20 in monocyte-derived DCs	Luft et al., 2002; Scandella et al., 2002
Inhibits early stages of B cell activation and Ig class switching	Simkin et al., 1987
Limits migration of DCs via induction of tissue inhibitor of proteinase-1	Baratelli et al., 2004
Increases the expression of IL-10, thrombospondin and IDO in DCs	Kaliński et al., 1997; Doyen et al., 2003; Braun et al., 2005
Promotes the maturation of DCs with an impaired ability to induce CTL-, Th1- and NK cell-mediated type 1 immunity	Kaliński et al., 1998, 1999; Gustafsson et al., 2008
Suppresses the level of bioactive IL-12p70	Kaliński et al., 1998
Blocks the ability of DCs to attract naïve T cells	Muthuswamy et al., 2010
Suppresses the production of IL-12 in monocytes and DCs	van der Pouw Kraan et al., 1995; Kaliński et al., 1997, 1998
Blocks the expression of the IL-12 receptor in monocytes and DCs	Wu et al., 1998
Promotes the development of IL-17 producing T cells	Sheibanie et al., 2007; Woolard et al., 2008; Boniface et al., 2009; Esaki et al., 2010
Inhibits cytotoxic T lymphocyte (CTL) activity	Lala et al., 1988; Parhar and Lala, 1988; Specht et al., 2001
Blocks activation of CTL responses by DCs by inhibiting IL-12 secretion	Watchmaker et al., 2010
Promotes IgE production	Carini et al., 1981
Promotes the development of regulatory T cells	Baratelli et al., 2005; Bergmann et al., 2007
Promotes the interaction of DCs with regulatory T cells	Muthuswamy et al., 2008
Required for the development of tumor associated suppressive macrophages and myeloid-derived suppressor cells	Heusinkveld et al., 2011; Obermajer et al., 2011
Induces the expression of IL-10 in tissue macrophages	Huang et al., 1998; Stolina et al., 2000
Suppress the production of retinoic acid in gut-associated DCs	Stock et al., 2011

major glycoproteins in human seminal plasma that have been identified as endogenous ligands for DC-SIGN, an immune lectin associated with DCs. They include clusterin, galectin-3 binding protein, prostatic acid phosphatase, and protein C inhibitor (Clark et al., 2012). The exact function of these glycoproteins remains to be determined, but studies with parasites and other persistent pathogens that express $Lewis^x$ and $Lewis^y$ sequences suggest that they are likely involved in the induction of tolerance to the developing human *in utero* (Garcia-Vallejo and van Kooyk, 2009).

OTHER FACTORS IN SEMINAL PLASMA

A number of other seminal plasma factors display immunosuppressive effects *in vitro*. The primary assay that has been employed to assess this effect is the inhibition of phytohemagglutinin (PHA)-induced proliferation of T lymphocytes. Prostasomes are a group of 40–500 nm membranous vesicles secreted by the prostate into human semen. Prostasomes inhibit PHA-induced proliferation by 69% in a dose dependent manner (Kelly et al., 1991). However, human prostasomes contain galelectin-3, which could compete with phytohemagglutinin for binding to galactose-terminated glycans on T cell glycoproteins (Jones et al., 2010), so it is possible that this effect could involve a simple lectin blockade rather than a specific immunosuppressive effect. Whether prostasomes mediate a specific immunosuppressive effect remains to be verified.

Still another study indicated that human seminal plasma components with a MW >3.5 kDa also inhibit PHA-induced

T lymphocyte proliferation (Ochsenkuhn et al., 2006). While seminal plasma glycoproteins could also inhibit PHA binding to T lymphocytes via non-specific lectin blockade, an antibody directed against TGF-β has been shown to inhibit this immunosuppressive activity by 50%, indicating that this specific cytokine could be partially responsible for this effect (Ochsenkuhn et al., 2006). The immune deviating effects of seminal plasma glycoproteins certainly deserve further attention.

Polyamines in seminal plasma have also been implicated in the suppression of immune responses in the female reproductive tract (Allen and Roberts, 1986). Spermine is present in millimolar concentrations in human seminal plasma (Agostinelli et al., 2007). Supplementation of lymphocyte cultures with spermine leads to a cytotoxic effect that mimics that seen after addition of seminal plasma (Allen and Roberts, 1987). Spermine inhibits LAK cell activity directed against cervical carcinoma cells by up to 60% at concentrations exceeding 10 nM (Evans et al., 1995). Although the precise mechanisms underlying this finding remain unclear, the deamination of spermine by amine oxidases generates both hydrogen peroxides and aldehydes that promote apoptosis (Agostinelli et al., 2007).

CONCLUSIONS

Humans have a complex immune system consisting of both innate and adaptive arms and immune cells have developed intricate means of recognizing each other that involve HLA class I and class II molecules. Further complexity is introduced by the diversification of these molecules into many haplotypes to enable exceptionally precise recognition of self in the immunological context. However, this diversity may come at a cost, as it makes the paternal antigen-expressing human fetus the equivalent of a foreign organ transplant within the immunocompetent gravid female (Reisner et al., 2011). The maternal immune system cannot simply be inactivated to allow for reproduction because of the incumbent risk of infection, particularly that arising in the complex microbiologic milieu of the lower genital tract. A compromise state must therefore be established that will allow selective immune privilege for gametes and the developing fetus within the context of an otherwise immunocompetent female reproductive system. A reasonable, though not fully potent, immune response to pathogens must persist to protect the mother from infection.

The pathways that promote immune privilege are best understood in the eye (Streilein, 2003; Niederkorn, 2012). Niederkorn recently proposed an attractive hypothesis that suggests that metastatic uveal melanoma cells found in the liver have "plagiarized" the blueprints employed for ocular immune privilege to create "*ad hoc*" immune privileged regions in this distant site (Niederkorn, 2012). Obviously, there are enormous advantages for metastatic cells if this hypothesis is correct, as it likely is.

The proposal was made some time ago that a similar type of immune privilege exists for human gametes and the uteroplacental unit (Clark et al., 1996, 1997). Both SLEX and the Lewis^y carbohydrate sequences were originally defined as tumor-associated carbohydrate antigens, based on their specific expression on cancer cells but not on their progenitor cells (Abe et al., 1983; Fukushima et al., 1984). A recent study has confirmed the profligate expression of SLEX sequences on the human ZP (Pang et al., 2011). Lewis^y sequences are similarly expressed on human sperm and seminal plasma glycoproteins (Pang et al., 2007, 2009). The strong possibility exists that pathogens can also hijack those elements of immune privilege utilized in the reproductive system of humans to evade the immune response. For example, variants of *H. pylori* that express Lewis^x/Lewis^y sequences on their lipopolysaccharides promote tolerance, whereas non-expressors evoke the severe inflammatory responses that result in the pathological symptoms associated with this bacterial pathogen (Bergman et al., 2006). HIV infection substantially increases the percentage of CD4$^+$ and CD8$^+$ T cells that express the Lewis^y sequence (Adachi et al., 1988; Kashiwagi et al., 1994), a characteristic that may allow for relatively unfettered viral proliferation.

It is a substantial challenge to understand how carbohydrate sequences act as functional groups to mediate immunomodulatory effects at the fetomaternal interface. One major obstacle has been the inability to sequence glycans from small amounts of glycoproteins, such as MHC class I molecules. Major advances in mass spectrometry have recently led to the complete structural analysis of native human ZP glycans by Dell and coworkers, a feat performed with only 5 μg of purified ZP (Pang et al., 2011). HLA-G expressed on EVTB is differentially glycosylated when compared to classical MHC molecules (McMaster et al., 1998). If HLA-G glycans act as crucial functional groups, then aberrant glycosylation could result in the pathological effects observed during pregnancy. Current molecular and proteomic strategies could never detect these glycobiological changes.

How the information in such carbohydrate signals is transmitted in immune cells must also be defined. Siglecs usually bear specific ITIM that mediate immune modulatory effects via conventional signaling mechanisms (Crocker et al., 2007). The two exceptions (Siglec H and Siglec 14) activate immune cells by interacting with the membrane protein, DAP12 (Angata et al., 2006; Blasius et al., 2006). Other C-type lectin-like receptor complexes are also expressed on NK cells, including the CD94/NKG2 family receptors that employ ITIM/ITAM (immunoreceptor tyrosine-based motif) dependent pathways (Borrego et al., 2006). Carbohydrate ligands for CD94 and NKG2 have not been defined, but neither have the glycans associated with naturally occurring MHC class I molecules that could interact with these lectins. The effects of galectins are more subtle and complex, because they can form lattices of different glycoproteins that promote signaling via both lectin-like and protein-protein interactions (Lau and Dennis, 2008). Since there are many galectins, the potential for establishing functional pathways by organizing such glycoprotein complexes on cell surfaces is substantial (Than et al., 2012).

In summary, these findings suggest that investigation of the pathways that evoke immune privileged states in humans could lead to an understanding of the mechanisms that enable pathogens and tumor cells to evade the immune response. Once such pathways are defined, they can hopefully be readily targeted for therapeutic intervention. The role of carbohydrate recognition in such processes is now beginning to be fully appreciated.

REFERENCES

Abe, K., McKibbin, J. M., and Hakomori, S. (1983). The monoclonal antibody directed to difucosylated type 2 chain (Fucα1-2Galβ1-4[Fucα1-3]GlcNAc; Y Determinant). *J. Biol. Chem.* 258, 11793–11797.

Adachi, M., Hayami, M., Kashiwagi, N., Mizuta, T., Ohta, Y., Gill, M. J., et al. (1988). Expression of Ley antigen in human immunodeficiency virus-infected human T cell lines and in peripheral lymphocytes of patients with acquired immune deficiency syndrome (AIDS) and AIDS-related complex (ARC). *J. Exp. Med.* 167, 323–331.

Agostinelli, E., Tempera, G., Molinari, A., Salvi, M., Battaglia, V., Toninello, A., et al. (2007). The physiological role of biogenic amines redox reactions in mitochondria. New perspectives in cancer therapy. *Amino Acids* 33, 175–187.

Allen, R. D., and Roberts, T. K. (1986). The relationship between the immunosuppressive and cytotoxic effects of human seminal plasma. *Am. J. Reprod. Immunol. Microbiol.* 11, 59–64.

Allen, R. D., and Roberts, T. K. (1987). Role of spermine in the cytotoxic effects of seminal plasma. *Am. J. Reprod. Immunol. Microbiol.* 13, 4–8.

Aluvihare, V. R., Kallikourdis, M., and Betz, A. G. (2004). Regulatory T cells mediate maternal tolerance to the fetus. *Nat. Immunol.* 5, 266–271.

Angata, T., Hayakawa, T., Yamanaka, M., Varki, A., and Nakamura, M. (2006). Discovery of Siglec-14, a novel sialic acid receptor undergoing concerted evolution with Siglec-5 in primates. *FASEB J.* 20, 1964–1973.

Angata, T., and Varki, A. (2000). Cloning, characterization, and phylogenetic analysis of siglec-9, a new member of the CD33-related group of siglecs. Evidence for co-evolution with sialic acid synthesis pathways. *J. Biol. Chem.* 275, 22127–22135.

Apps, R., Gardner, L., and Moffett, A. (2008). A critical look at HLA-G. *Trends Immunol.* 29, 313–321.

Apps, R., Sharkey, A., Gardner, L., Male, V., Kennedy, P., Masters, L., et al. (2011). *Ex vivo* functional responses to HLA-G differ between blood and decidual NK cells. *Mol. Hum. Reprod.* 17, 577–586.

Arruvito, L., Sanz, M., Banham, A. H., and Fainboim, L. (2007). Expansion of CD4+CD25+and FOXP3+ regulatory T cells during the follicular phase of the menstrual cycle: implications for human reproduction. *J. Immunol.* 178, 2572–2578.

Avril, T., Floyd, H., Lopez, F., Vivier, E., and Crocker, P. R. (2004). The membrane-proximal immunoreceptor tyrosine-based inhibitory motif is critical for the inhibitory signaling mediated by Siglecs-7 and -9, CD33-related Siglecs expressed on human monocytes and NK cells. *J. Immunol.* 173, 6841–6849.

Baban, B., Chandler, P., McCool, D., Marshall, B., Munn, D. H., and Mellor, A. L. (2004). Indoleamine 2, 3-dioxygenase expression is restricted to fetal trophoblast giant cells during murine gestation and is maternal genome specific. *J. Reprod. Immunol.* 61, 67–77.

Bankhurst, A. D. (1982). The modulation of human natural killer cell activity by prostaglandins. *J. Clin. Lab. Immunol.* 7, 85–91.

Baratelli, F., Lin, Y., Zhu, L., Yang, S. C., Heuze-Vourc'h, N., Zeng, G., et al. (2005). Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human CD4+ T cells. *J. Immunol.* 175, 1483–1490.

Baratelli, F. E., Heuze-Vourc'h, N., Krysan, K., Dohadwala, M., Riedl, K., Sharma, S., et al. (2004). Prostaglandin E2-dependent enhancement of tissue inhibitors of metalloproteinases-1 production limits dendritic cell migration through extracellular matrix. *J. Immunol.* 173, 5458–5466.

Barondes, S. H., Castronovo, V., Cooper, D. N., Cummings, R. D., Drickamer, K., Feizi, T., et al. (1994). Galectins: a family of animal beta-galactoside-binding lectins. *Cell* 76, 597–598.

Basso, O., Christensen, K., and Olsen, J. (2001). Higher risk of pre-eclampsia after change of partner. An effect of longer interpregnancy intervals? *Epidemiology* 12, 624–629.

Bast, R. C. Jr., Feeney, M., Lazarus, H., Nadler, L. M., Colvin, R. B., and Knapp, R. C. (1981). Reactivity of a monoclonal antibody with human ovarian carcinoma. *J. Clin. Invest.* 68, 1331–1337.

Bast, R. C. Jr., Klug, T. L., St. John, E., Jenison, E., Niloff, J. M., Lazarus, H., et al. (1983). A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. *N. Engl. J. Med.* 309, 883–887.

Beer, A. E., and Billingham, R. E. (1971). Immunobiology of mammalian reproduction. *Adv. Immunol.* 14, 1–84.

Benirschke, K. (1994). Anatomical relationship between fetus and mother. *Ann. N.Y. Acad. Sci.* 731, 9–20.

Bergman, M., Del Prete, G., van Kooyk, Y., and Appelmelk, B. (2006). Helicobacter pylori phase variation, immune modulation and gastric autoimmunity. *Nat. Rev. Microbiol.* 4, 151–159.

Bergmann, C., Strauss, L., Zeidler, R., Lang, S., and Whiteside, T. L. (2007). Expansion of human T regulatory type 1 cells in the microenvironment of cyclooxygenase 2 overexpressing head and neck squamous cell carcinoma. *Cancer Res.* 67, 8865–8873.

Bian, C. F., Zhang, Y., Sun, H., Li, D. F., and Wang, D. C. (2011). Structural basis for distinct binding properties of the human galectins to Thomsen-Friedenreich antigen. *PLoS ONE* 6:e25007. doi: 10.1371/journal.pone.0025007

Blaschitz, A., Juch, H., Volz, A., Hutter, H., Daxboeck, C., Desoye, G., et al. (2005). The soluble pool of HLA-G produced by human trophoblasts does not include detectable levels of the intron 4-containing HLA-G5 and HLA-G6 isoforms. *Mol. Hum. Reprod.* 11, 699–710.

Blasius, A. L., Cella, M., Maldonado, J., Takai, T., and Colonna, M. (2006). Siglec-H is an IPC-specific receptor that modulates type I IFN secretion through DAP12. *Blood* 107, 2474–2476.

Blixt, O., Head, S., Mondala, T., Scanlan, C., Huflejt, M. E., Alvarez, R., et al. (2004). Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. *Proc. Natl. Acad. Sci. U.S.A.* 101, 17033–17038.

Blois, S. M., Ilarregui, J. M., Tometten, M., Garcia, M., Orsal, A. S., Cordo-Russo, R., et al. (2007). A pivotal role for galectin-1 in fetomaternal tolerance. *Nat. Med.* 13, 1450–1457.

Bluestone, J. A., and Tang, Q. (2005). How do CD4+CD25+ regulatory T cells control autoimmunity? *Curr. Opin. Immunol.* 17, 638–642.

Bohn, H., Kraus, W., and Winckler, W. (1983). Purification and characterization of two new soluble placental tissue proteins (PP13 and PP17). *Oncodev. Biol. Med.* 4, 343–350.

Boniface, K., Bak-Jensen, K. S., Li, Y., Blumenschein, W. M., McGeachy, M. J., McClanahan, T. K., et al. (2009). Prostaglandin E2 regulates Th17 cell differentiation and function through cyclic AMP and EP2/EP4 receptor signaling. *J. Exp. Med.* 206, 535–548.

Borrego, F., Masilamani, M., Marusina, A. I., Tang, X., and Coligan, J. E. (2006). The CD94/NKG2 family of receptors: from molecules and cells to clinical relevance. *Immunol. Res.* 35, 263–278.

Braun, D., Longman, R. S., and Albert, M. L. (2005). A two-step induction of indoleamine 2, 3 dioxygenase (IDO) activity during dendritic-cell maturation. *Blood* 106, 2375–2381.

Burton, G. J., and Jauniaux, E. (2004). Placental oxidative stress: from miscarriage to preeclampsia. *J. Soc. Gynecol. Investig.* 11, 342–352.

Carini, C., Hudspith, B. N., and Brostoff, J. (1981). Effect of prostaglandins and cyclic nucleotides on growth and immunoglobulin secretion of two IgE myeloma cell lines. *Br. J. Cancer* 43, 257–260.

Chazara, O., Xiong, S., and Moffett, A. (2011). Maternal KIR and fetal HLA-C: a fine balance. *J. Leukoc. Biol.* 90, 703–716.

Chiu, P. C., Chung, M. K., Tsang, H. Y., Koistinen, R., Koistinen, H., Seppala, M., et al. (2005). Glycodelin-S in human seminal plasma reduces cholesterol efflux and inhibits capacitation of spermatozoa. *J. Biol. Chem.* 280, 25580–25589.

Clark, G. F. (2010). The mammalian zona pellucida: a matrix that mediates both gamete binding and immune recognition? *Syst. Biol. Reprod. Med.* 56, 349–364.

Clark, G. F., Dell, A., Morris, H. R., Patankar, M., Oehninger, S., and Seppala, M. (1997). Viewing AIDS from a glycobiological perspective: potential linkages to the human fetoembryonic defence system hypothesis. *Mol. Hum. Reprod.* 3, 5–13.

Clark, G. F., Grassi, P., Pang, P. C., Panico, M., Lafrenz, D., Drobnis, E. Z., et al. (2012). Tumor biomarker glycoproteins in the seminal plasma of healthy human males are endogenous ligands for DC-SIGN. *Mol. Cell. Proteomics* 11:M111.008730. doi: 10.1074/mcp.M111.008730

Clark, G. F., Oehninger, S., Patankar, M. S., Koistinen, R., Dell, A., Morris, H. R., et al. (1996). A role for glycoconjugates in human development: the human feto- embryonic defence system hypothesis. *Hum. Reprod.* 11, 467–473.

Crocker, P. R., Paulson, J. C., and Varki, A. (2007). Siglecs and their roles in the immune system. *Nat. Rev. Immunol.* 7, 255–266.

Dalton, C. F., Laird, S. M., Serle, E., Saravelos, H., Warren, M. A., Li, T. C., et al. (1995). The measurement of CA 125 and placental protein 14 in uterine flushings in women with recurrent

miscarriage; relation to endometrial morphology. *Hum. Reprod.* 10, 2680–2684.

Dell, A., Morris, H. R., Easton, R. L., Panico, M., Patankar, M., Oehniger, S., et al. (1995). Structural analysis of the oligosaccharides derived from glycodelin, a human glycoprotein with potent immunosuppressive and contraceptive activities. *J. Biol. Chem.* 270, 24116–24126.

Doyen, V., Rubio, M., Braun, D., Nakajima, T., Abe, J., Saito, H., et al. (2003). Thrombospondin 1 is an autocrine negative regulator of human dendritic cell activation. *J. Exp. Med.* 198, 1277–1283.

Drake, P. M., Gunn, M. D., Charo, I. F., Tsou, C. L., Zhou, Y., Huang, L., et al. (2001). Human placental cytotrophoblasts attract monocytes and CD56(bright) natural killer cells via the actions of monocyte inflammatory protein 1alpha. *J. Exp. Med.* 193, 1199–1212.

Drobnis, E. Z., and Overstreet, J. W. (1992). Natural history of mammalian spermatozoa in the female reproductive tract. *Oxf. Rev. Reprod. Biol.* 14, 1–45.

Dym, M. (1973). The fine structure of the monkey (Macaca) Sertoli cell and its role in maintaining the blood-testis barrier. *Anat. Rec.* 175, 639–656.

Earle, K. E., Tang, Q., Zhou, X., Liu, W., Zhu, S., Bonyhadi, M. L., et al. (2005). *In vitro* expanded human CD4+CD25+ regulatory T cells suppress effector T cell proliferation. *Clin. Immunol.* 115, 3–9.

Easton, R. L., Patankar, M. S., Clark, G. F., Morris, H. R., and Dell, A. (2000). Pregnancy-associated changes in the glycosylation of Tamm-Horsfall glycoprotein. Expression of sialyl Lewisx sequence on core 2 type O-glycans derived from uromodulin. *J. Biol. Chem.* 275, 21928–21938.

Esaki, Y., Li, Y., Sakata, D., Yao, C., Segi-Nishida, E., Matsuoka, T., et al. (2010). Dual roles of PGE2-EP4 signaling in mouse experimental autoimmune encephalomyelitis. *Proc. Natl. Acad. Sci. U.S.A.* 107, 12233–12238.

Evans, C. H., Lee, T. S., and Flugelman, A. A. (1995). Spermine-directed immunosuppression of cervical carcinoma cell sensitivity to a majority of lymphokine-activated killer lymphocyte cytotoxicity. *Nat. Immun.* 14, 157–163.

Ewoldsen, M. A., Ostlie, N. S., and Warner, C. M. (1987). Killing of mouse blastocyst stage embryos by cytotoxic T lymphocytes directed to major histocompatibility complex antigens. *J. Immunol.* 138, 2764–2770.

Favier, B., Lemaoult, J., and Carosella, E. D. (2007a). Functions of HLA-G in the immune system. *Tissue Antigens* 69(Suppl. 1), 150–152.

Favier, B., Lemaoult, J., Rouas-Freiss, N., Moreau, P., Menier, C., and Carosella, E. D. (2007b). Research on HLA-G: an update. *Tissue Antigens* 69, 207–211.

Fedder, J. (1996). Nonsperm cells in human semen: with special reference to seminal leukocytes and their possible influence on fertility. *Arch. Androl.* 36, 41–65.

Fijak, M., and Meinhardt, A. (2006). The testis in immune privilege. *Immunol. Rev.* 213, 66–81.

Fox, C. A., Meldrum, S. J., and Watson, B. W. (1973). Continuous measurement by radio-telemetry of vaginal pH during human coitus. *J. Reprod. Fertil.* 33, 69–75.

Foxall, C., Watson, S. R., Dowbenko, D., Fennie, C., Lasky, L. A., Kiso, M., et al. (1992). The three members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl Lewis^x oligosaccharide. *J. Cell Biol.* 117, 895–902.

Fukuda, M., Hiraoka, N., and Yeh, J. C. (1999). C-type lectins and sialyl Lewis X oligosaccharides. Versatile roles in cell-cell interaction. *J. Cell Biol.* 147, 467–470.

Fukushima, K., Hirota, M., Terasaki, P. I., Wakisaka, A., Togashi, H., Chia, D., et al. (1984). Characterization of sialosylated Lewis^x as a new tumor-associated antigen. *Cancer Res.* 44, 5279–5285.

Garcia-Vallejo, J. J., and van Kooyk, Y. (2009). Endogenous ligands for C-type lectin receptors: the true regulators of immune homeostasis. *Immunol. Rev.* 230, 22–37.

Georgiades, P., Ferguson-Smith, A. C., and Burton, G. J. (2002). Comparative developmental anatomy of the murine and human definitive placentae. *Placenta* 23, 3–19.

Ghiringhelli, F., Menard, C., Martin, F., and Zitvogel, L. (2006). The role of regulatory T cells in the control of natural killer cells: relevance during tumor progression. *Immunol. Rev.* 214, 229–238.

Goldblatt, M. W. (1935). Properties of human seminal plasma. *J. Physiol.* 84, 208–218.

Gomi, K., Zhu, F. G., and Marshall, J. S. (2000). Prostaglandin E2 selectively enhances the IgE-mediated productin of IL-6 and granulocyte-macrophase colony-stimulating factor by mast cells through an EP1/EP3-dependent mechanism. *J. Immunol.* 165, 6545–6552.

Goto, T., Herberman, R. B., Maluish, A., and Strong, D. M. (1983). Cyclic AMP as a mediator of prostaglandin E-induced suppression of human natural killer cell activity. *J. Immunol.* 130, 1350–1355.

Grinnell, B. W., Hermann, R. B., and Yan, S. B. (1994). Human protein C inhibits selectin-mediated cell adhesion: role of unique fucosylated oligosaccharide. *Glycobiology* 4, 221–225.

Gubbels, J. A., Felder, M., Horibata, S., Belisle, J. A., Kapur, A., Holden, H., et al. (2010). MUC16 provides immune protection by inhibiting synapse formation between NK and ovarian tumor cells. *Mol. Cancer* 9:11. doi: 10.1186/1476-4598-9-11

Gupta, S. K., Mountain, L., and Alexander, N. J. (1988). Seminal plasma antigens detected by immunoblotting with human sera from vasectomized males. *J. Reprod. Immunol.* 12, 263–276.

Gustafsson, K., Ingelsten, M., Bergqvist, L., Nystrom, J., Andersson, B., and Karlsson-Parra, A. (2008). Recruitment and activation of natural killer cells *in vitro* by a human dendritic cell vaccine. *Cancer Res.* 68, 5965–5971.

He, J., and Baum, L. G. (2006). Endothelial cell expression of galectin-1 induced by prostate cancer cells inhibits T-cell transendothelial migration. *Lab. Invest.* 86, 578–590.

Head, J. R., and Billingham, R. E. (1985). Immunologically privileged sites in transplantation immunology and oncology. *Perspect. Biol. Med.* 29, 115–131.

Hession, C., Decker, J. M., Sherblom, A. P., Kumar, S., Yue, C. C., Mattaliano, R. J., et al. (1987). Uromodulin (Tamm-Horsfall glycoprotein): a renal ligand for lymphokines. *Science* 237, 1479–1484.

Heusinkveld, M., de Vos van Steenwijk, P. J., Goedemans, R., Ramwadhdoebe, T. H., Gorter, A., Welters, M. J., et al. (2011). M2 macrophages induced by prostaglandin E2 and IL-6 from cervical carcinoma are switched to activated M1 macrophages by CD4+ Th1 cells. *J. Immunol.* 187, 1157–1165.

Hu, Z. Q., Asano, K., Seki, H., and Shimanura, T. (1995). An essential role of prostaglandin E on mouse mast cell induction. *J. Immunol.* 155, 2134–2142.

Huang, M., Stolina, M., Sharma, S., Mao, J. T., Zhu, L., Miller, P. W., et al. (1998). Non-small cell lung cancer cyclooxygenase-2-dependent regulation of cytokine balance in lymphocytes and macrophages: up-regulation of interleukin 10 and down-regulation of interleukin 12 production. *Cancer Res.* 58, 1208–1216.

Hubbard, L. L., Ballinger, M. N., Thomas, P. E., Wilke, C. A., Standiford, T. J., Kobayashi, K. S., et al. (2010). A role for IL-1 receptor-associated kinase-M in prostaglandin E2-induced immunosuppression post-bone marrow transplantation. *J. Immunol.* 184, 6299–6308.

Hunt, J. S. (2006). Stranger in a strange land. *Immunol. Rev.* 213, 36–47.

Hutter, H., and Dohr, G. (1998). HLA expression on immature and mature human germ cells. *J. Reprod. Immunol.* 38, 101–122.

Hutter, H., Hammer, A., Blaschitz, A., Hartmann, M., Ebbesen, P., Dohr, G., et al. (1996). Expression of HLA class I molecules in human first trimester and term placenta trophoblast. *Cell Tissue Res.* 286, 439–447.

Ilarregui, J. M., Croci, D. O., Bianco, G. A., Toscano, M. A., Salatino, M., Vermeulen, M. E., et al. (2009). Tolerogenic signals delivered by dendritic cells to T cells through a galectin-1-driven immunoregulatory circuit involving interleukin 27 and interleukin 10. *Nat. Immunol.* 10, 981–991.

Ish-Shalom, E., Gargir, A., Andre, S., Borovsky, Z., Ochanuna, Z., Gabius, H. J., et al. (2006). alpha2, 6-Sialylation promotes binding of placental protein 14 via its Ca2+-dependent lectin activity: insights into differential effects on CD45RO and CD45RA T cells. *Glycobiology* 16, 173–183.

Jeschke, U., Wang, X., Briese, V., Friese, K., and Stahn, R. (2003). Glycodelin and amniotic fluid transferrin as inhibitors of E-selectin-mediated cell adhesion. *Histochem. Cell Biol.* 119, 345–354.

Jones, J. L., Saraswati, S., Block, A. S., Lichti, C. F., Mahadevan, M., and Diekman, A. B. (2010). Galectin-3 is associated with prostasomes in human semen. *Glycoconj. J.* 27, 227–236.

Jonuleit, H., Kuhn, U., Muller, G., Steinbrink, K., Paragnik, L., Schmitt, E., et al. (1997). Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf

serum-free conditions. *Eur. J. Immunol.* 27, 3135–3142.

Joshi, P. C., Zhou, X., Cuchens, M., and Jones, Q. (2001). Prostaglandin E2 suppressed IL-15-mediated human NK cell function through down-regulation of common gamma-chain. *J. Immunol.* 166, 885–891.

Juch, H., Blaschitz, A., Dohr, G., and Hutter, H. (2012). HLA class I expression in the human placenta. *Wien. Med. Wochenschr.* 162, 196–200.

Julkunen, M., Rutanen, E. M., Koskimies, A., Ranta, T., Bohn, H., and Seppala, M. (1985). Distribution of placental protein 14 in tissues and body fluids during pregnancy. *Br. J. Obstet. Gynaecol.* 92, 1145–1151.

Kaliński, P., Hilkens, C. M., Snijders, A., Snijdewint, F. G., and Kapsenberg, M. L. (1997). IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 2 cytokine production in maturing human naive T helper cells. *J. Immunol.* 159, 28–35.

Kaliński, P., Hilkens, C. M., Wierenga, E. A., and Kapsenberg, M. L. (1999). T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. *Immunol. Today* 20, 561–567.

Kaliński, P., Schuitemaker, J. H., Hilkens, C. M., and Kapsenberg, M. L. (1998). Prostaglandin E2 induces the final maturation of IL-12-deficient CD1a+CD83+ dendritic cells: the levels of IL-12 are determined during the final dendritic cell maturation and are resistant to further modulation. *J. Immunol.* 161, 2804–2809.

Kamimura, S., Eguchi, K., Yonezawa, M., and Sekiba, K. (1991). Localization and developmental change of indoleamine 2, 3-dioxygenase activity in the human placenta. *Acta Med. Okayama* 45, 135–139.

Karre, K. (1991). MHC gene control of the natural killer system at the level of the target and the host. *Semin. Cancer Biol.* 2, 295–309.

Kashiwagi, N., Gill, M. J., Adachi, M., Church, D., Wong, S. J., Poon, M. C., et al. (1994). Lymphocyte membrane modifications induced by HIV infection. *Tohoku J. Exp. Med.* 173, 115–131.

Kelly, R. W., Holland, P., Skibinski, G., Harrison, C., McMillan, L., Hargreave, T., et al. (1991). Extracellular organelles (prostasomes) are immunosuppressive components of human semen. *Clin. Exp. Immunol.* 86, 550–556.

Kelly, R. W., Taylor, P. L., Hearn, J. P., Short, R. V., Martin, D. E., and Marston, J. H. (1976). 19-Hydroxyprostaglandin E1 as a major component of the semen of primates. *Nature* 260, 544–545.

Kho, E. M., McCowan, L. M., North, R. A., Roberts, C. T., Chan, E., Black, M. A., et al. (2009). Duration of sexual relationship and its effect on preeclampsia and small for gestational age perinatal outcome. *J. Reprod. Immunol.* 82, 66–73.

King, A., Allan, D. S., Bowen, M., Powis, S. J., Joseph, S., Verma, S., et al. (2000). HLA-E is expressed on trophoblast and interacts with CD94/NKG2 receptors on decidual NK cells. *Eur. J. Immunol.* 30, 1623–1631.

King, A., Birkby, C., and Loke, Y. W. (1989). Early human decidual cells exhibit NK activity against the K562 cell line but not against first trimester trophoblast. *Cell. Immunol.* 118, 337–344.

King, A., Burrows, T., and Loke, Y. W. (1996). Human uterine natural killer cells. *Nat. Immun.* 15, 41–52.

King, A., Burrows, T., Verma, S., Hiby, S., and Loke, Y. W. (1998). Human uterine lymphocytes. *Hum. Reprod. Update* 4, 480–485.

King, A., and Loke, Y. W. (1990). Human trophoblast and JEG choriocarcinoma cells are sensitive to lysis by IL-2-stimulated decidual NK cells. *Cell. Immunol.* 129, 435–448.

Krensky, A. M., Weiss, A., Crabtree, G., Davis, M. M., and Parham, P. (1990). T-lymphocyte-antigen interactions in transplant rejection. *N. Engl. J. Med.* 322, 510–517.

Kui Wong, N., Easton, R. L., Panico, M., Sutton-Smith, M., Morrison, J. C., Lattanzio, F. A., et al. (2003). Characterization of the oligosaccharides associated with the human ovarian tumor marker CA125. *J. Biol. Chem.* 278, 28619–28634.

Kuo, P. L., Hung, J. Y., Huang, S. K., Chou, S. H., Cheng, D. E., Jong, Y. J., et al. (2011). Lung cancer-derived galectin-1 mediates dendritic cell anergy through inhibitor of DNA binding 3/IL-10 signaling pathway. *J. Immunol.* 186, 1521–1530.

Lala, P. K., Kennedy, T. G., and Parhar, R. S. (1988). Suppression of lymphocyte alloreactivity by early gestational human decidua. II. Characterization of the suppressor mechanisms. *Cell. Immunol.* 116, 411–422.

Lau, K. S., and Dennis, J. W. (2008). N-Glycans in cancer progression. *Glycobiology* 18, 750–760.

Lee, C. L., Lam, E. Y., Lam, K. K., Koistinen, H., Seppala, M., Ng, E. H., et al. (2012). Glycodelin-A stimulates interleukin-6 secretion by human monocytes and macrophages through L-selectin and the extracellular signal-regulated kinase pathway. *J. Biol. Chem.* 287, 36999–37009.

Lee, C. L., Pang, P. C., Yeung, W. S., Tissot, B., Panico, M., Lao, T. T., et al. (2009). Effects of differential glycosylation of glycodelins on lymphocyte survival. *J. Biol. Chem.* 284, 15084–15096.

Leibovitz, A., Baumoehl, Y., and Segal, R. (2004). Increased incidence of pathological and clinical prostate cancer with age: age related alterations of local immune surveillance. *J. Urol.* 172, 435–437.

Li, N., Wang, T., and Han, D. (2012). Structural, cellular and molecular aspects of immune privilege in the testis. *Front. Immun.* 3:152. doi: 10.3389/fimmu.2012.00152

Lombardo, F., Gandini, L., Dondero, F., and Lenzi, A. (2001). Antisperm immunity in natural and assisted reproduction. *Hum. Reprod. Update* 7, 450–456.

Luft, T., Jefford, M., Luetjens, P., Toy, T., Hochrein, H., Masterman, K. A., et al. (2002). Functionally distinct dendritic cell (DC) populations induced by physiologic stimuli: prostaglandin E(2) regulates the migratory capacity of specific DC subsets. *Blood* 100, 1362–1372.

Maegawa, M., Kamada, M., Irahara, M., Yamamoto, S., Yoshikawa, S., Kasai, Y., et al. (2002). A repertoire of cytokines in human seminal plasma. *J. Reprod. Immunol.* 54, 33–42.

Mailliard, R. B., Alber, S. M., Shen, H., Watkins, S. C., Kirkwood, J. M., Herberman, R. B., et al. (2005). IL-18-induced CD83+CCR7+ NK helper cells. *J. Exp. Med.* 202, 941–953.

McIlroy, A., Caron, G., Blanchard, S., Fremaux, I., Duluc, D., Delneste, Y., et al. (2006). Histamine and prostaglandin E up-regulate the production of Th2-attracting chemokines (CCL17 and CCL22) and down-regulate IFN-gamma-induced CXCL10 production by immature human dendritic cells. *Immunology* 117, 507–516.

McMaster, M., Zhou, Y., Shorter, S., Kapasi, K., Geraghty, D., Lim, K. H., et al. (1998). HLA-G isoforms produced by placental cytotrophoblasts and found in amniotic fluid are due to unusual glycosylation. *J. Immunol.* 160, 5922–5928.

Medawar, P. (1953). Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. *Symp. Soc. Exp. Biol.* 7, 320–338.

Meekins, J. W., Luckas, M. J., Pijnenborg, R., and McFadyen, I. R. (1997). Histological study of decidual spiral arteries and the presence of maternal erythrocytes in the intervillous space during the first trimester of normal human pregnancy. *Placenta* 18, 459–464.

Morris, H. R., Dell, A., Easton, R. L., Panico, M., Koistinen, H., Koistinen, R., et al. (1996). Gender-specific glycosylation of human glycodelin affects its contraceptive activity. *J. Biol. Chem.* 271, 32159–32167.

Motran, C. C., Molinder, K. M., Liu, S. D., Poirier, F., and Miceli, M. C. (2008). Galectin-1 functions as a Th2 cytokine that selectively induces Th1 apoptosis and promotes Th2 function. *Eur. J. Immunol.* 38, 3015–3027.

Mukhopadhyay, D., Sundereshan, S., Rao, C., and Karande, A. A. (2001). Placental protein 14 induces apoptosis in T cells but not in monocytes. *J. Biol. Chem.* 276, 28268–28273.

Muldoon, L. L., Alvarez, J. I., Begley, D. J., Boado, R. J., Del Zoppo, G. J., Doolittle, N. D., et al. (2013). Immunologic privilege in the central nervous system and the blood-brain barrier. *J. Cereb. Blood Flow Metab.* 33, 13–21.

Munn, D. H., and Armstrong, E. (1993). Cytokine regulation of human monocyte differentiation *in vitro*: the tumor-cytotoxic phenotype induced by macrophage colony-stimulating factor is developmentally regulated by gamma-interferon. *Cancer Res.* 53, 2603–2613.

Munn, D. H., Zhou, M., Attwood, J. T., Bondarev, I., Conway, S. J., Marshall, B., et al. (1998). Prevention of allogeneic fetal rejection by tryptophan catabolism *Science* 281, 1191–1193.

Muthuswamy, R., Mueller-Berghaus, J., Haberkorn, U., Reinhart, T. A., Schadendorf, D., and Kalinski, P. (2010). PGE2 transiently enhances DC expression of CCR7 but inhibits the ability of DCs to produce CCL19 and attract naive T cells. *Blood* 116, 1454–1459.

Muthuswamy, R., Urban, J., Lee, J. J., Reinhart, T. A., Bartlett, D., and Kalinski, P. (2008). Ability of mature dendritic cells to interact with regulatory T cells is imprinted during maturation. *Cancer Res.* 68, 5972–5978.

Nakayama, T., Mutsuga, N., Yao, L., and Tosato, G. (2006). Prostaglandin

E2 promotes degranulation-independent release of MCP-1 from mast cells. *J. Leukoc. Biol.* 79, 95–104.

Neaves, W. B., and Billingham, R. E. (1979). The lymphatic drainage of the rat prostate and its status as an immunologically privileged site. *Transplantation* 27, 127–132.

Niederkorn, J. Y. (2012). Ocular immune privilege and ocular melanoma: parallel universes or immunological plagiarism? *Front. Immun.* 3:148. doi: 10.3389/fimmu.2012.00148

Nocera, M., and Chu, T. M. (1993). Transforming growth factor beta as an immunosuppressive protein in human seminal plasma. *Am. J. Reprod. Immunol.* 30, 1–8.

Nocera, M., and Chu, T. M. (1995). Characterization of latent transforming growth factor-beta from human seminal plasma. *Am. J. Reprod. Immunol.* 33, 282–291.

Norling, L. V., Sampaio, A. L., Cooper, D., and Perretti, M. (2008). Inhibitory control of endothelial galectin-1 on *in vitro* and *in vivo* lymphocyte trafficking. *FASEB J.* 22, 682–690.

North, S. J., Hitchen, P. G., Haslam, S. M., and Dell, A. (2009). Mass spectrometry in the analysis of N-linked and O-linked glycans. *Curr. Opin. Struct. Biol.* 19, 498–506.

Obermajer, N., Muthuswamy, R., Lesnock, J., Edwards, R. P., and Kalinski, P. (2011). Positive feedback between PGE2 and COX2 redirects the differentiation of human dendritic cells toward stable myeloid-derived suppressor cells. *Blood* 118, 5498–5505.

Ochsenkuhn, R., O'Connor, A. E., Hirst, J. J., Gordon Baker, H. W., De Kretser, D. M., and Hedger, M. P. (2006). The relationship between immunosuppressive activity and immunoregulatory cytokines in seminal plasma: influence of sperm autoimmunity and seminal leukocytes. *J. Reprod. Immunol.* 71, 57–74.

Okamoto, N., Uchida, A., Takakura, K., Kariya, Y., Kanzaki, H., Riittinen, L., et al. (1991). Suppression by human placental protein 14 of natural killer cell activity. *Am. J. Reprod. Immunol.* 26, 137–142.

Pandya, I. J., and Cohen, J. (1985). The leukocytic reaction of the human uterine cervix to spermatozoa. *Fertil. Steril.* 43, 417–421.

Pang, P. C., Chiu, P. C., Lee, C. L., Chang, L. Y., Panico, M., Morris, H. R., et al. (2011). Human sperm binding is mediated by the sialyl-Lewis^x oligosaccharide on the zona pellucida. *Science* 333, 1761–1764.

Pang, P. C., Tissot, B., Drobnis, E. Z., Morris, H. R., Dell, A., and Clark, G. F. (2009). Analysis of the human seminal plasma glycome reveals the presence of immunomodulatory carbohydrate functional groups. *J. Proteome Res.* 8, 4906–4915.

Pang, P. C., Tissot, B., Drobnis, E. Z., Sutovsky, P., Morris, H. R., Clark, G. F., et al. (2007). Expression of bisecting type and Lewisx/Lewisy terminated N-glycans on human sperm. *J. Biol. Chem.* 282, 36593–36602.

Parhar, R. S., and Lala, P. K. (1988). Prostaglandin E2-mediated inactivation of various killer lineage cells by tumor-bearing host macrophages. *J. Leukoc. Biol.* 44, 474–484.

Patankar, M. S., Yu, J., Morrison, J. C., Belisle, J. A., Lattanzio, F. A., Deng, Y., et al. (2005). Potent suppression of natural killer cell response mediated by the ovarian tumor marker CA125. *Gynecol. Oncol.* 99, 704–713.

Pennington, K. A., Schlitt, J. M., Jackson, D. L., Schulz, L. C., and Schust, D. J. (2012). Preeclampsia: multiple approaches for a multifactorial disease. *Dis. Model. Mech.* 5, 9–18.

Perillo, N. L., Pace, K. E., Seilhamer, J. J., and Baum, L. G. (1995). Apoptosis of T cells mediated by galectin-1. *Nature* 378, 736–739.

Pockley, A. G., and Bolton, A. E. (1989). Placental protein 14 (PP14) inhibits the synthesis of interleukin-2 and the release of soluble interleukin-2 receptors from phytohaemagglutinin-stimulated lymphocytes. *Clin. Exp. Immunol.* 77, 252–256.

Pockley, A. G., Mowles, E. A., Stoker, R. J., Westwood, O. M., Chapman, M. G., and Bolton, A. E. (1988). Suppression of *in vitro* lymphocyte reactivity to phytohemagglutinin by placental protein 14. *J. Reprod. Immunol.* 13, 31–39.

Rajagopalan, S., and Long, E. O. (1999). A human histocompatibility leukocyte antigen (HLA)-G-specific receptor expressed on all natural killer cells. *J. Exp. Med.* 189, 1093–1100.

Reisner, Y., Hagin, D., and Martelli, M. F. (2011). Haploidentical hematopoietic transplantation: current status and future perspectives. *Blood* 118, 6006–6017.

Rieser, C., Bock, G., Klocker, H., Bartsch, G., and Thurnher, M. (1997). Prostaglandin E2 and tumor necrosis factor alpha cooperate to activate human dendritic cells: synergistic activation of interleukin 12 production. *J. Exp. Med.* 186, 1603–1608.

Rumke, P., and Hellinga, G. (1959). Autoantibodies against spermatozoa in sterile men. *Am. J. Clin. Pathol.* 32, 357–363.

Saari, T., Jahnukainen, K., and Pollanen, P. (1996). Autoantigenicity of the basal compartment of seminiferous tubules in the rat. *J. Reprod. Immunol.* 31, 65–79.

Samuelsson, B. (1963). Isolation and identification of prostaglandins from human seminal plasma. 18. prostaglandins and related factors. *J. Biol. Chem.* 238, 3229–3234.

Sargent, I. L. (2005). Does 'soluble' HLA-G really exist? Another twist to the tale. *Mol. Hum. Reprod.* 11, 695–698.

Sasaki, Y., Sakai, M., Miyazaki, S., Higuma, S., Shiozaki, A., and Saito, S. (2004). Decidual and peripheral blood CD4+CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. *Mol. Hum. Reprod.* 10, 347–353.

Scandella, E., Men, Y., Gillessen, S., Forster, R., and Groettrup, M. (2002). Prostaglandin E2 is a key factor for CCR7 surface expression and migration of monocyte-derived dendritic cells. *Blood* 100, 1354–1361.

Setchell, B. P. (1967). The blood-testicular fluid barrier in sheep. *J. Physiol.* 189, 63P–65P.

Sharkey, D. J., Macpherson, A. M., Tremellen, K. P., Mottershead, D. G., Gilchrist, R. B., and Robertson, S. A. (2012a). TGF-beta mediates proinflammatory seminal fluid signaling in human cervical epithelial cells. *J. Immunol.* 189, 1024–1035.

Sharkey, D. J., Tremellen, K. P., Jasper, M. J., Gemzell-Danielsson, K., and Robertson, S. A. (2012b). Seminal fluid induces leukocyte recruitment and cytokine and chemokine mRNA expression in the human cervix after coitus. *J. Immunol.* 188, 2445–2454.

Sheibanie, A. F., Yen, J. H., Khayrullina, T., Emig, F., Zhang, M., Tuma, R., et al. (2007). The proinflammatory effect of prostaglandin E2 in experimental inflammatory bowel disease is mediated through the IL-23→IL-17 axis. *J. Immunol.* 178, 8138–8147.

Shima, T., Sasaki, Y., Itoh, M., Nakashima, A., Ishii, N., Sugamura, K., et al. (2010). Regulatory T cells are necessary for implantation and maintenance of early pregnancy but not late pregnancy in allogeneic mice. *J. Reprod. Immunol.* 85, 121–129.

Simkin, N. J., Jelinek, D. F., and Lipsky, P. E. (1987). Inhibition of human B cell responsiveness by prostaglandin E2. *J. Immunol.* 138, 1074–1081.

Smith, R. J. (1977). Modulation of phagocytosis by and lysosomal enzyme secretion from guinea-pig neutrophils: effect of nonsteroid anti-inflammatory agents and prostaglindins. *J. Pharmacol. Exp. Ther.* 200, 647–657.

Specht, C., Bexten, S., Kolsch, E., and Pauels, H. G. (2001). Prostaglandins, but not tumor-derived IL-10, shut down concomitant tumor-specific CTL responses during murine plasmacytoma progression. *Int. J. Cancer* 91, 705–712.

Stock, A., Booth, S., and Cerundolo, V. (2011). Prostaglandin E2 suppresses the differentiation of retinoic acid-producing dendritic cells in mice and humans. *J. Exp. Med.* 208, 761–773.

Stolina, M., Sharma, S., Lin, Y., Dohadwala, M., Gardner, B., Luo, J., et al. (2000). Specific inhibition of cyclooxygenase 2 restores antitumor reactivity by altering the balance of IL-10 and IL-12 synthesis. *J. Immunol.* 164, 361–370.

Stowell, S. R., Karmakar, S., Stowell, C. J., Dias-Baruffi, M., McEver, R. P., and Cummings, R. D. (2007). Human galectin-1, -2, and -4 induce surface exposure of phosphatidylserine in activated human neutrophils but not in activated T cells. *Blood* 109, 219–227.

Streilein, J. W. (1995). Unraveling immune privilege. *Science* 270, 1158–1159.

Streilein, J. W. (2003). Ocular immune privilege: therapeutic opportunities from an experiment of nature. *Nat. Rev. Immunol.* 3, 879–889.

Sublett, J. W., and Bernstein, J. A. (2011). Seminal plasma hypersensitivity reactions: an updated review. *Mt. Sinai J. Med.* 78, 803–809.

Tamm, I., and Horsfall, F. L. (1950). Characterization and separation of an inhibitor of viral hemagglutination present in urine. *Proc. Soc. Exp. Biol. Med.* 74, 108–114.

Taylor, P. L., and Kelly, R. W. (1974). 19-Hydroxylated E prostaglandins as the major prostaglandins of human semen. *Nature* 250, 665–667.

Templeton, A. A., Cooper, I., and Kelly, R. W. (1978). Prostaglandin concentrations in the semen of fertile men. *J. Reprod. Fertil.* 52, 147–150.

Than, N. G., Romero, R., Kim, C. J., McGowen, M. R., Papp, Z., and

Wildman, D. E. (2012). Galectins: guardians of eutherian pregnancy at the maternal-fetal interface. *Trends Endocrinol. Metab.* 23, 23–31.

Thompson, L. A., Barratt, C. L., Bolton, A. E., and Cooke, I. D. (1992). The leukocytic reaction of the human uterine cervix. *Am. J. Reprod. Immunol.* 28, 85–89.

Tirado-Gonzalez, I., Freitag, N., Barrientos, G., Shaikly, V., Nagaeva, O., Strand, M., et al. (2013). Galectin-1 influences trophoblast immune evasion and emerges as a predictive factor for the outcome of pregnancy. *Mol. Hum. Reprod.* 19, 43–53.

Tirado-Gonzalez, I., Munoz-Fernandez, R., Prados, A., Leno-Duran, E., Martin, F., Abadia-Molina, A. C., et al. (2012). Apoptotic DC-SIGN+ cells in normal human decidua. *Placenta* 33, 257–263.

Toscano, M. A., Bianco, G. A., Ilarregui, J. M., Croci, D. O., Correale, J., Hernandez, J. D., et al. (2007). Differential glycosylation of TH1, TH2 and TH-17 effector cells selectively regulates susceptibility to cell death. *Nat. Immunol.* 8, 825–834.

Tung, K. S., Teuscher, C., and Meng, A. L. (1981). Autoimmunity to spermatozoa and the testis. *Immunol. Rev.* 55, 217–255.

van der Pouw Kraan, T. C., Boeije, L. C., Smeenk, R. J., Wijdenes, J., and Aarden, L. A. (1995). Prostaglandin-E2 is a potent inhibitor of human interleukin 12 production. *J. Exp. Med.* 181, 775–779.

van Dooremaal, J. C. (1873). Die Entwicklung der in fremden Grund versetzten lebenden Geweba. *Albrecht von Graefes Arch. Ophthalmol.* 19, 358–373.

van Liempt, E., Bank, C. M., Mehta, P., Garcia-Vallejo, J. J., Kawar, Z. S., Geyer, R., et al. (2006). Specificity of DC-SIGN for mannose- and fucose-containing glycans. *FEBS Lett.* 580, 6123–6131.

von Euler, U. (1935). Über die Spezifische Blutdrucksenkende Substanz des Menschlichen Prostata- und Samenblasensekretes. *Wien Klin Wochenschr* 14, 1182–1183.

von Euler, U. S. (1936). On the specific vasodilating and plain muscle stimulating substances from the accessory genital glands in man and certain animals (prostaglandins and vesiglandin). *J. Physiol. (Lond.)* 88, 213–234.

Wakefield, L. M., Smith, D. M., Masui, T., Harris, C. C., and Sporn, M. B. (1987). Distribution and modulation of the cellular receptor for transforming growth factor-beta. *J. Cell Biol.* 105, 965–975.

Walker, C., Kristense, F., Bettens, F., and Deweck, A. L. (1983). Lymphokine regulation of activated (G1) lymphocytes, I: prostaglandin E2-induced inhibition of interleukin 2 production. *J. Immunol.* 130, 1770–1773.

Walker, W., and Rotondo, D. (2004). Prostaglandin E2 is a potent regulator of interleukin-12- and interleukin-18-induced natural killer cell interferon-gamma synthesis. *Immunology* 111, 298–305.

Watchmaker, P. B., Berk, E., Muthuswamy, R., Mailliard, R. B., Urban, J. A., Kirkwood, J. M., et al. (2010). Independent regulation of chemokine responsiveness and cytolytic function versus CD8+ T cell expansion by dendritic cells. *J. Immunol.* 184, 591–597.

Weller, C. L., Collington, S. J., Hartnell, A., Conroy, D. M., Kaise, T., Barker, J. E., et al. (2007). Chemotactic action of prostaglandin E2 on mouse mast cells acting via the PGE2 receptor 3. *Proc. Natl. Acad. Sci. U.S.A.* 104, 11712–11717.

Woolard, M. D., Hensley, L. L., Kawula, T. H., and Frelinger, J. A. (2008). Respiratory Francisella tularensis live vaccine strain infection induces Th17 cells and prostaglandin E2, which inhibits generation of gamma interferon-positive T cells. *Infect. Immun.* 76, 2651–2659.

Wu, C. Y., Wang, K., McDyer, J. F., and Seder, R. A. (1998). Prostaglandin E2 and dexamethasone inhibit IL-12 receptor expression and IL-12 responsiveness. *J. Immunol.* 161, 2723–2730.

Yaniv, E., Borovsky, Z., Mishan-Eisenberg, G., and Rachmilewitz, J. (2003). Placental protein 14 regulates selective B cell responses. *Cell. Immunol.* 222, 156–163.

Yin, B. W., Dnistrian, A., and Lloyd, K. O. (2002). Ovarian cancer antigen CA125 is encoded by the MUC16 mucin gene. *Int. J. Cancer* 98, 737–740.

Yu, Y., and Chadee, K. (1998). Prostaglandin E2 stimulates IL-8 gene expression in human colonic epithelial cells by a posttranscriptional mechanism. *J. Immunol.* 161, 3746–3752.

Yule, T. D., Montoya, G. D., Russell, L. D., Williams, T. M., and Tung, K. S. (1988). Autoantigenic germ cells exist outside the blood testis barrier. *J. Immunol.* 141, 1161–1167.

Zinkernagel, R. M., and Doherty, P. C. (1974). Restriction of *in vitro* T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. *Nature* 248, 701–702.

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Structural, cellular and molecular aspects of immune privilege in the testis

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The testis presents a special immunological environment, considering its property of immune privilege that tolerates allo- and auto-antigens. Testicular immune privilege was once believed to be mainly based on the sequestration of antigens from the immune system by the blood–testis barrier in the seminiferous epithelium. Substantial evidence supports the view that the combination of physical structure, testicular cells, and cytokines controls immune responses in the testis to preserve the structural and functional integrity of testicular immune privilege. Both systemic immune tolerance and local immunosuppression help maintain the immune privilege status. Constitutive expression of anti-inflammatory factors in testicular cells is critical for local immunosuppression. However, the testis locally generates an efficient innate immune system against pathogens. Disruption of these mechanisms may lead to orchitis and impair fertility. This review article highlights the current understanding of structural, cellular, and molecular mechanisms underlying the unique immune environment of the testis, particularly its immune privilege status.

Keywords: immune privilege, testis, Sertoli cell

INTRODUCTION

Immune privilege implies a special status of some sites in a mammalian body, where allo- and auto-antigens are tolerated (Mellor and Munn, 2006). This phenomenon emerged more than a century ago in the eye and the brain of rabbits and rodents during exploration of tumor rejection after transplantation (Simpson, 2006). Later studies revealed various remarkable immune privilege sites including the eye, brain, testis, and pregnant uterus.

The testis is a distinct immune privilege site. The concept of immune privilege in mammals includes two aspects: some tissues induce tolerance after their transplantation to an allogenic recipient and some tissues readily accept foreign cells without the induction of immune rejection. The testis exhibits both two aspects of immune privilege (Fijak and Meinhardt, 2006). A large number of novel proteins are expressed in developing germ cells during spermatogenesis. Therefore, sperm production represents challenges to the immune system since sperms are unique to the body and appear long after the establishment of immune competence. However, the testis tolerates these unique antigens. The testis itself confers protection since auto-antigens induce strong autoimmune responses when they are injected elsewhere in the body (Tung et al., 1981). Initial consideration of the testis as an immune privileged site was substantiated experimentally when allografts placed into the interstitial space of the rat testis survived indefinitely (Head et al., 1983). Transplantation of spermatogonial stem cells into germ cell-depleted testes, even at the interspecies level, can restore spermatogenesis (Brinster, 2002). Similarly, ectopically transplanted allogenic testes under the kidney capsule or subderma of animals resist rejection without systemic immunosuppression in animals (Kuopio et al., 1989; Ma et al., 2004).

The mechanisms of testicular immune privilege have been gradually updated based on the progress of numerous investigations (Fijak et al., 2011). Testicular immune privilege was initially proposed to be attributed to the absence of lymphatic drainage until evidence of the existence of afferent lymphatic vessels in the testis was obtained (Barker and Billingham, 1977). Subsequently, the sequestration of antigens and antibodies from the immune system by the blood–testis barrier (BTB) was believed to play a central role in the maintenance of immune privilege status. A growing body of evidence supports the view that systemic immune tolerance and localized active immunosuppression are involved in the regulation of testicular immune privilege. Therefore, multiple mechanisms, including the special structure of the testis, the immunosuppressive properties of local cells, and paracrine and endocrine cytokines, could control the immune privilege of the testis (Meinhardt et al., 1998; Meinhardt and Hedger, 2011).

STRUCTURAL BASIS OF TESTICULAR IMMUNE PRIVILEGE

The testis is structurally complex regarding the BTB and the many different cell types it contains. While the testis structure is not fully responsible for the immune privilege, it is involved partially in the maintenance of the special testicular immune environment.

STRUCTURE OF THE TESTIS

The structure of the adult mammalian testis is highly organized and complex consisting of two distinct regions: the seminiferous tubules and the interstitial spaces between tubules (**Figure 1**). The testis has two major functions: the generation of sperms (spermatogenesis), and the synthesis of sex steroid hormones (steroidogenesis). Spermatogenesis takes place within the seminiferous tubules, and steroidogenesis is fulfilled by Leydig cells located in the interstitial spaces. The sex steroid hormones, mainly testosterone in the testis, are critical for normal spermatogenesis.

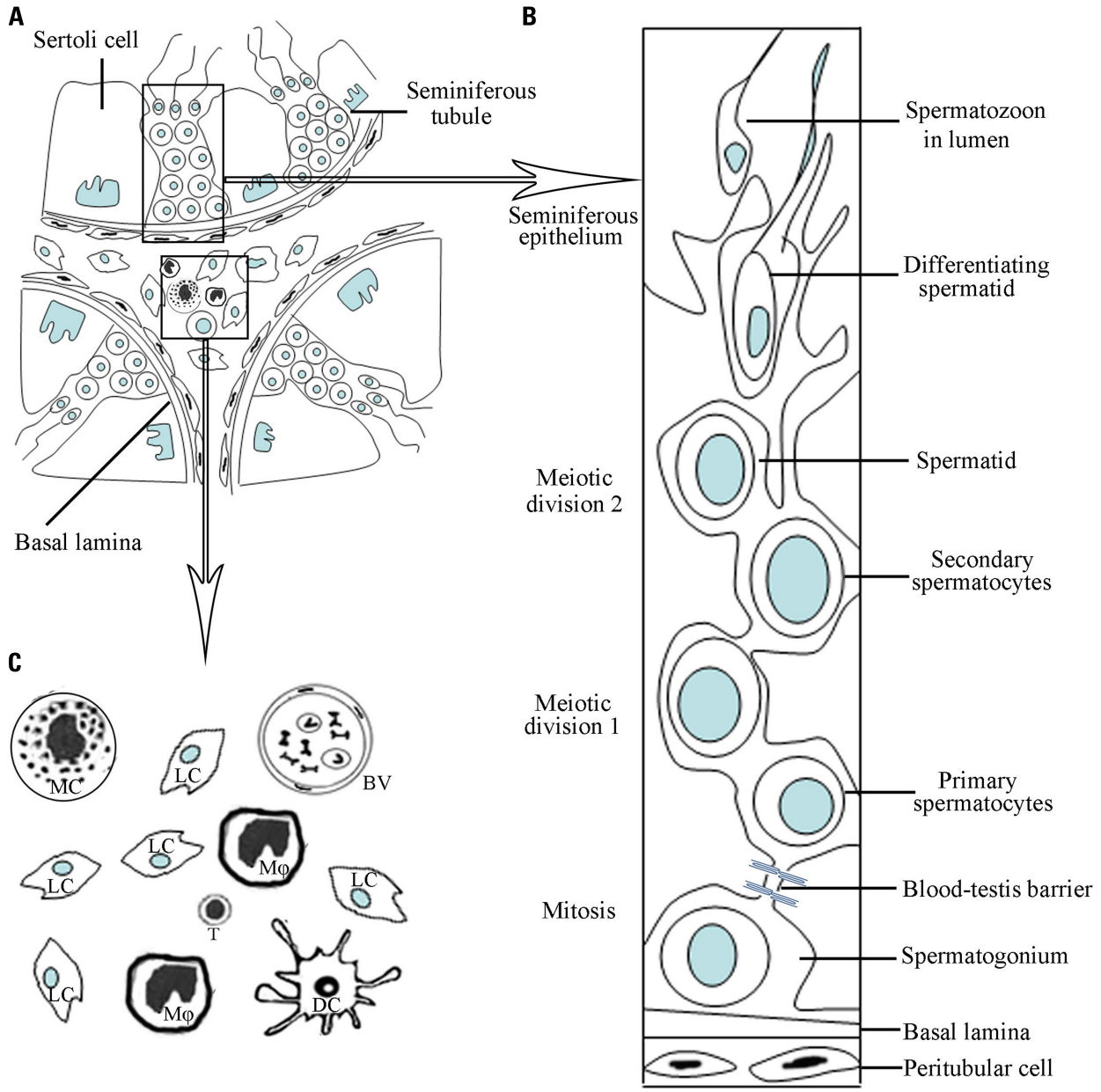

FIGURE 1 | A schematic drawing of micrographs of mammalian testicular structure and cells. (A) The testis consists of two distinct regions, namely, seminiferous tubules and interstitial compartments. **(B)** Within the seminiferous tubules, the different stages of developing germ cells are in intimate association with Sertoli cells, forming the seminiferous epithelium. The blood–testis barrier is formed by junctions between neighboring Sertoli cells near the basement membrane. **(C)** Amplification of the interstitial compartment shows cell types. Leydig cells (Lc) are majority of the interstitial cells. There are various types of immune cells including mainly macrophages (Mφ) and minor dendritic cells (DC), T lymphocytes (T) and mast cells (MC). The blood vessels (BV) exist in the interstitial compartment.

The seminiferous tubules, which are highly coiled, originate and terminate at the rete testis. The tubules are surrounded by myoid peritubular cells (MPCs), which, together with Sertoli cells (SCs), secrete components of the basement membranes that enclose the seminiferous epithelium. Columnar SCs extend from the basal lamina to the lumen of the tubules. These SCs are responsible for physical support of the germ cells, providing them with essential nutrients and growth factors.

Besides Leydig cells in the testicular interstitial spaces, there are also blood and lymphatic vessels, as well as various immune cells including macrophages, mast cells, dendritic cells (DCs), and lymphocytes (**Figure 1**). The interstitium represents the first line of testicular defense against pathogens from the bloodstream. The rete testis, which is a transition zone between the testis and the epididymitis, is the exit of the testis. The BTB is terminated in the rete testis and spermatozoa are no longer protected

from autoimmune attacks in the rete testis. Thus, certain forms of autoimmune orchitis are primarily observed in the rete testis (Itoh et al., 2005).

BLOOD–TESTIS BARRIER

The BTB is created near the basement membrane by various junctions including tight junctions (TJs), basal ectoplasanic specializations, gap junctions, and demosome-like junctions between two adjacent SCs (Su et al., 2011). The BTB is anatomically much more complex than other blood–tissue barriers, such as the blood–brain and blood–retina barriers, which are constructed exclusively of TJs between endothelial cells. Moreover, the BTB can be comprised of three components, namely anatomical, physiological, and immunological barriers (Mital et al., 2011). The junctions that restrict passage of molecules and cells into or out of the BTB form the anatomical barrier. The physiological barrier comprises transporters that regulate the passage of substances, creating a microenvironment for spermatogenesis. The immunological barrier limits the access of systemic immunity and sequesters the majority of the auto-antigenic germ cells. A functional BTB relies on the complex interaction between the three components.

The immune privilege status of the testis was initially believed to be mainly based on the sequestration of antigens and antibodies by the BTB. This view has been challenged by the observation that the testicular interstitial spaces, which are located outside the BTB, are also immune-privileged (Setchell, 1990). Moreover, although the BTB sequesters most of the auto-antigenic germ cells in the adluminal compartment of the testis, preleptotene spermatocytes and spermatogonia located outside the BTB also express antigenic molecules (Yule et al., 1988). Therefore, the BTB is partially responsible for the testicular immune privilege. A growing body of evidence shows that the actively immunosuppressive mechanisms in the testis play important roles in maintaining the testicular immune privilege status (Meinhardt and Hedger, 2011). Thus, both the BTB and local immunoregulatory mechanisms contribute to the testicular immune privilege.

TESTICULAR IMMUNE PRIVILEGE IN DIFFERENT SPECIES

The testicular immune privilege depends on the species. Allografts and xenografts can survive in the testes of some species, such as rat and mouse (Head and Billingham, 1985). By contrast, similar studies on ram and monkey have not been successful (Setchell et al., 1995). Moreover, the testes of some species do not have privilege properties as donor tissues. Mouse testis allografts under the kidney capsule survive long time (Bellgrau et al., 1995), but adult rat testes grafted to the kidney were rejected (Statter et al., 1988). These differences suggest that the factors controlling testicular immune privilege are variable among species.

An intact testis is not necessary for the immune privilege. In fact, SCs display an inherent immunosuppressive role that supports the survival of cells from other tissues, such as pancreatic islet cells, when they were co-transplanted (Selawry and Cameron, 1993; Suarez-Pinzon et al., 2000). While these previous studies support that the genetic elements play roles in the maintenance of testicular immune privilege, some common mechanisms underlying the immune privilege of the testis should be existed in different species. Notably, the immune privilege of the testis does not mean the absence of immune responses in this tissue, rather, such responses are only reduced.

CELLULAR MECHANISMS OF TESTICULAR IMMUNE PRIVILEGE

The testis is composed of various cell types including immune cells and testis-specific cells (**Figure 1**). Most types of immune cells, which contain predominantly macrophages, can be found in the interstitial spaces and are important for maintaining the special immunological environment of the testis. Tissue-specific cell types include the somatic cells: SCs, Leydig cells, and MPCs, as well as the different stages of developing germ cells. A growing body of evidence indicates that the testis-specific cells exhibit immunological functions, thus contributing to the testicular immune privilege.

IMMUNE CELLS IN THE TESTIS

While the testis is a remarkable immune privilege site, it is well connected to afferent lymph nodes. Therefore, the testis has most types of immune cells, including macrophages, T lymphocytes, DCs, and mast cells. These immune cells are important in the maintenance of the special testicular immune environment.

Macrophages

Macrophages represent a major population among immune cells in the interstitial space of the testis. Macrophages represent about 20–25% of the interstitial cells of rats under physiological conditions (Hedger, 2002). At least two subsets of macrophages can be discerned. One of them can be defined by the expression of a surface antigen that is recognized by antibody ED2, while the other subset expresses a lysosomal glycoprotein recognized by antibody ED1. $ED2^+$ macrophages, which are considered to be resident cells in the testis, represent the majority (∼80%) of testicular macrophages. $ED1^+$ macrophages, which presumably derive from circulating monocytes/macrophages that have only recently arrived in the testis, represent a minor (∼20%) proportion of all testicular macrophages. The balance between these two macrophage subsets in rats can be disrupted under orchitis conditions (Breucker, 1978; Rival et al., 2008). Clinical studies have shown that macrophage numbers are increased in the testes of patients with aspermatogenesis of different etiologies (Frungieri et al., 2002a). These observations suggest that testicular macrophages associate with the immune homeostasis in the testis and normal spermatogenesis.

Aside from the common features of macrophages in other organs, testicular macrophages display tissue-specific functions. Testicular macrophages, which have close physical interaction with Leydig cells, show important roles in the development and steroidogenesis of Leydig cells in adult rats (Gaytan et al., 1994; Hutson, 2006). Moreover, testicular macrophages also influence SC functions and spermatogenesis by producing soluble factors (Cohen et al., 1999). As a major immune cell population, the macrophages in the testis are believed to be critical in maintaining the testicular immune environment, particularly its immune privilege properties. Testicular macrophages display a reduced capacity for producing inflammatory factors compared with macrophages

from other tissues (Kern et al., 1995) and exhibit immunosuppressive properties (Kern and Maddocks, 1995), which should contribute to the maintenance of testicular immune privilege.

However, the two types of testicular macrophages exhibit different immune properties. ED2$^+$ resident macrophages, which are believed to participate to maintain the immune privilege status in the testis, do not initiate inflammatory responses in LPS-challenged rats (Gerdprasert et al., 2002b). In contrast, ED1$^+$ macrophages migrate into the testis during acute and chronic inflammation and prominently initiate the inflammatory process by releasing inflammatory cytokines with the potential to overcome the immune privilege and mount innate immunity to defense pathogens (Gerdprasert et al., 2002a; Rival et al., 2008). LPS-induced acute inflammation results in only a temporary influx of new ED1$^+$ macrophages, which can be resolved within a day or two (Gerdprasert et al., 2002a). In contrast, the increased number of ED1$^+$ cells is maintained for long periods of time during chronic inflammation, such as autoimmune orchitis (Theas et al., 2008). These observations give rise to several important questions (Fijak and Meinhardt, 2006). What factors are responsible for the recruitment and resolution of ED1$^+$ macrophages under acute inflammatory conditions? What causes the persistent elevated macrophage numbers in the testis with autoimmune orchitis? What factors regulate the balance of ED1$^+$ inflammatory cells and ED2$^+$ resident immunosuppressive macrophages in the testis under physiologic conditions? The roles of ED1$^+$ macrophages in the pathogenesis of autoimmune orchitis remain largely unclear.

Dendritic cells

Dendritic cells are bone marrow-derived specialized antigen-presenting cells (APCs), and induce activation and differentiation of lymphocytes in response to antigens. DCs not only activate lymphocyte responses to allo-antigens but also inhibit autoimmune responses by tolerating T cells to auto-antigens, thereby mounting immune responses against invading pathogens and minimizing the responses to auto-antigens (Banchereau and Steinman, 1998). DCs represent a minor population ($\sim 1 \times 10^5$) of the interstitial cells in normal rat testis (Sanchez et al., 2006), numbering about one-tenth of the macrophages (Meinhardt et al., 1998). Since DCs are the most important immunoregulatory cell types, they could play roles in regulating testicular immune responses. However, testicular DCs have not been paid enough attentions because their minor ratio in the testis. The number of DCs in the testis significantly increases in experimental autoimmune orchitis (EAO) models (Sanchez et al., 2006), suggesting that DCs may participate in the development of testicular autoimmunity. The mechanisms underlying the functions of DCs in EAO remain elusive. Various heat shock proteins (HSPs), including HSP60 and HSP70, which are abundant in male germ cells, have recently been characterized as testicular auto-antigens in EAO (Fijak et al., 2005). They may initiate the testicular DC-mediated activation of auto-reactive lymphocytes. HSP70 has been reported to promote APC function and converts T cell tolerance to autoimmunity *in vivo* (Millar et al., 2003). Therefore, the immature DCs, which normally participate in maintaining immune privilege, can be hypothesized to mature by sensing self-antigens, such as HSPs, and mature DCs may convert immune privilege by the local activation and expansion of auto-reactive T cells (Fijak et al., 2011). The role of DCs in regulating the testis immunity is worthy of further investigation.

Lymphocytes

The testis has afferent lymphatic vessels (Barker and Billingham, 1977). Approximately 15% of the testicular immune cells in adult rat are T cells with predominant CD8$^+$ cells, whereas B cells are not found in the normal testis (Hedger and Meinhardt, 2000). Testicular lymphocyte numbers are increased in EAO models (Lustig et al., 1993) and infertile patients with sperm autoimmunity (el-Demiry et al., 1987). In EAO, CD4$^+$ and CD8$^+$ cell numbers dramatically increase at the onset of disease. CD4$^+$ cell numbers decrease and CD8$^+$ cells remain consistent during disease progression. These data suggest that CD4$^+$ cells may be involved in the initiation of the chronic phage of EAO. Interestingly, the two subsets of lymphocytes contain regulatory T cells (Tregs), which inhibit antigen-specific immune responses (Andre et al., 2009).

Studies on pancreatic islet cell allografts in mouse testes shown that activated T cells are destroyed and graft antigen-specific Tregs are produced when they enter the testis environment (Dai et al., 2005; Nasr et al., 2005). CD4$^+$CD25$^+$ Tregs are critical for peripheral tolerance. Tregs may control immune privilege within organs by preventing autoimmunity induction in regions where antigen-specific Tregs continuously encounter tissue antigens (Samy et al., 2005). This mechanism controls tolerogenic versus autoimmune response to sperm in vasectomy (Wheeler et al., 2011). Tregs are found within the testicular interstitium under physiological conditions (Jacobo et al., 2009) and may contribute to the testicular immune privilege.

Mast cells

Mast cells are another immune cell population with considerable numbers in the testis. Mast cells in mammalian testis regulate steroidogenesis by Leydig cells (Aguilar et al., 1995). The increased mast cell numbers in the testis is associated with male infertility (Hussein et al., 2005). Mast cells secret serine protease tryptase, which promotes the proliferation of fibroblasts and synthesis of collagen (Abe et al., 1998), leading to fibrosis, sclerosis, thickening hyalinization of tissues, all of which are the features frequently found in the testis of infertile patients (Apa et al., 2002). Fibrosis results in granuloma formation. Mast cells involve in the granuloma formation in the testis through proteinase-activated receptor-2 (PAR2) activation. PAR2 is localized to the MPCs, macrophages, and acrosomes of spermatids in rat testis, and involved in the development of testicular inflammation (Iosub et al., 2006). PAR2 expression in EAO models is upregulated and associated with granuloma formation. Mast cells are 10-fold higher in number and distributed around granulomas in the testis of EAO compared to normal animals. The mast cells release tryptases into the interstitial spaces in EAO models, thus activating PAR2 to induce cell proliferation and cytokine production. Upregulation of monocyte chemoattractant protein-1 (MCP-1) could, at least in part, be responsible for the massive infiltration of macrophages into the testis. Similarly, evidence shows that testicular fibrosis is related to PAR2 activation by mast cells

(Frungieri et al., 2002b). Most of the studies on the mast cells focus on their role in regulating testicular inflammation. The role of mast cells in testicular immune privilege remains unknown. The relatively low number and restricted distribution of mast cells in the normal testis are believed to be one of mechanisms underlying immune privilege. Prevention of mast cell activation may be a strategy to maintain the immune privilege status of the testis.

TISSUE-SPECIFIC CELLS OF THE TESTIS

Aside from the immune cells, growing evidence shows that the testis tissue-specific cells exhibit immunological functions and contribute to the maintenance of the testicular immune environment.

Leydig cells

Leydig cells represent the majority of the cell population in the interstitial compartment of the testis. Leydig cells are critical endocrine cells that produce androgens for both the seminiferous tubule compartment to regulate spermatogenesis and peripheral circulation to extra-testicular androgen-target organs (Diemer et al., 2003). Several studies have shown that rat Leydig cells exhibit high antiviral activities in response to viral infections (Dejucq et al., 1998; Melaine et al., 2003), whereas human Leydig cells display relatively weak antiviral abilities (Le Tortorec et al., 2008). The mechanisms of Leydig cell-initiated antiviral responses remain elusive.

Leydig cells may also regulate testicular immunity through affecting the immune cell functions. It has been known that Leydig cells regulate the expansion of testicular macrophages and lymphocyte numbers in the testis (Raburn et al., 1993; Hedger and Meinhardt, 2000). Androgens have immunosuppressive roles, contributing to the immunological differences between sexes (Cutolo et al., 2004). A blockade of androgen production in Leydig cell rapidly rejects intratesticular allografts, suggesting the role of androgens in regulating immune privilege (Head and Billingham, 1985). The intratesticular testosterone concentration is 10-fold higher than the serum concentration far greater than necessary for the maintenance of normal spermatogenesis (Jarow et al., 2005). The high local testosterone concentration is likely involved in the maintenance of the testicular immune privilege. Testosterones seem to play immunosuppressive functions by regulating the balance between pro- and anti-inflammatory cytokine expression in SCs, Leydig cells, and MPCs, but they should not directly affect testicular leukocytes since androgen receptors have not been found in testicular immune cells. The role of testosterones in regulating testicular immune responses is worthwhile to be further investigated.

Myoid peritubular cells

Myoid peritubular cells surround the seminiferous tubules, and build a wall supporting the integrity of the tubules (**Figure 1**). MPCs contain contractile elements that help transport the immotile spermatozoa into the epididymis (Maekawa et al., 1996). MPCs secrete the components of the basal lamina that enclose the contents of the seminiferous epithelium. Several layers of MPCs build the walls of the tubules in human males, whereas only one layer of MPCs in rodents. The functions of MPCs in regulating spermatogenesis are largely unexplored. Previous studies have shown that MPCs can directly, or more likely indirectly, regulate spermatogenesis and testis development via secreted factors (Verhoeven et al., 2000). A recent study indicated that MPCs express androgen receptors and mediate androgen actions on fetal SC proliferation (Scott et al., 2007). Based on their localization and structure, MPCs are believed to participate in the maintenance of the testicular immune environment. A role of MPCs in testicular inflammation has emerged, especially in EAO (Schuppe and Meinhardt, 2005). MPCs release a number of cytokines, including transforming growth factor β-2 (TGFβ-2), MCP-1, and leukemia inhibitory factor. MCP-1 should, at least in part, account for the recruitment of inflammatory monocytes and/or macrophages to inflame the testis. Human MPCs express TNF-α receptors 1 and 2, which mediate the expression of other inflammatory molecules, including IL-6 and COX-2 (Schell et al., 2008). The roles of MPCs in the testicular inflammatory responses must be further clarified.

Sertoli cells

Sertoli cells are only somatic cells within the seminiferous tubules and acquire a columnar sharp extending from the basal lamina toward the lumen of the tubules (**Figure 1**). SCs constitute the main structural element of the seminiferous epithelium and are responsible for the physical support of germ cells, aside from providing essential nutrients and growth factors. Moreover, immune activities of SCs in the testis are emerging.

Sertoli cells were recognized to have immunosuppressive activities two decades ago (Wyatt et al., 1988; De Cesaris et al., 1992). They are able to provide an immunoprotective environment for some allografts and xenografts in co-transplantation experiments (Sanberg et al., 1996; Suarez-Pinzon et al., 2000). This immunoprotective environment can not be due to the physical barrier formed by SCs in the testis, rather, it seems to be provided by the inherent properties of the cells. Factors secreted by SCs, and the molecules expressed on their surfaces are most likely participated in the immunoprotection of SCs. Molecule mechanisms underlying the immunological functions of SCs will be discussed in late sections of this review.

More than half of all developing germ cells undergo apoptosis during spermatogenesis, and the cytoplasmic portions of elongated spermatids are shed and form residual bodies in the last stage of spermatogenesis. Phagocyte removal of the apoptotic germ cells and residual bodies by SCs are critical for healthy germ cells to proceed through spermatogenesis. The importance of removal of the apoptotic cells and residual bodies for normal spermatogenesis can be hypothesized as follows (Nakanishi and Shiratsuchi, 2004): (1) elimination of apoptotic cells provides appropriate space in the seminiferous epithelium for normal spermatogenesis; (2) apoptotic cells and residual bodies must be removed prior to secondary necrosis that may release noxious contents for healthy cells; and (3) the uptake of residual bodies and apoptosis cells endows SCs into producing factors necessary for spermatogenesis. However, direct evidence showing the meaning of phagocytic removal of apoptotic cells and residual bodies by SCs is still missing.

Phagocytosis is a fundamental cellular process that serves multiple functions in immunity (Greenberg and Grinstein, 2002).

The significance of phagocytic removal of apoptotic germ cells by SCs in maintaining testicular immune environment has not been revealed. Growing evidence shows that endogenous Toll-like receptor (TLR) ligands released from damaged tissues and necrotic cells can induce non-infectious inflammation through TLR activation (Piccinini and Midwood, 2010). Numerous endogenous TLR ligands high-mobility group box1 (HMGB1) and different HSPs have been identified (Vabulas et al., 2001; Curtin et al., 2009; Wheeler et al., 2009). HMGB1 and HSPs are abundantly expressed in male germ cells (Biggiogera et al., 1996; Zetterstrom et al., 2006). Therefore, one can hypothesize that necrotic germ cells and broken down residual bodies under some pathological conditions may release endogenous TLR ligands, inducing non-infectious inflammation. This hypothesis is agreement with the previous observations that physical trauma and chemical noxae, which induce germ cell apoptosis, associate with chronic testicular orchitis (Schuppe et al., 2008). A recent study showed that an impaired removal of apoptotic germ cells induce non-infectious testicular inflammation, thus favoring autoimmunity in the testis (Schuppe et al., 2008; Pelletier et al., 2009). The meaning of phagocytic removal of apoptotic germ cells by SCs in maintaining testicular immune environment should be an interesting topic to be investigated.

Germ cells

Sperm production is a major function of the testis, and developing germ cells represent the majority of testicular cells. However, the roles of germ cells in regulating testicular immune environment have yet to be extensively investigated. Previous studies demonstrated that male germ cells secrete various inflammatory cytokines, including IL-1α (Haugen et al., 1994) and TNF-α (De et al., 1993). Moreover, spermatogonia produce antiviral proteins in response to interferon (IFN)-α and IFN-γ (Melaine et al., 2003). In physiology, these cytokines may play roles in controlling the efficiency of spermatogenesis by inhibiting germ cell apoptosis (Pentikainen et al., 2001). In pathology, inflammatory cytokines are up-regulated in the testis of EAO models and impair spermatogenesis through stimulation of inflammation and induction of germ cell apoptosis (Rival et al., 2006; Theas et al., 2008).

Most intriguingly, Fas ligand (FasL) is abundantly expressed in the meiotic and post-meiotic germ cells (D'Alessio et al., 2001). FasL-expressing cells may contribute to immune privilege by inducing apoptosis of Fas-bearing lymphocytes (Suda et al., 1993). Recent studies have revealed that some stages of spermatogenic cells express TLRs, which will be discussed in a late section of this paper. The role of male germ cells in maintaining the special testicular immune environment is emerging, and worthy of further investigation.

MOLECULAR ASPECTS OF TESTICULAR IMMUNE PRIVILEGE

The testicular cells express and secret numerous immunoregulatory molecules that play important roles in regulating immune responses in the testis (**Figure 2**). Various immunosuppressive molecules, such as androgens, programmed death ligand-1 (PD-L1), FasL, growth arrest-specific gene product 6 (Gas6), and protein S (ProS) are produced by testicular cells. To overcome immune privilege, the testis must mount appropriate local innate response against invading pathogens. TLRs in testicular cells play important roles in initiating testicular innate immune responses.

EFFECT OF HORMONES ON THE TESTICULAR IMMUNE RESPONSES

Macrophages and Leydig cells represent major immune and endocrine cells in the interstitial spaces, these two cell populations regulate each other's development (Hedger, 2002). Luteinizing hormone may control macrophage expansion in the testis during puberty and maintenance of macrophages in the adult testis by acting on Leydig cells (Raburn et al., 1993). Evidence shows that follicle-stimulating hormone regulates maturation of testicular macrophages via SCs (Duckett et al., 1997). Moreover, the testicular endocrine environment can affect other type of immune cells in the testis (Hedger and Meinhardt, 2000). The immune cells in the testis should be regulated by the function of a mature testis, rather than the direct effects of the hormones on the immune cells that lack hormone receptors (Meinhardt and Hedger, 2011).

Production of androgens (mainly testosterone) is a major function of Leydig cells. Androgens exhibit immunosuppressive activities that contribute to different immune responses between the sexes (Cutolo et al., 2004). Testosterone reduces TLR4 expression in macrophages (Rettew et al., 2008). Administration of testosterone suppresses autoimmune diseases (Cutolo, 2009; Gold and Voskuhl, 2009). Accordingly, gonadotropin-releasing hormone antagonists significantly reduce the percentage of Treg cells, and increase the proportion of NK cells in human males (Page et al., 2006). These data suggest that androgens play roles in maintaining the balance between autoimmunity and tolerance. Substantial evidence of the link between androgens and testicular immune privilege was found in investigations using mice conditional knockout androgen receptors in SCs. SC-specific deletion of the androgen receptor in mice disrupts testicular immune privilege (Meng et al., 2011), possibly because androgens regulate SC TJs (McCabe et al., 2010). In agreement with these the data above, an earlier study indicated that androgens regulate the permeability of the BTB by regulating the expression of a SC TJ protein, CLDN3 (Meng et al., 2005). Taken together, androgens play critical roles in maintaining the integrity of testicular immune privilege by regulating local microenvironments.

Fas/FasL SYSTEM

FasL-induced lymphocyte apoptosis was once thought as a critical mechanism underlying the testicular immune privilege. This concept was contradicted by different studies. FasL suppresses immune responses by inducing apoptosis of Fas-bearing activated lymphocytes (Dhein et al., 1995). The testis is a major source of FasL mRNA in rodents (Suda et al., 1993), and the Fas/FasL system was demonstrated to be important in the maintenance of testicular immune privilege based on observations that SCs expressing FasL induce apoptosis of Fas-bearing lymphocytes (Bellgrau et al., 1995; Takeda et al., 1998; **Figure 2**). This conclusion was challenged by other studies. Genetically modified islets expressing FasL did not protect the islets from immune rejection (Allison et al., 1997). Neutralizing antibodies against FasL did not significantly reduce

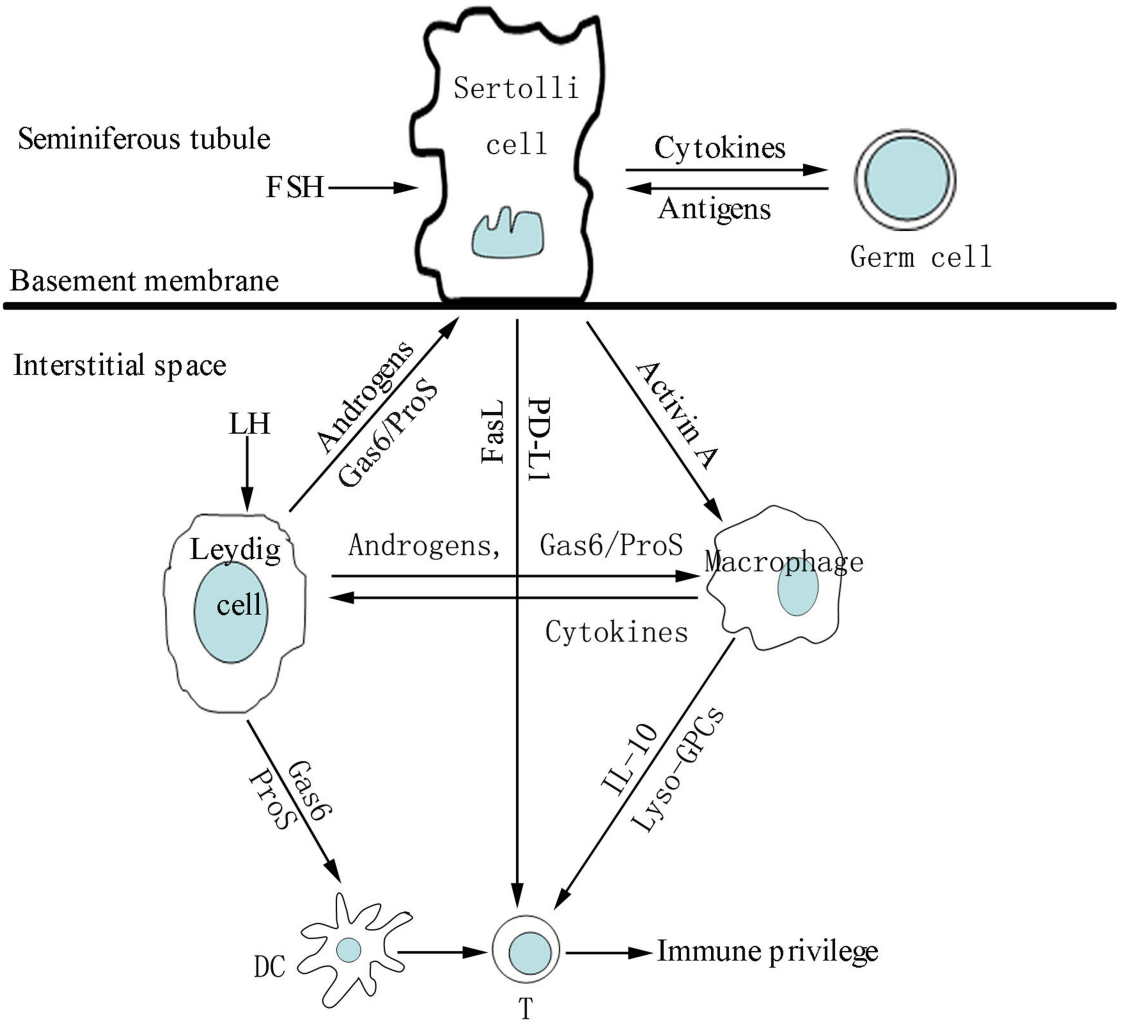

FIGURE 2 | A schematic drawing illustrating intercellular communication via paracrine immunosuppressive molecules that favor immune privilege in the testis. Under the influence of follicle-stimulating hormone (FSH), androgens and germ cell antigens, Sertoli cells secrete immunosuppressive molecules that inhibit inflammatory responses of macrophages and T lymphocytes (T) in the interstitial compartments. Sertoli, Leydig cells and macrophages regulate activities of the other immune cells including dendritic cells (DC), T and mast cells (MC) via paracrine immunosuppressive molecules, favoring testicular immune privilege property.

the survival of islets in non-obese diabetic mice that received co-grafts of SCs and islets (Korbutt et al., 2000).

In fact, convincing evidence showed that testicular FasL is expressed not in SCs but in meiotic and post-meiotic germ cells (D'Alessio et al., 2001). However, it remains to be clarified whether the germ cells expressing FasL contribute to the immune privileged status within the seminiferous tubules. Discrepant distribution of FasL as indicated in these previous studies might be caused by cell contamination and usage of non-specific antibodies. The involvement of Fas/FasL in the immunosuppressive properties of SCs should be reconsidered.

LOCAL IMMUNOSUPPRESSIVE MILIEU

While the role of Fas/FasL system in maintaining the testicular immune privilege has been in doubt, other immunosuppressive molecules have been demonstrated to play roles in suppressing immune responses in the testis. A recent study shows that lyso-glycerophosphatidylcholines (lyso-GPCs) in interstitial fluid inhibit T cell activity, contributing to testicular immunosuppression (Foulds et al., 2008). However, the mechanisms underlying the lyso-GPC-mediated immune suppression remain unclear. Programmed death receptor-1 (PD-1) is a transmembrane protein, and PD-L1 (also named B7-H1) is a functional ligand of PD-1. PD-1 is expressed in T cells, and its activation by PD-L1 mediates T cell tolerance (Keir et al., 2006). A recent study demonstrated that PD-L1 is constitutively expressed in the testis and contributes to the long-term survival of islet allografts transplanted in the testis, suggesting that the PD-1/PD-L1 system may contribute to testicular immune privilege (Cheng et al., 2009).

Another attention deserves to be paid to the role of Gas6/ProS-TAM system in maintaining testicular immune environment. TAM receptors are the latest identified subfamily of receptor

tyrosine kinases, which include three members, namely, Tyro3, Axl and Mer (TAM; Hafizi and Dahlback, 2006). Two close relative vitamin K-dependent proteins, the product of Gas6 and ProS (a blood anticoagulant cofactor), are biological ligands of TAM receptors (Hafizi and Dahlback, 2006). TAM triple knockout mice are male infertile due to progressive loss of germ cells (Lu et al., 1999; Chen et al., 2009). Gene targeting mutation studies have shown that TAM receptors are essential regulators of the immune homeostasis by regulating negatively TLR-initiated innate immune responses in DCs and macrophages (Rothlin et al., 2007; Lemke and Rothlin, 2008). We previously demonstrated that TAM receptors are abundantly expressed in mouse SCs and Leydig cells, whereas Gas6 and ProS are prominently expressed in Leydig cells (Wang et al., 2005). We also provided recently evidence that TAM signaling suppresses TLR-initiated testicular innate immune responses in SCs and Leydig cells (Sun et al., 2010; Shang et al., 2011). Based on these findings, we speculate that the Gas6/TAM system may be critical in regulating the immune privileged status of the testis. It is worthwhile to investigate this possibility.

TLR-INITIATED TESTICULAR INNATE IMMUNE RESPONSES

An immune privileged status does not indicate that the site has no effective immune response. Actually, the testis tolerates invading antigens. TLR-mediated innate immune responses in testicular cells play a critical role in the protection of the testis from infections.

Toll-like receptors are pattern recognition receptors that recognize pathogen-associated molecular patterns (PAMPs). The activation of TLRs by PAMPs represents one of the most important mechanisms of immune responses against pathogens (Takeda and Akira, 2005). TLRs can be also recognized and activated by endogenous ligands that can be released from damaged tissues and necrotic cells, which are termed damage-associated molecular patterns (DAMPs; Seong and Matzinger, 2004). The expression and significance of TLRs in the testicular functions have recently been recognized (Hedger, 2011). Various TLRs are expressed in the testis of different species including humans (Fujita et al., 2011) and murines (Bhushan et al., 2008). Most studies on TLR functions in the testis have been performed using murine models. Recent studies demonstrated that mouse SCs express functional TLR2 to TLR6 (Riccioli et al., 2006; Starace et al., 2008; Wu et al., 2008), which were confirmed in rat SCs (Bhushan et al., 2008; Winnall et al., 2011). Using isolated testicular cells, we also demonstrated *in vitro* that TLR3 and TLR4 in mouse Leydig cells, and TLR3 in spermatogenic cells trigger innate immunity in response to ligand stimulation (Shang et al., 2011; Tao et al., 2012). TLR2 and TLR4 were observed in human sperms, and responded to bacterial endotoxins (Fujita et al., 2011). The observations imply that TLRs in non-immune testicular cells may play important roles for the testis to mount appropriate local innate immune responses to invading microbial pathogens. Notably, SCs are relatively less sensitive to ultra-pure LPS or Poly(I:C) compared with macrophages based on the expression levels of inflammatory cytokines, although both cell types express comparable levels of TLR3 and TLR4. Inhibitory effect of Gas6/ProS-TAM system on TLR signaling in both SCs and Leydig cells should be responsible, at least in part, for the less sensitive to TLR stimulation. Therefore, the interplay of TLRs and TAM receptors could be important in the maintenance of immunological balance in the testis. We are collecting more evidence to confirm this hypothesis. It should be note that most of the studies on expression and function of TLRs in the testicular cells are performed *in vitro* using primary cells and purified ligands. Role of TLRs in defense against invading pathogens in the testis should be confirmed *in vivo* when animals are infected with microbes using TLR knockout mice.

PARACRINE AND ENDOCRINE CYTOKINE REGULATION OF TESTICULAR INFLAMMATION

Numerous paracrine and autocrine cytokines in the testis are critical for normal testicular functions. Most of these cytokines belong to immunoregulatory factors including those with immunosuppressive or pro-inflammatory activities, such as TGF-β family (Avallet et al., 1994; O'Bryan et al., 2005). TGF-βs contribute to testicular immune privilege through their immunosuppressive activities (Pollanen et al., 1993). TGF-β1 facilitates SCs to support graft survival in co-transplantation experiments (Suarez-Pinzon et al., 2000).

Major pro-inflammatory factors, including IL-1, IL-6, and TNF-α, are expressed in the testis and regulate testicular functions under physiological conditions. IL-1 is an important mediator of inflammation in the testis. A recent study showed that IL-1α facilitates BTB opening by affecting the actin cytoskeleton (Sarkar et al., 2008). IL-1β is feebly produced by the testis under physiological conditions. However, an upregulation of IL-1β has been observed under inflammatory conditions in the testis, which may be harmful to spermatogenesis (Guazzone et al., 2009). IL-6 promotes potently inflammatory events via the expansion and activation of T cells. Most testicular cells, including somatic cells and germ cells in normal rats, produce IL-6 (Potashnik et al., 2005). IL-6 production is upregulated in LPS-induced acute testicular inflammation model. Moreover, IL-6 expression in testicular macrophages is significantly increased in EAO (Rival et al., 2006). Circulating monocytes ($ED1^+$) that arrive at the testis notably express IL-6 at high levels compared with resident testicular macrophages ($ED2^+$), suggesting the distinct roles of the two types of macrophages in mediating inflammatory responses. An *in vitro* study has shown that exogenous IL-6 induces germ cell apoptosis (Theas et al., 2003). TNF-α is the most potent proinflammatory cytokine. TNF-α is prominently synthesized by germ cells within normal seminiferous tubules (De et al., 1993). Various types of interstitial cells, such as macrophages and mast cells, synthesize TNF-α (Xiong and Hales, 1993). TNF-α protects germ cells from apoptosis at a physiologically low concentrations in normal testes. In oppositely, TNF-α behaves as an apoptotic factor that induces germ cell death under inflammatory conditions (Theas et al., 2008). These three pro-inflammatory cytokines can be induced by TLR activation in testicular cells, including SCs, Leydig, and germ cells. Moreover, antiviral cytokines including IFN-α, -β are produced by these testis-specific cells through TLR activation (Wu et al., 2008; Sun et al., 2010; Shang et al., 2011; Wang et al., 2012). These cytokines could participate in the host defense against pathogens through the regulation of immune responses

or the direct killing of invading pathogens *in vivo*. They may also impair spermatogenesis under the inflammatory conditions.

Various chemokines may regulate immune responses in the testis. Chemokines are a large family of small cytokines with chemoattractive activities. They can be grouped into the two major subfamilies, namely, CC ligands (CCLs) and CXC ligands (CXCLs). Among the CCL subfamilies, CCL2 (MCP-1) is present in the testis at physiologically low levels (Gerdprasert et al., 2002b). MCP-1 is expressed by Leydig and MPCs and can be regulated by IL-1, TNF-α, and IFN-β. In response to TLR activation, MCP-1 is upregulated in SCs (Riccioli et al., 2006). Injection of LPS *in vivo* induces elevated MCP-1 levels in the testicular fluid of rats (Gerdprasert et al., 2002b), increased MCP-1 levels have been observed in the testicular fluid of and EAO model (Guazzone et al., 2003). Among the CXCL subfamilies, CXCL10 is expressed in rat Leydig cells and upregulated by IL-1α, TNF-α, and IFN-γ (Hu et al., 1998). In addition, Sendai viruses induce CXCL1 and CXCL10 in rat testicular macrophages, SCs, Leydig cells, and MPCs (Aubry et al., 2000). Chemokines are not detected in rat germ cells. Increased chemokines could facilitate leukocyte infiltrations and promote the inflammatory reactions in the testis.

In contrast to inflammatory cytokines, IL-10, another anti-flammatory cytokine, may reduce inflammation, autoimmunity, and spermatogenic damages in the mouse model of EAO (Watanabe et al., 2005). During inflammation, testicular resident macrophages produce IL-10. Production of immunosuppressive cytokines supports the speculation that testicular immune cells contribute to the testicular immune privileged status.

CONCLUDING REMARKS

While the testis is a remarkable immune privileged site, chronic orchitis and autoimmunity are important etiological factors of male infertility. Further investigation on the immunoregulation of the testis and the link between testicular inflammatory disorders and male infertility will have important implications for interventions of inflammatory disorder-related male infertility.

Mechanisms underlying testicular immune privilege remain largely misunderstood. A local innate immune system of the testis apparently plays an important role in the initiation of the testicular immune responses. Negative regulation of innate immunity in the testis must contribute to testicular immune privilege, which could be the focus of future research. TLR expression and function have recently been revealed in many testis-specific cells. These findings suggest that both immune cells and local tissue-specific cells possess innate immune protective roles against pathogens. Therefore, local cell-initiated inflammatory responses represent a topic that is worthy of further investigation. Endogenous TLR ligand-triggered non-infectious inflammatory conditions in the testis must be considered, because a large portion of spermatogenic cells undergo apoptosis under physiological and pathological conditions, which may release endogenous TLR ligands if the apoptotic cells are not removed timely. The manner by which systemic and local inflammations impair male fertility is another issue that remains unclear. Understanding of the fundamental aspects described above may provide novel insights into the development of prevention and treatment approaches for testicular inflammation-related male infertility.

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REFERENCES

Abe, M., Kurosawa, M., Ishikawa, O., Miyachi, Y., and Kido, H. (1998). Mast cell tryptase stimulates both human dermal fibroblast proliferation and type I collagen production. *Clin. Exp. Allergy* 28, 1509–1517.

Aguilar, R., Anton, F., Bellido, C., Aguilar, E., and Gaytan, F. (1995). Testicular serotonin is related to mast cells but not to Leydig cells in the rat. *J. Endocrinol.* 146, 15–21.

Allison, J., Georgiou, H. M., Strasser, A., and Vaux, D. L. (1997). Transgenic expression of CD95 ligand on islet beta cells induces a granulocytic infiltration but does not confer immune privilege upon islet allografts. *Proc. Natl. Acad. Sci. U.S.A.* 94, 3943–3947.

Andre, S., Tough, D. F., Lacroix-Desmazes, S., Kaveri, S. V., and Bayry, J. (2009). Surveillance of antigen-presenting cells by CD4+ CD25+ regulatory T cells in autoimmunity: immunopathogenesis and therapeutic implications. *Am. J. Pathol.* 174, 1575–1587.

Apa, D. D., Cayan, S., Polat, A., and Akbay, E. (2002). Mast cells and fibrosis on testicular biopsies in male infertility. *Arch. Androl.* 48, 337–344.

Aubry, F., Habasque, C., Satie, A. P., Jegou, B., and Samson, M. (2000). Expression and regulation of the CXC-chemokines, GRO/KC and IP-10/mob-1 in rat seminiferous tubules. *Eur. Cytokine Netw.* 11, 690–698.

Avallet, O., Vigier, M., Leduque, P., Dubois, P. M., and Saez, J. M. (1994). Expression and regulation of transforming growth factor-beta 1 messenger ribonucleic acid and protein in cultured porcine Leydig and Sertoli cells. *Endocrinology* 134, 2079–2087.

Banchereau, J., and Steinman, R. M. (1998). Dendritic cells and the control of immunity. *Nature* 392, 245–252.

Barker, C. F., and Billingham, R. E. (1977). Immunologically privileged sites. *Adv. Immunol.* 25, 1–54.

Bellgrau, D., Gold, D., Selawry, H., Moore, J., Franzusoff, A., and Duke, R. C. (1995). A role for CD95 ligand in preventing graft rejection. *Nature* 377, 630–632.

Bhushan, S., Tchatalbachev, S., Klug, J., Fijak, M., Pineau, C., Chakraborty, T., and Meinhardt, A. (2008). Uropathogenic Escherichia coli block MyD88-dependent and activate MyD88-independent signaling pathways in rat testicular cells. *J. Immunol.* 180, 5537–5547.

Biggiogera, M., Tanguay, R. M., Marin, R., Wu, Y., Martin, T. E., and Fakan, S. (1996). Localization of heat shock proteins in mouse male germ cells: an immunoelectron microscopical study. *Exp. Cell Res.* 229, 77–85.

Breucker, H. (1978). Macrophages, a normal component in seasonally involuting testes of the swan, *Cygnus olor*. *Cell Tissue Res.* 193, 463–471.

Brinster, R. L. (2002). Germline stem cell transplantation and transgenesis. *Science* 296, 2174–2176.

Chen, Y., Wang, H., Qi, N., Wu, H., Xiong, W., Ma, J., Lu, Q., and Han, D. (2009). Functions of TAM RTKs in regulating spermatogenesis and male fertility in mice. *Reproduction* 138, 655–666.

Cheng, X., Dai, H., Wan, N., Moore, Y., Vankayalapati, R., and Dai, Z. (2009). Interaction of programmed death-1 and programmed death-1 ligand-1 contributes to testicular immune privilege. *Transplantation* 87, 1778–1786.

Cohen, P. E., Nishimura, K., Zhu, L., and Pollard, J. W. (1999). Macrophages: important accessory cells for reproductive function. *J. Leukoc. Biol.* 66, 765–772.

Curtin, J. F., Liu, N., Candolfi, M., Xiong, W., Assi, H., Yagiz, K., Edwards, M. R., Michelsen, K. S., Kroeger, K. M., Liu, C., Muhammad, A. K., Clark, M. C., Arditi, M., Comin-Anduix, B., Ribas, A., Lowenstein, P. R., and Castro, M. G. (2009). HMGB1 mediates endogenous TLR2 activation and brain tumor regression. *PLoS Med.* 6, e10. doi: 10.1371/journal.pmed.1000010

Cutolo, M. (2009). Androgens in rheumatoid arthritis: when are they effectors? *Arthritis Res. Ther.* 11, 126.

Cutolo, M., Sulli, A., Capellino, S., Villaggio, B., Montagna, P., Seriolo, B., and Straub, R. H. (2004). Sex hormones influence on the immune system: basic and clinical

aspects in autoimmunity. *Lupus* 13, 635–638.

D'Alessio, A., Riccioli, A., Lauretti, P., Padula, F., Muciaccia, B., De Cesaris, P., Filippini, A., Nagata, S., and Ziparo, E. (2001). Testicular FasL is expressed by sperm cells. *Proc. Natl. Acad. Sci. U.S.A.* 98, 3316–3321.

Dai, Z., Nasr, I. W., Reel, M., Deng, S., Diggs, L., Larsen, C. P., Rothstein, D. M., and Lakkis, F. G. (2005). Impaired recall of CD8 memory T cells in immunologically privileged tissue. *J. Immunol.* 174, 1165–1170.

De, S. K., Chen, H. L., Pace, J. L., Hunt, J. S., Terranova, P. F., and Enders, G. C. (1993). Expression of tumor necrosis factor-alpha in mouse spermatogenic cells. *Endocrinology* 133, 389–396.

De Cesaris, P., Filippini, A., Cervelli, C., Riccioli, A., Muci, S., Starace, G., Stefanini, M., and Ziparo, E. (1992). Immunosuppressive molecules produced by Sertoli cells cultured *in vitro*: biological effects on lymphocytes. *Biochem. Biophys. Res. Commun.* 186, 1639–1646.

Dejucq, N., Lienard, M. O., Guillaume, E., Dorval, I., and Jegou, B. (1998). Expression of interferons-alpha and -gamma in testicular interstitial tissue and spermatogonia of the rat. *Endocrinology* 139, 3081–3087.

Dhein, J., Walczak, H., Baumler, C., Debatin, K. M., and Krammer, P. H. (1995). Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). *Nature* 373, 438–441.

Diemer, T., Hales, D. B., and Weidner, W. (2003). Immune-endocrine interactions and Leydig cell function: the role of cytokines. *Andrologia* 35, 55–63.

Duckett, R. J., Hedger, M. P., Mclachlan, R. I., and Wreford, N. G. (1997). The effects of gonadotropin-releasing hormone immunization and recombinant follicle-stimulating hormone on the Leydig cell and macrophage populations of the adult rat testis. *J. Androl.* 18, 417–423.

el-Demiry, M. I., Hargreave, T. B., Busuttil, A., Elton, R., James, K., and Chisholm, G. D. (1987). Immunocompetent cells in human testis in health and disease. *Fertil. Steril.* 48, 470–479.

Fijak, M., Bhushan, S., and Meinhardt, A. (2011). Immunoprivileged sites: the testis. *Methods Mol. Biol.* 677, 459–470.

Fijak, M., Iosub, R., Schneider, E., Linder, M., Respondek, K., Klug, J., and Meinhardt, A. (2005). Identification of immunodominant autoantigens in rat autoimmune orchitis. *J. Pathol.* 207, 127–138.

Fijak, M., and Meinhardt, A. (2006). The testis in immune privilege. *Immunol. Rev.* 213, 66–81.

Foulds, L. M., Boysen, R. I., Crane, M., Yang, Y., Muir, J. A., Smith, A. I., De Kretser, D. M., Hearn, M. T., and Hedger, M. P. (2008). Molecular identification of lysoglycerophosphocholines as endogenous immunosuppressives in bovine and rat gonadal fluids. *Biol. Reprod.* 79, 525–536.

Frungieri, M. B., Calandra, R. S., Lustig, L., Meineke, V., Kohn, F. M., Vogt, H. J., and Mayerhofer, A. (2002a). Number, distribution pattern, and identification of macrophages in the testes of infertile men. *Fertil. Steril.* 78, 298–306.

Frungieri, M. B., Weidinger, S., Meineke, V., Köhn, F. M., and Mayerhofer, A. (2002b). Proliferative action of mast-cell tryptase is mediated by PAR2, COX2, prostaglandins, and PPARgamma: possible relevance to human fibrotic disorders. *Proc. Natl. Acad. Sci. U.S.A.* 99, 15072–15077.

Fujita, Y., Mihara, T., Okazaki, T., Shitanaka, M., Kushino, R., Ikeda, C., Negishi, H., Liu, Z., Richards, J. S., and Shimada, M. (2011). Toll-like receptors (TLR) 2 and 4 on human sperm recognize bacterial endotoxins and mediate apoptosis. *Hum. Reprod.* 26, 2799–2806.

Gaytan, F., Bellido, C., Aguilar, E., and Van Rooijen, N. (1994). Requirement for testicular macrophages in Leydig cell proliferation and differentiation during prepubertal development in rats. *J. Reprod. Fertil.* 102, 393–399.

Gerdprasert, O., O'Bryan, M. K., Muir, J. A., Caldwell, A. M., Schlatt, S., De Kretser, D. M., and Hedger, M. P. (2002a). The response of testicular leukocytes to lipopolysaccharide-induced inflammation: further evidence for heterogeneity of the testicular macrophage population. *Cell Tissue Res.* 308, 277–285.

Gerdprasert, O., O'Bryan, M. K., Nikolic-Paterson, D. J., Sebire, K., De Kretser, D. M., and Hedger, M. P. (2002b). Expression of monocyte chemoattractant protein-1 and macrophage colony-stimulating factor in normal and inflamed rat testis. *Mol. Hum. Reprod.* 8, 518–524.

Gold, S. M., and Voskuhl, R. R. (2009). Estrogen and testosterone therapies in multiple sclerosis. *Prog. Brain Res.* 175, 239–251.

Greenberg, S., and Grinstein, S. (2002). Phagocytosis and innate immunity. *Curr. Opin. Immunol.* 14, 136–145.

Guazzone, V. A., Jacobo, P., Theas, M. S., and Lustig, L. (2009). Cytokines and chemokines in testicular inflammation: a brief review. *Microsc. Res. Tech.* 72, 620–628.

Guazzone, V. A., Rival, C., Denduchis, B., and Lustig, L. (2003). Monocyte chemoattractant protein-1 (MCP-1/CCL2) in experimental autoimmune orchitis. *J. Reprod. Immunol.* 60, 143–157.

Hafizi, S., and Dahlback, B. (2006). Signalling and functional diversity within the Axl subfamily of receptor tyrosine kinases. *Cytokine Growth Factor Rev.* 17, 295–304.

Haugen, T. B., Landmark, B. F., Josefsen, G. M., Hansson, V., and Hogset, A. (1994). The mature form of interleukin-1 alpha is constitutively expressed in immature male germ cells from rat. *Mol. Cell. Endocrinol.* 105, R19–23.

Head, J. R., and Billingham, R. E. (1985). Immune privilege in the testis. II. Evaluation of potential local factors. *Transplantation* 40, 269–275.

Head, J. R., Neaves, W. B., and Billingham, R. E. (1983). Immune privilege in the testis. I. Basic parameters of allograft survival. *Transplantation* 36, 423–431.

Hedger, M. P. (2002). Macrophages and the immune responsiveness of the testis. *J. Reprod. Immunol.* 57, 19–34.

Hedger, M. P. (2011). Toll-like receptors and signalling in spermatogenesis and testicular responses to inflammation – a perspective. *J. Reprod. Immunol.* 88, 130–141.

Hedger, M. P., and Meinhardt, A. (2000). Local regulation of T cell numbers and lymphocyte-inhibiting activity in the interstitial tissue of the adult rat testis. *J. Reprod. Immunol.* 48, 69–80.

Hu, J., You, S., Li, W., Wang, D., Nagpal, M. L., Mi, Y., Liang, P., and Lin, T. (1998). Expression and regulation of interferon-gamma-inducible protein 10 gene in rat Leydig cells. *Endocrinology* 139, 3637–3645.

Hussein, M. R., Abou-Deif, E. S., Bedaiwy, M. A., Said, T. M., Mustafa, M. G., Nada, E., Ezat, A., and Agarwal, A. (2005). Phenotypic characterization of the immune and mast cell infiltrates in the human testis shows normal and abnormal spermatogenesis. *Fertil. Steril.* 83, 1447–1453.

Hutson, J. C. (2006). Physiologic interactions between macrophages and Leydig cells. *Exp. Biol. Med. (Maywood)* 231, 1–7.

Iosub, R., Klug, J., Fijak, M., Schneider, E., Frohlich, S., Blumbach, K., Wennemuth, G., Sommerhoff, C. P., Steinhoff, M., and Meinhardt, A. (2006). Development of testicular inflammation in the rat involves activation of proteinase-activated receptor-2. *J. Pathol.* 208, 686–698.

Itoh, M., Terayama, H., Naito, M., Ogawa, Y., and Tainosho, S. (2005). Tissue microcircumstances for leukocytic infiltration into the testis and epididymis in mice. *J. Reprod. Immunol.* 67, 57–67.

Jacobo, P., Guazzone, V. A., Jarazo-Dietrich, S., Theas, M. S., and Lustig, L. (2009). Differential changes in CD4+ and CD8+ effector and regulatory T lymphocyte subsets in the testis of rats undergoing autoimmune orchitis. *J. Reprod. Immunol.* 81, 44–54.

Jarow, J. P., Wright, W. W., Brown, T. R., Yan, X., and Zirkin, B. R. (2005). Bioactivity of androgens within the testes and serum of normal men. *J. Androl.* 26, 343–348.

Keir, M. E., Liang, S. C., Guleria, I., Latchman, Y. E., Qipo, A., Albacker, L. A., Koulmanda, M., Freeman, G. J., Sayegh, M. H., and Sharpe, A. H. (2006). Tissue expression of PD-L1 mediates peripheral T cell tolerance. *J. Exp. Med.* 203, 883–895.

Kern, S., and Maddocks, S. (1995). Indomethacin blocks the immunosuppressive activity of rat testicular macrophages cultured *in vitro*. *J. Reprod. Immunol.* 28, 189–201.

Kern, S., Robertson, S. A., Mau, V. J., and Maddocks, S. (1995). Cytokine secretion by macrophages in the rat testis. *Biol. Reprod.* 53, 1407–1416.

Korbutt, G. S., Suarez-Pinzon, W. L., Power, R. F., Rajotte, R. V., and Rabinovitch, A. (2000). Testicular Sertoli cells exert both protective and destructive effects on syngeneic islet grafts in non-obese diabetic mice. *Diabetologia* 43, 474–480.

Kuopio, T., Savouras, P. O., Pelliniemi, L. J., and Huhtaniemi, I. T. (1989). Transplantation of newborn rat testis under the kidney capsule of adult host as a model to study the structure and function of Leydig cells. *J. Androl.* 10, 335–345.

Le Tortorec, A., Denis, H., Satie, A. P., Patard, J. J., Ruffault, A., Jegou, B., and Dejucq-Rainsford, N. (2008). Antiviral responses of human Leydig cells to mumps virus infection or poly I:C stimulation. *Hum. Reprod.* 23, 2095–2103.

Lemke, G., and Rothlin, C. V. (2008). Immunobiology of the TAM receptors. *Nat. Rev. Immunol.* 8, 327–336.

Lu, Q., Gore, M., Zhang, Q., Camenisch, T., Boast, S., Casagranda, F., Lai, C., Skinner, M. K., Klein, R., Matsushima, G. K., Earp, H. S., Goff, S. P., and Lemke, G. (1999). Tyro-3 family receptors are essential regulators of

mammalian spermatogenesis. *Nature* 398, 723–728.

Lustig, L., Lourtau, L., Perez, R., and Doncel, G. F. (1993). Phenotypic characterization of lymphocytic cell infiltrates into the testes of rats undergoing autoimmune orchitis. *Int. J. Androl.* 16, 279–284.

Ma, P., Ge, Y., Wang, S., Ma, J., Xue, S., and Han, D. (2004). Spermatogenesis following syngeneic testicular transplantation in Balb/c mice. *Reproduction* 128, 163–170.

Maekawa, M., Kamimura, K., and Nagano, T. (1996). Peritubular myoid cells in the testis: their structure and function. *Arch. Histol. Cytol.* 59, 1–13.

McCabe, M. J., Tarulli, G. A., Meachem, S. J., Robertson, D. M., Smooker, P. M., and Stanton, P. G. (2010). Gonadotropins regulate rat testicular tight junctions *in vivo*. *Endocrinology* 151, 2911–2922.

Meinhardt, A., Bacher, M., Metz, C., Bucala, R., Wreford, N., Lan, H., Atkins, R., and Hedger, M. (1998). Local regulation of macrophage subsets in the adult rat testis: examination of the roles of the seminiferous tubules, testosterone, and macrophage-migration inhibitory factor. *Biol. Reprod.* 59, 371–378.

Meinhardt, A., and Hedger, M. P. (2011). Immunological, paracrine and endocrine aspects of testicular immune privilege. *Mol. Cell. Endocrinol.* 335, 60–68.

Melaine, N., Lienard, M. O., Guillaume, E., Ruffault, A., Dejucq-Rainsford, N., and Jegou, B. (2003). Production of the antiviral proteins 2′5′oligoadenylate synthetase, PKR and Mx in interstitial cells and spermatogonia. *J. Reprod. Immunol.* 59, 53–60.

Mellor, A. L., and Munn, D. H. (2006). Immune privilege: a recurrent theme in immunoregulation. *Immunol. Rev.* 213, 5–11.

Meng, J., Greenlee, A. R., Taub, C. J., and Braun, R. E. (2011). Sertoli cell-specific deletion of the androgen receptor compromises testicular immune privilege in mice. *Biol. Reprod.* 85, 254–260.

Meng, J., Holdcraft, R. W., Shima, J. E., Griswold, M. D., and Braun, R. E. (2005). Androgens regulate the permeability of the blood–testis barrier. *Proc. Natl. Acad. Sci. U.S.A.* 102, 16696–16700.

Millar, D. G., Garza, K. M., Odermatt, B., Elford, A. R., Ono, N., Li, Z., and Ohashi, P. S. (2003). Hsp70 promotes antigen-presenting cell function and converts T-cell tolerance to autoimmunity *in vivo*. *Nat. Med.* 9, 1469–1476.

Mital, P., Hinton, B. T., and Dufour, J. M. (2011). The blood–testis and blood–epididymis barriers are more than just their tight junctions. *Biol. Reprod.* 84, 851–858.

Nakanishi, Y., and Shiratsuchi, A. (2004). Phagocytic removal of apoptotic spermatogenic cells by Sertoli cells: mechanisms and consequences. *Biol. Pharm. Bull.* 27, 13–16.

Nasr, I. W., Wang, Y., Gao, G., Deng, S., Diggs, L., Rothstein, D. M., Tellides, G., Lakkis, F. G., and Dai, Z. (2005). Testicular immune privilege promotes transplantation tolerance by altering the balance between memory and regulatory T cells. *J. Immunol.* 174, 6161–6168.

O'Bryan, M. K., Gerdprasert, O., Nikolic-Paterson, D. J., Meinhardt, A., Muir, J. A., Foulds, L. M., Phillips, D. J., De Kretser, D. M., and Hedger, M. P. (2005). Cytokine profiles in the testes of rats treated with lipopolysaccharide reveal localized suppression of inflammatory responses. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288, R1744–R1755.

Page, S. T., Plymate, S. R., Bremner, W. J., Matsumoto, A. M., Hess, D. L., Lin, D. W., Amory, J. K., Nelson, P. S., and Wu, J. D. (2006). Effect of medical castration on CD4+ CD25+ T cells, CD8+ T cell IFN-gamma expression, and NK cells: a physiological role for testosterone and/or its metabolites. *Am. J. Physiol. Endocrinol. Metab.* 290, E856–E863.

Pelletier, R. M., Yoon, S. R., Akpovi, C. D., Silvas, E., and Vitale, M. L. (2009). Defects in the regulatory clearance mechanisms favor the breakdown of self-tolerance during spontaneous autoimmune orchitis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, R743–R762.

Pentikainen, V., Erkkila, K., Suomalainen, L., Otala, M., Pentikainen, M. O., Parvinen, M., and Dunkel, L. (2001). TNFalpha down-regulates the Fas ligand and inhibits germ cell apoptosis in the human testis. *J. Clin. Endocrinol. Metab.* 86, 4480–4488.

Piccinini, A. M., and Midwood, K. S. (2010). DAMPening inflammation by modulating TLR signalling. *Mediat. Inflamm.* 2010, 672395.

Pollanen, P., Von Euler, M., Jahnukainen, K., Saari, T., Parvinen, M., Sainio-Pollanen, S., and Soder, O. (1993). Role of transforming growth factor beta in testicular immunosuppression. *J. Reprod. Immunol.* 24, 123–137.

Potashnik, H., Elhija, M. A., Lunenfeld, E., Potashnik, G., Schlatt, S., Nieschlag, E., and Huleihel, M. (2005). Interleukin-6 expression during normal maturation of the mouse testis. *Eur. Cytokine Netw.* 16, 161–165.

Raburn, D. J., Coquelin, A., Reinhart, A. J., and Hutson, J. C. (1993). Regulation of the macrophage population in postnatal rat testis. *J. Reprod. Immunol.* 24, 139–151.

Rettew, J. A., Huet-Hudson, Y. M., and Marriott, I. (2008). Testosterone reduces macrophage expression in the mouse of toll-like receptor 4, a trigger for inflammation and innate immunity. *Biol. Reprod.* 78, 432–437.

Riccioli, A., Starace, D., Galli, R., Fuso, A., Scarpa, S., Palombi, F., De Cesaris, P., Ziparo, E., and Filippini, A. (2006). Sertoli cells initiate testicular innate immune responses through TLR activation. *J. Immunol.* 177, 7122–7130.

Rival, C., Theas, M. S., Guazzone, V. A., and Lustig, L. (2006). Interleukin-6 and IL-6 receptor cell expression in testis of rats with autoimmune orchitis. *J. Reprod. Immunol.* 70, 43–58.

Rival, C., Theas, M. S., Suescun, M. O., Jacobo, P., Guazzone, V., Van Rooijen, N., and Lustig, L. (2008). Functional and phenotypic characteristics of testicular macrophages in experimental autoimmune orchitis. *J. Pathol.* 215, 108–117.

Rothlin, C. V., Ghosh, S., Zuniga, E. I., Oldstone, M. B., and Lemke, G. (2007). TAM receptors are pleiotropic inhibitors of the innate immune response. *Cell* 131, 1124–1136.

Samy, E. T., Parker, L. A., Sharp, C. P., and Tung, K. S. (2005). Continuous control of autoimmune disease by antigen-dependent polyclonal CD4+CD25+ regulatory T cells in the regional lymph node. *J. Exp. Med.* 202, 771–781.

Sanberg, P. R., Borlongan, C. V., Saporta, S., and Cameron, D. F. (1996). Testis-derived Sertoli cells survive and provide localized immunoprotection for xenografts in rat brain. *Nat. Biotechnol.* 14, 1692–1695.

Sanchez, J. F., Crooks, D. R., Lee, C. T., Schoen, C. J., Amable, R., Zeng, X., Florival-Victor, T., Morales, N., Truckenmiller, M. E., Smith, D. R., and Freed, W. J. (2006). GABAergic lineage differentiation of AF5 neural progenitor cells *in vitro*. *Cell Tissue Res.* 324, 1–8.

Sarkar, O., Mathur, P. P., Cheng, C. Y., and Mruk, D. D. (2008). Interleukin 1 alpha (IL1A) is a novel regulator of the blood–testis barrier in the rat. *Biol. Reprod.* 78, 445–454.

Schell, C., Albrecht, M., Mayer, C., Schwarzer, J. U., Frungieri, M. B., and Mayerhofer, A. (2008). Exploring human testicular peritubular cells: identification of secretory products and regulation by tumor necrosis factor-alpha. *Endocrinology* 149, 1678–1686.

Schuppe, H. C., and Meinhardt, A. (2005). Immune privilege and inflammation of the testis. *Chem. Immunol. Allergy* 88, 1–14.

Schuppe, H. C., Meinhardt, A., Allam, J. P., Bergmann, M., Weidner, W., and Haidl, G. (2008). Chronic orchitis: a neglected cause of male infertility? *Andrologia* 40, 84–91.

Scott, H. M., Hutchison, G. R., Mahood, I. K., Hallmark, N., Welsh, M., De Gendt, K., Verhoeven, G., O'shaughnessy, P., and Sharpe, R. M. (2007). Role of androgens in fetal testis development and dysgenesis. *Endocrinology* 148, 2027–2036.

Selawry, H. P., and Cameron, D. F. (1993). Sertoli cell-enriched fractions in successful islet cell transplantation. *Cell Transplant.* 2, 123–129.

Seong, S. Y., and Matzinger, P. (2004). Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. *Nat. Rev. Immunol.* 4, 469–478.

Setchell, B. P. (1990). The testis and tissue transplantation: historical aspects. *J. Reprod. Immunol.* 18, 1–8.

Setchell, B. P., Granholm, T., and Ritzen, E. M. (1995). Failure of thyroid allografts to function in the testes of cynomolgous monkeys. *J. Reprod. Immunol.* 28, 75–80.

Shang, T., Zhang, X., Wang, T., Sun, B., Deng, T., and Han, D. (2011). Toll-like receptor-initiated testicular innate immune responses in mouse Leydig cells. *Endocrinology* 152, 2827–2836.

Simpson, E. (2006). A historical perspective on immunological privilege. *Immunol. Rev.* 213, 12–22.

Starace, D., Galli, R., Paone, A., De Cesaris, P., Filippini, A., Ziparo, E., and Riccioli, A. (2008). Toll-like receptor 3 activation induces antiviral immune responses in mouse sertoli cells. *Biol. Reprod.* 79, 766–775.

Statter, M. B., Foglia, R. P., Parks, D. E., and Donahoe, P. K. (1988). Fetal and postnatal testis shows immunoprivilege as donor tissue. *J. Urol.* 139, 204–210.

Su, L., Mruk, D. D., and Cheng, C. Y. (2011). Drug transporters, the blood–testis barrier, and spermatogenesis. *J. Endocrinol.* 208, 207–223.

Suarez-Pinzon, W., Korbutt, G. S., Power, R., Hooton, J., Rajotte, R. V.,

and Rabinovitch, A. (2000). Testicular sertoli cells protect islet beta-cells from autoimmune destruction in NOD mice by a transforming growth factor-beta1-dependent mechanism. *Diabetes* 49, 1810–1818.

Suda, T., Takahashi, T., Golstein, P., and Nagata, S. (1993). Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. *Cell* 75, 1169–1178.

Sun, B., Qi, N., Shang, T., Wu, H., Deng, T., and Han, D. (2010). Sertoli cell-initiated testicular innate immune response through toll-like receptor-3 activation is negatively regulated by Tyro3, Axl, and mer receptors. *Endocrinology* 151, 2886–2897.

Takeda, K., and Akira, S. (2005). Toll-like receptors in innate immunity. *Int. Immunol.* 17, 1–14.

Takeda, Y., Gotoh, M., Dono, K., Nishihara, M., Grochowiecki, T., Kimura, F., Yoshida, T., Ohta, Y., Ota, H., Ohzato, H., Umeshita, K., Takeda, T., Matsuura, N., Sakon, M., Kayagaki, N., Yagita, H., Okumura, K., Miyasaka, M., and Monden, M. (1998). Protection of islet allografts transplanted together with Fas ligand expressing testicular allografts. *Diabetologia* 41, 315–321.

Tao, W., Xiaoyan, Z., Qiaoyuan, C., Tingting, D., Yue, Z., Nan, L., Tao, S., Yongmei, C., and Daishu, H. (2012). Toll-like receptor 3-initiated antiviral responses in mouse male germ cells *in vitro*. *Biol. Reprod.* 86, 1–10.

Theas, M. S., Rival, C., Jarazo-Dietrich, S., Jacobo, P., Guazzone, V. A., and Lustig, L. (2008). Tumour necrosis factor-alpha released by testicular macrophages induces apoptosis of germ cells in autoimmune orchitis. *Hum. Reprod.* 23, 1865–1872.

Theas, S., Rival, C., and Lustig, L. (2003). Germ cell apoptosis in autoimmune orchitis: involvement of the Fas-FasL system. *Am. J. Reprod. Immunol.* 50, 166–176.

Tung, K. S., Teuscher, C., and Meng, A. L. (1981). Autoimmunity to spermatozoa and the testis. *Immunol. Rev.* 55, 217–255.

Vabulas, R. M., Ahmad-Nejad, P., Da Costa, C., Miethke, T., Kirschning, C. J., Hacker, H., and Wagner, H. (2001). Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. *J. Biol. Chem.* 276, 31332–31339.

Verhoeven, G., Hoeben, E., and De Gendt, K. (2000). Peritubular cell-Sertoli cell interactions: factors involved in PmodS activity. *Andrologia* 32, 42–45.

Wang, H., Chen, Y., Ge, Y., Ma, P., Ma, Q., Ma, J., Xue, S., and Han, D. (2005). Immunoexpression of Tyro 3 family receptors – Tyro 3, Axl, and Mer – and their ligand Gas6 in postnatal developing mouse testis. *J. Histochem. Cytochem.* 53, 1355–1364.

Wang, T., Zhang, X., Chen, Q., Deng, T., Zhang, Y., Li, N., Shang, T., Chen, Y., and Han, D. (2012). Toll-like receptor 3-initiated antiviral responses in mouse male germ cells *in vitro*. *Biol. Reprod.* 86, 1–10.

Watanabe, M., Kashiwakura, Y., Kusumi, N., Tamayose, K., Nasu, Y., Nagai, A., Shimada, T., Daida, H., and Kumon, H. (2005). Adeno-associated virus-mediated human IL-10 gene transfer suppresses the development of experimental autoimmune orchitis. *Gene Ther.* 12, 1126–1132.

Wheeler, D. S., Chase, M. A., Senft, A. P., Poynter, S. E., Wong, H. R., and Page, K. (2009). Extracellular Hsp72, an endogenous DAMP, is released by virally infected airway epithelial cells and activates neutrophils via Toll-like receptor (TLR)-4. *Respir. Res.* 10, 31.

Wheeler, K., Tardif, S., Rival, C., Luu, B., Bui, E., Del Rio, R., Teuscher, C., Sparwasser, T., Hardy, D., and Tung, K. S. (2011). Regulatory T cells control tolerogenic versus autoimmune response to sperm in vasectomy. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7511–7516.

Winnall, W. R., Muir, J. A., and Hedger, M. P. (2011). Differential responses of epithelial Sertoli cells of the rat testis to Toll-like receptor 2 and 4 ligands: implications for studies of testicular inflammation using bacterial lipopolysaccharides. *Innate Immun.* 17, 123–136.

Wu, H., Wang, H., Xiong, W., Chen, S., Tang, H., and Han, D. (2008). Expression patterns and functions of toll-like receptors in mouse sertoli cells. *Endocrinology* 149, 4402–4412.

Wyatt, C. R., Law, L., Magnuson, J. A., Griswold, M. D., and Magnuson, N. S. (1988). Suppression of lymphocyte proliferation by proteins secreted by cultured Sertoli cells. *J. Reprod. Immunol.* 14, 27–40.

Xiong, Y., and Hales, D. B. (1993). Expression, regulation, and production of tumor necrosis factor-alpha in mouse testicular interstitial macrophages *in vitro*. *Endocrinology* 133, 2568–2573.

Yule, T. D., Montoya, G. D., Russell, L. D., Williams, T. M., and Tung, K. S. (1988). Autoantigenic germ cells exist outside the blood testis barrier. *J. Immunol.* 141, 1161–1167.

Zetterstrom, C. K., Strand, M. L., and Soder, O. (2006). The high mobility group box chromosomal protein 1 is expressed in the human and rat testis where it may function as an antibacterial factor. *Hum. Reprod.* 21, 2801–2809.

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A new paradigm for an old story: the role of regulatory B cells in cancer

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A common feature between cancer escape and autoimmune diseases is an inappropriate involvement of the regulatory immune system, albeit for opposing purposes. While autoimmune disease is a reflection of the failure to control responses to self, cancer is a result of an exaggerated use of these controls to abrogate antitumor effector responses. Although the importance of regulatory B cells [Bregs, the definition first used by Mizoguchi to describe B cells exerting protection from colitis in mice (Mizoguchi et al., 1997)] in protection from autoimmunity is now accepted, their involvement in cancer escape remains poorly understood. The conundrum of Bregs is that, if their numbers are low (in analogy with Tregs), their existence and importance may be concealed by the overwhelming response of effector B cells. For example, aberrant activation of B cells promotes autoimmune diseases, such as rheumatoid arthritis (RA), type 1 diabetes mellitus (T1D), multiple sclerosis (MS), and systemic lupus erythematosus (SLE). As such, the depletion of B cells with anti-CD20 antibody rituximab impairs antigen-specific $CD4^+$ T cell activation (Bouaziz et al., 2007) and ameliorates RA, MS, and T1D (Townsend et al., 2010). Yet, treatment with rituximab can also exacerbate the disease in some patients with ulcerative colitis, or even induce other diseases, such as psoriasis with psoriatic arthropathy and colitis in patients with Graves disease and non-Hodgkin lymphoma, respectively (Dass et al., 2007; Goetz et al., 2007; Mielke et al., 2008). The increased numbers of B cells in peripheral blood of transplant patients is positively associated with a rare but long-term drug-free clinical tolerance (Newell et al., 2010; Pallier et al., 2010; Sagoo et al., 2010). Although these clinical examples clearly indicate the importance of B cells, a current issue is how to segregate the role of Bregs from suppressive activity of B cells that has been known for more than 30 years. As first proposed by Morris and Moller in late 1960s (Morris and Moller, 1968), B cell-produced immunoglobulin can elicit immune suppression by directly triggering ITIM-mediated suppressive signaling in target cells upon binding with inhibitory FcγRIIB (Ravetch and Bolland, 2001) or by indirectly modulating dendritic cells (DCs) via activating FcγR (Morris and Moller, 1968).

The first evidence of suppressive B cells (Bregs?) that functioned independently of their immunoglobulin was shown by Shimamura et al. (1982) about 30 years ago. Confirming this, the absence of B cells was linked with exacerbated autoimmune responses in mice deficient in B cells, such as mice that lack mature B cells (Wolf et al., 1996) and CD19 B cells (Yanaba et al., 2008). To date, the protection from autoimmune diseases in mice was linked with several unique subsets of IL-10-producing Bregs, such as $CD1d^{High}$ B1b cells ($CD5^-$ $B220^{Low}$ $CD11b^+$ IgM^+ $CD1d^{High}$; Mizoguchi et al., 2002), B10 regulatory cells (IL-10-producing $CD1d^{High}$ $CD5^+$ B cells; Yanaba et al., 2008), and $CD1d^{High}$ Tim-1^+ $CD5^+$ Bregs (Ding et al., 2011). Although little is known about human Bregs, protection from SLE was recently linked with an impairment of regulatory activity of $CD19^+$ $CD24^{High}$ $CD38^{High}$ B cells (Blair et al., 2010). Moreover, a rare subset of IL-10-producing memory $CD24^{hi}$ $CD27^+$ B cells that functions like murine B10 cells was also shown to exist in humans (Iwata et al., 2011). Humans also have IL-10 and TGFβ-producing $CD25^{hi}$ $CD27^{hi}$ $CD86^{hi}$ $CD1d^{hi}$ B cells that can suppress proliferation of autologous T cells and induce the generation of $Foxp3^+$ CTLA-4^+ Tregs (Kessel et al., 2012).

The majority of protective effects of Bregs requires IL-10 (Mizoguchi et al., 2002; Byrne and Halliday, 2005; Matsushita et al., 2008; Yanaba et al., 2008; Blair et al., 2010), a cytokine also utilized in other B cell-mediated suppression. For example, IL-10 is also abundantly produced and utilized by $CD5^+$ B1 cells and MZ B cells to ameliorate collagen-induced arthritis in mice (O'Garra and Howard, 1992; Brummel and Lenert, 2005; Lenert et al., 2005; Evans et al., 2007) and by LPS-stimulated B cells to protect from autoimmune responses in mice by rendering T cells anergic (Parekh et al., 2003; Lampropoulou et al., 2008) and tolerogenic (Fuchs and Matzinger, 1992). The boundaries between Bregs and IL-10 producing B cells can often be obscure, raising question whether IL-10 is a primary mediator of suppressive activity or a factor that promotes homeostasis of Bregs. As for murine and human B1 cells (Balabanian et al., 2002; Gary-Gouy et al., 2002), IL-10 may promote survival and proliferation of Bregs. On the other hand, full suppressive power of Bregs and concomitant IL-10 production often requires activation, for example, by chronic inflammation or by engagement of their toll-like receptors (TLRs) or CD40 (Mizoguchi et al., 2002; Gray et al., 2007; Lampropoulou et al., 2008). This leads to production of other immunomodulatory factors (TGFβ and galectin-1) and upregulation of surface antigens, such as PD-1 and CTLA-4. As a result, activated Bregs can either directly induce apoptosis and anergy of effector Th1 cells and $CD8^+$ T cells (Zuniga et al., 2001; Parekh et al., 2003; Frommer et al., 2008; Tretter et al., 2008) or indirectly by converting Tregs (Reichardt et al., 2007; Sun et al., 2008; Sayi et al., 2011; Scapini et al., 2011) and modulating DCs (Byrne and Halliday, 2005; Watt et al., 2007).

Although cancer often uses homeostatic regulatory machinery to escape from immune surveillance, surprisingly the process seems does not involve Bregs that protect from autoimmune diseases. As such, the role of Bregs in cancer escape is poorly appreciated. Instead, B cells are mostly known for their "pathogenic" antitumor properties (Lanzavecchia, 1985; Candolfi et al., 2011). The presence of $CD20^+$ B cells in metastatic lymph nodes is a sign of favorable outcome in patients with head and neck cancer (Pretscher et al., 2009); and depletion of CD20-expressing B cells increases tumor burden in the lungs of mice intravenously injected with B16-F10 melanoma after (Sorrentino et al., 2011). Despite this, B cells also participate in carcinogenesis of methylcholanthrene-induced (Brodt and Gordon, 1978, 1982) or transplanted tumors (Monach et al., 1993); and syngeneic tumors progress poorly in μMT mice deficient in B cells unless replenished with $B220^+$ B cells (Qin et al., 1998; Olkhanud et al., 2011). Cancer-promoting B cells appear to exert a multitude of functions, such as production of immunoglobulins and cytokines (Townsend et al., 2010). As in autoimmunity, the immunoglobulin deposition induces FcR- and complement-mediated chronic inflammation needed for carcinogenesis (Zusman et al., 1996; de Visser et al., 2005). Activated B cells produce TGFβ (Parekh et al., 2003; Lampropoulou et al., 2008), and immunoglobulin can serve as a carrier for TGFβ and thereby mediate suppression of cellular immune responses (Stach and Rowley, 1993; Rowley and Stach, 1998). Tumor-infiltrating B cells also produce lymphotoxin α/β and promote androgen-independent growth of prostate cancer cells by inducing the nuclear translocation of IKKα and activation of STAT3 (Ammirante et al., 2010). Pro-tumorigenic activity of B cells also requires production of IL-10 (Inoue et al., 2006) and TNFα (Schioppa et al., 2011) to presumably mediate Th2 polarization and inhibition of the cytotoxic activity of $CD8^+$ T and NK cells. Importantly, B cells isolated from tumor-bearing mice inhibit $CD4^+$ T cell-mediated help for CTLs (Qin et al., 1998).

To date, there are only two clearly defined examples of cancer escape-promoting Bregs are reported. First, murine B10 cells can abrogate monocyte activity and reduce surface expression of FcγR in IL-10-dependent fashion (Horikawa et al., 2011). As a result, the presence of B10 cells inhibits the therapeutic efficacy of anti-CD20 antibody against lymphoma. On the other hand, we recently discovered a unique subset of tumor-evoked Bregs (tBregs) that actively facilitates breast cancer escape and metastasis in BALB/C mice bearing 4T1 carcinoma cells (Olkhanud et al., 2011). In fact, the cancer cells themselves induce the generation of TGFβ-producing tBregs from normal B cells. As a result, tBreg then convert non-Treg $CD4^+$ T cells into metastasis-promoting $FoxP3^+$ Tregs (Olkhanud et al., 2011), which in turn inactivate antitumor NK cells and protect metastasizing cancer cells in the lungs (Olkhanud et al., 2009). We believe that the tBreg-like cells also exist in humans, as they can be readily generated *ex vivo* by treating normal human donor B cells with conditioned media of human cancer lines, such as breast, ovarian, and colon carcinomas (Olkhanud et al., 2011). tBregs differ phenotypically and functionally from other Bregs involved in autoimmune responses (Mizoguchi et al., 2002; Matsushita et al., 2008; Yanaba et al., 2008) and LPS- or BCR-activated B cells (Fuchs and Matzinger, 1992; Hussain and Delovitch, 2007). tBregs resemble B2 cells (IgD^{High}) but express constitutively active Stat3 and surface markers like $CD25^{High}$ $B7\text{-}H1^{High}$ $CD81^{High}$ $CD86^{High}$ $CCR6^{High}$ and $CD62L^{Low}$ $IgM^{Int/Low}$ and poorly proliferate. They do not express CD27 and CD5 or up regulate CD1d, and their suppressive activity does not require IL-10 or other known suppressive pathways, such as B7-H1-PD-1, Fas-FasL, and IL27/IL35. Treatment with *S. aureus* Cowan 1 antigen can also generate suppressive $CD25^+$ B cells that induce anergy of activated T cells by competing for IL-2 (Tretter et al., 2008). However, unlike them, tBregs regulate both resting and activated T cells (both $CD4^+$ and $CD8^+$ T cells) acting independently of IL-2 and without inducing cell death.

Since cancer actively converts tBregs from normal B cells, the clinical implication of this is that, as long as cancer persists, it will induce their generation and thereby initiate the chain of suppressive events. Thus, strategies that abrogate any step of this process are expected to inhibit cancer escape and metastasis, a primary cause of patients' bad disease outcome. However, the success of a strategy will also depend on the use of tailored approaches, ideally, ones that only inactivate tBregs, while protecting or promoting "good" B cells needed for optimal cancer eradication. For example, 4T1 breast cancer metastasis is abrogated by antibody that targets IL2Rα expressed on Tregs and tBregs (Olkhanud et al., 2009, 2011). Despite this, no clinical benefit was elicited in patients with renal cell carcinoma treated with B cell-depleting anti-CD20 antibody rituximab (Aklilu et al., 2004). Although this result questions the role of Bregs in human cancers, our recent data indicate that tBregs can escape anti-CD20 antibody due to low levels of CD20 expression. As a result, treatment with anti-CD20 antibody preferentially depletes "good" and activated B cells, while enriching for tBregs and thereby enhancing cancer escape and metastasis (Bodogai et al., MS in preparation). Overall, although plethora of conventional B cells can often conceal and hamper analysis of small population of Bregs, the use of tailored and unique methodologies clearly indicates their existence and importance in mediation of cancer escape. It is time to unequivocally accept Bregs and tBregs as true members of the regulatory immune network.

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REFERENCES

Aklilu, M., Stadler, W. M., Markiewicz, M., Vogelzang, N. J., Mahowald, M., Johnson, M., and Gajewski, T. F. (2004). Depletion of normal B cells with rituximab as an adjunct to IL-2 therapy for renal cell carcinoma and melanoma. *Ann. Oncol.* 15, 1109–1114.

Ammirante, M., Luo, J. L., Grivennikov, S., Nedospasov, S., and Karin, M. (2010). B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. *Nature* 464, 302–305.

Balabanian, K., Foussat, A., Bouchet-Delbos, L., Couderc, J., Krzysiek, R., Amara, A., Baleux, F., Portier, A., Galanaud, P., and Emilie, D. (2002). Interleukin-10 modulates the sensitivity of peritoneal B lymphocytes to chemokines with opposite effects on stromal cell-derived factor-1 and B-lymphocyte chemoattractant. *Blood* 99, 427–436.

Blair, P. A., Norena, L. Y., Flores-Borja, F., Rawlings, D. J., Isenberg, D. A., Ehrenstein, M. R., and Mauri, C. (2010). CD19(+) CD24(hi) CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. *Immunity* 32, 129–140.

Bouaziz, J. D., Yanaba, K., Venturi, G. M., Wang, Y., Tisch, R. M., Poe, J. C., and Tedder, T. F. (2007). Therapeutic B cell depletion impairs adaptive and autoreactive CD4+ T cell activation in mice. *Proc. Natl. Acad. Sci. U.S.A.* 104, 20878–20883.

Brodt, P., and Gordon, J. (1978). Anti-tumor immunity in B lymphocyte-deprived mice. I. Immunity to a chemically induced tumor. *J. Immunol.* 121, 359–362.

Brodt, P., and Gordon, J. (1982). Natural resistance mechanisms may play a role in protection against chemical carcinogenesis. *Cancer Immunol. Immunother.* 13, 125–127.

Brummel, R., and Lenert, P. (2005). Activation of marginal zone B cells from lupus mice with type A(D) CpG-oligodeoxynucleotides. *J. Immunol.* 174, 2429–2434.

Byrne, S. N., and Halliday, G. M. (2005). B cells activated in lymph nodes in response to ultraviolet irradiation or by interleukin-10 inhibit dendritic cell induction of immunity. *J. Invest. Dermatol.* 124, 570–578.

Candolfi, M., Curtin, J. F., Yagiz, K., Assi, H., Wibowo, M. K., Alzadeh, G. E., Foulad, D., Muhammad, A. K., Salehi, S., Keech, N., Puntel, M., Liu, C., Sanderson, N. R., Kroeger, K. M., Dunn, R., Martins, G., Lowenstein, P. R., and Castro, M. G. (2011). B cells are critical to T-cell-mediated antitumor immunity induced by a combined immune-stimulatory/conditionally cytotoxic therapy for glioblastoma. *Neoplasia* 13, 947–960.

Dass, S., Vital, E. M., and Emery, P. (2007). Development of psoriasis after B cell depletion with rituximab. *Arthritis Rheum.* 56, 2715–2718.

de Visser, K. E., Korets, L. V., and Coussens, L. M. (2005). De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. *Cancer Cell* 7, 411–423.

Ding, Q., Yeung, M., Camirand, G., Zeng, Q., Akiba, H., Yagita, H., Chalasani, G., Sayegh, M. H., Najafian, N., and Rothstein, D. M. (2011). Regulatory B cells are identified by expression of TIM-1 and can be induced through TIM-1 ligation to promote tolerance in mice. *J. Clin. Invest.* 121, 3645–3656.

Evans, J. G., Chavez-Rueda, K. A., Eddaoudi, A., Meyer-Bahlburg, A., Rawlings, D. J., Ehrenstein, M. R., and Mauri, C. (2007). Novel suppressive function of transitional 2 B cells in experimental arthritis. *J. Immunol.* 178, 7868–7878.

Frommer, F., Heinen, T. J., Wunderlich, F. T., Yogev, N., Buch, T., Roers, A., Bettelli, E., Müller, W., Anderton, S. M., and Waisman, A. (2008). Tolerance without clonal expansion: self-antigen-expressing B cells program self-reactive T cells for future deletion. *J. Immunol.* 181, 5748–5759.

Fuchs, E. J., and Matzinger, P. (1992). B cells turn off virgin but not memory T cells. *Science* 258, 1156–1159.

Gary-Gouy, H., Harriague, J., Bismuth, G., Platzer, C., Schmitt, C., and Dalloul, A. H. (2002). Human CD5 promotes B-cell survival through stimulation of autocrine IL-10 production. *Blood* 100, 4537–4543.

Goetz, M., Atreya, R., Ghalibafian, M., Galle, P. R., and Neurath, M. F. (2007). Exacerbation of ulcerative colitis after rituximab salvage therapy. *Inflamm. Bowel Dis.* 13, 1365–1368.

Gray, M., Miles, K., Salter, D., Gray, D., and Savill, J. (2007). Apoptotic cells protect mice from autoimmune inflammation by the induction of regulatory B cells. *Proc. Natl. Acad. Sci. U.S.A.* 104, 14080–14085.

Horikawa, M., Minard-Colin, V., Matsushita, T., and Tedder, T. F. (2011). Regulatory B cell production of IL-10 inhibits lymphoma depletion during CD20 immunotherapy in mice. *J. Clin. Invest.* 121, 4268–4280.

Hussain, S., and Delovitch, T. L. (2007). Intravenous transfusion of BCR-activated B cells protects NOD mice from type 1 diabetes in an IL-10-dependent manner. *J. Immunol.* 179, 7225–7232.

Inoue, S., Leitner, W. W., Golding, B., and Scott, D. (2006). Inhibitory effects of B cells on antitumor immunity. *Cancer Res.* 66, 7741–7747.

Iwata, Y., Matsushita, T., Horikawa, M., Dilillo, D. J., Yanaba, K., Venturi, G. M., Szabolcs, P. M., Bernstein, S. H., Magro, C. M., Williams, A. D., Hall, R. P., St Clair, E. W., and Tedder, T. F. (2011). Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. *Blood* 117, 530–541.

Kessel, A., Haj, T., Peri, R., Snir, A., Melamed, D., Sabo, E., and Toubi, E. (2012). Human CD19(+) CD25(high) B regulatory cells suppress proliferation of CD4(+) T cells and enhance Foxp3 and CTLA-4 expression in T-regulatory cells. *Autoimmun. Rev.* 11, 670–677.

Lampropoulou, V., Hoehlig, K., Roch, T., Neves, P., Calderon Gomez, E., Sweenie, C. H., Hao, Y., Freitas, A. A., Steinhoff, U., Anderton, S. M., and Fillatreau, S. (2008). TLR-activated B cells suppress T cell-mediated autoimmunity. *J. Immunol.* 180, 4763–4773.

Lanzavecchia, A. (1985). Antigen-specific interaction between T and B cells. *Nature* 314, 537–539.

Lenert, P., Brummel, R., Field, E. H., and Ashman, R. F. (2005). TLR-9 activation of marginal zone B cells in lupus mice regulates immunity through increased IL-10 production. *J. Clin. Immunol.* 25, 29–40.

Matsushita, T., Yanaba, K., Bouaziz, J. D., Fujimoto, M., and Tedder, T. F. (2008). Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. *J. Clin. Invest.* 118, 3420–3430.

Mielke, F., Schneider-Obermeyer, J., and Dorner, T. (2008). Onset of psoriasis with psoriatic arthropathy during rituximab treatment of non-Hodgkin lymphoma. *Ann. Rheum. Dis.* 67, 1056–1057.

Mizoguchi, A., Mizoguchi, E., Smith, R. N., Preffer, F. I., and Bhan, A. K. (1997). Suppressive role of B cells in chronic colitis of T cell receptor alpha mutant mice. *J. Exp. Med.* 186, 1749–1756.

Mizoguchi, A., Mizoguchi, E., Takedatsu, H., Blumberg, R. S., and Bhan, A. K. (2002). Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. *Immunity* 16, 219–230.

Monach, P. A., Schreiber, H., and Rowley, D. A. (1993). CD4+ and B lymphocytes in transplantation immunity. II. Augmented rejection of tumor allografts by mice lacking B cells. *Transplantation* 55, 1356–1361.

Morris, A., and Moller, G. (1968). Regulation of cellular antibody synthesis effect of adoptively transferred antibody-producing spleen cells on cellular antibody synthesis. *J. Immunol.* 101, 439–445.

Newell, K. A., Asare, A., Kirk, A. D., Gisler, T. D., Bourcier, K., Suthanthiran, M., Burlingham, W. J., Marks, W. H., Sanz, I., Lechler, R. I., Hernandez-Fuentes, M. P., Turka, L. A., Seyfert-Margolis, V. L., and Immune Tolerance Network ST507 Study Group. (2010). Identification of a B cell signature associated with renal transplant tolerance in humans. *J. Clin. Invest.* 120, 1836–1847.

O'Garra, A., and Howard, M. (1992). Cytokines and Ly-1 (B1) B cells. *Int. Rev. Immunol.* 8, 219–234.

Olkhanud, P. B., Baatar, D., Bodogai, M., Hakim, F., Gress, R., Anderson, R. L., Deng, J., Xu, M., Briest, S., and Biragyn, A. (2009). Breast cancer lung metastasis requires expression of chemokine receptor CCR4 and regulatory T cells. *Cancer Res.* 69, 5996–6004.

Olkhanud, P. B., Damdinsuren, B., Bodogai, M., Gress, R. E., Sen, R., Wejksza, K., Malchinkhuu, E., Wersto, R. P., and Biragyn, A. (2011). Tumor-evoked regulatory B cells promote breast cancer metastasis by converting resting CD4 T cells to T-regulatory cells. *Cancer Res.* 71, 3505–3515.

Pallier, A., Hillion, S., Danger, R., Giral, M., Racape, M., Degauque, N., Dugast, E., Ashton-Chess, J., Pettré, S., Lozano, J. J., Bataille, R., Devys, A., Cesbron-Gautier, A., Braudeau, C., Larrose, C., Soulillou, J. P., and Brouard, S. (2010). Patients with drug-free long-term graft function display increased numbers of peripheral B cells with a memory and inhibitory phenotype. *Kidney Int.* 78, 503–513.

Parekh, V. V., Prasad, D. V., Banerjee, P. P., Joshi, B. N., Kumar, A., and Mishra, G. C. (2003). B cells activated by lipopolysaccharide, but not by anti-Ig and anti-CD40 antibody, induce anergy in CD8+ T cells: role of TGF-beta 1. *J. Immunol.* 170, 5897–5911.

Pretscher, D., Distel, L. V., Grabenbauer, G. G., Wittlinger, M., Buettner, M., and Niedobitek, G. (2009). Distribution of immune cells in head and neck cancer: CD8+ T-cells and CD20+ B-cells in metastatic lymph nodes are associated with favourable outcome in patients with oro- and hypopharyngeal carcinoma. *BMC Cancer* 9, 292. doi: 10.1186/1471-2407-9-292

Qin, Z., Richter, G., Schuler, T., Ibe, S., Cao, X., and Blankenstein, T. (1998). B cells inhibit induction of T cell-dependent tumor immunity. *Nat. Med.* 4, 627–630.

Ravetch, J. V., and Bolland, S. (2001). IgG Fc receptors. *Annu. Rev. Immunol.* 19, 275–290.

Reichardt, P., Dornbach, B., Rong, S., Beissert, S., Gueler, F., Loser, K., and Gunzer, M. (2007). Naive B cells generate regulatory T cells in the presence of a mature immunologic synapse. *Blood* 110, 1519–1529.

Rowley, D. A., and Stach, R. M. (1998). B lymphocytes secreting IgG linked to latent transforming growth factor-beta prevent primary cytolytic T lymphocyte responses. *Int. Immunol.* 10, 355–363.

Sagoo, P., Perucha, E., Sawitzki, B., Tomiuk, S., Stephens, D. A., Miqueu, P., Chapman, S., Craciun, L., Sergeant, R., Brouard, S., Rovis, F., Jimenez, E., Ballow, A., Giral, M., Rebollo-Mesa, I., Le Moine, A., Braudeau, C., Hilton, R., Gerstmayer, B., Bourcier, K., Sharif, A., Krajewska, M., Lord, G. M., Roberts, I., Goldman, M., Wood, K. J., Newell, K., Seyfert-Margolis, V., Warrens, A. N., Janssen, U., Volk, H. D., Soulillou, J. P., Hernandez-Fuentes, M. P., and Lechler, R. I. (2010). Development of a cross-platform biomarker signature to detect renal transplant tolerance in humans. *J. Clin. Invest.* 120, 1848–1861.

Sayi, A., Kohler, E., Toller, I. M., Flavell, R. A., Muller, W., Roers, A., and Müller, A. (2011). TLR-2-activated B cells suppress *Helicobacter*-induced preneoplastic gastric immunopathology by inducing T regulatory-1 cells. *J. Immunol.* 186, 878–890.

Scapini, P., Lamagna, C., Hu, Y., Lee, K., Tang, Q., DeFranco, A. L., and Lowell, C. A. (2011). B cell-derived IL-10 suppresses inflammatory disease in Lyn-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 108, E823–E832.

Schioppa, T., Moore, R., Thompson, R. G., Rosser, E. C., Kulbe, H., Nedospasov, S., Mauri, C., Coussens, L. M., and Balkwill, F. R. (2011). B regulatory cells and the tumor-promoting actions of TNF-alpha during squamous carcinogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 108, 10662–10667.

Shimamura, T., Hashimoto, K., and Sasaki, S. (1982). Feedback suppression of the immune response in vivo. I. Immune B cells induce antigen-specific suppressor T cells. *Cell. Immunol.* 68, 104–113.

Sorrentino, R., Morello, S., Forte, G., Montinaro, A., de Vita, G., Luciano, A., Palma, G., Arra, C., Maiolino, P., Adcock, I. M., and Pinto, A. (2011). B cells contribute to the antitumor activity of CpG-oligodeoxynucleotide in a mouse model of metastatic lung carcinoma. *Am. J. Respir. Crit. Care Med.* 183, 1369–1379.

Stach, R. M., and Rowley, D. A. (1993). A first or dominant immunization. II. Induced immunoglobulin carries transforming growth factor beta and suppresses cytolytic T cell responses to unrelated alloantigens. *J. Exp. Med.* 178, 841–852.

Sun, J. B., Flach, C. F., Czerkinsky, C., and Holmgren, J. (2008). B lymphocytes promote expansion of regulatory T cells in oral tolerance: powerful induction by antigen coupled to cholera toxin B subunit. *J. Immunol.* 181, 8278–8287.

Townsend, M. J., Monroe, J. G., and Chan, A. C. (2010). B-cell targeted therapies in human autoimmune diseases: an updated perspective. *Immunol. Rev.* 237, 264–283.

Tretter, T., Venigalla, R. K., Eckstein, V., Saffrich, R., Sertel, S., Ho, A. D., and Lorenz, H. M. (2008). Induction of CD4+ T-cell anergy and apoptosis by activated human B cells. *Blood* 112, 4555–4564.

Watt, V., Ronchese, F., and Ritchie, D. (2007). Resting B cells suppress tumor immunity via an MHC class-II dependent mechanism. *J. Immunother.* 30, 323–332.

Wolf, S. D., Dittel, B. N., Hardardottir, F., and Janeway, C. A. Jr. (1996). Experimental autoimmune encephalomyelitis induction in genetically B cell-deficient mice. *J. Exp. Med.* 184, 2271–2278.

Yanaba, K., Bouaziz, J. D., Haas, K. M., Poe, J. C., Fujimoto, M., and Tedder, T. F. (2008). A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses. *Immunity* 28, 639–650.

Zuniga, E., Rabinovich, G. A., Iglesias, M. M., and Gruppi, A. (2001). Regulated expression of galectin-1 during B-cell activation and implications for T-cell apoptosis. *J. Leukoc. Biol.* 70, 73–79.

Zusman, T., Lisansky, E., Arons, E., Anavi, R., Bonnerot, C., Sautes, C., Fridman, W. H., Witz, I. P., and Ran, M. (1996). Contribution of the intracellular domain of murine Fc-gamma receptor type IIB1 to its tumor-enhancing potential. *Int. J. Cancer* 68, 219–227.

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Altered tryptophan metabolism as a paradigm for good and bad aspects of immune privilege in chronic inflammatory diseases

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The term "immune privilege" was coined to describe weak immunogenicity (hypo-immunity) that manifests in some transplant settings. We extended this concept to encompass hypo-immunity that manifests at local sites of inflammation relevant to clinical diseases. Here, we focus on emerging evidence that enhanced tryptophan catabolism is a key metabolic process that promotes and sustains induced immune privilege, and discuss the implications for exploiting this knowledge to improve treatments for hypo-immune and hyper-immune syndromes using strategies to manipulate tryptophan metabolism.

Keywords: IDO, inflammation, immunity, regulation, tolerance

INTRODUCTION

Immune privilege refers to the phenomenon of low immunogenicity associated with certain organs such as the anterior chamber of the eye, the brain, the testes, and the placenta (Munn and Mellor, 2006). Mechanistically, immune privilege probably arises from the combined effects of physical barriers to immunity and active immune regulatory processes that are particularly potent in some organs and tissues. The concept of immune privilege can also be extended to some chronic inflammatory syndromes where enhanced immune regulation (*hypo-immunity*) manifests as a factor in disease progression such as persistent infections and cancer (Mellor and Munn, 2008). In this sense, immune privilege is not a unique feature of certain specialized tissues; rather, it is a functional state induced by certain conditions in most – perhaps all – tissues as one consequence of chronic inflammation. In this review we focus on a specific molecular pathway that creates induced immune privilege in a range of disease settings involving expression of the inducible enzyme indoleamine 2,3 dioxygenase (IDO). When biochemically active the IDO enzyme catabolizes tryptophan to produce metabolites known collectively as kynurenines. Local metabolic effects of IDO-expressing cells exert profound effects on immune and tissue cells that suppress pro-inflammatory and immune stimulatory responses to a variety of insults. IDO is not the only molecular pathway able to create immune privilege in inflamed tissues, and several other metabolic pathways have been linked to regulatory outcomes recently. By focusing exclusively on the IDO pathway our aim is to use this particular metabolic pathway to illustrate "good" and "bad" aspects of immune privilege in relation to clinical disease syndromes.

A CONCEPTUAL MODEL OF INDUCED IMMUNE PRIVILEGE

A diverse range of primary insults stimulate local inflammation, including wounding, infections, pre-malignancies, radiation, vaccines, and chemical or biological toxins. However, the mucosal surfaces of the respiratory, gastro-intestinal-tracts, and reproductive tracts (especially the uterus during pregnancy) exhibit many features of inflamed tissues during homeostasis. The ability of insults to elicit potent innate and adaptive immunity varies considerably depending on which tissue experiences the primary insult. In this review we focus on natural and experimentally induced insults that stimulate inflammatory responses with potent immune regulatory components as examples of where induced immune privilege manifests. Previously, we proposed a model to explain how IDO enzyme activity induced in local tissues as a response to inflammatory cues may create immune privilege (Mellor and Munn, 2008). We proposed four key checkpoints that promote and maintain immune privilege in local inflamed tissues. In this section, we further develop this model in light of recent novel insights (**Figure 1**).

CHECKPOINT A

Innate immune responses to inflammatory insults

Typically, microbial infections induce rapid release of pro-inflammatory cytokines such as interferons (IFNαβ, IFNγ), tumor necrosis factor (TNFα), interleukins (IL-1β, IL-6, etc.), and chemokines by stromal cells and innate immune cells to provoke effective adaptive immunity. However some inflammatory stimuli – including some microbes – elicit innate immune responses that promote production of regulatory cytokines such as IL-10,

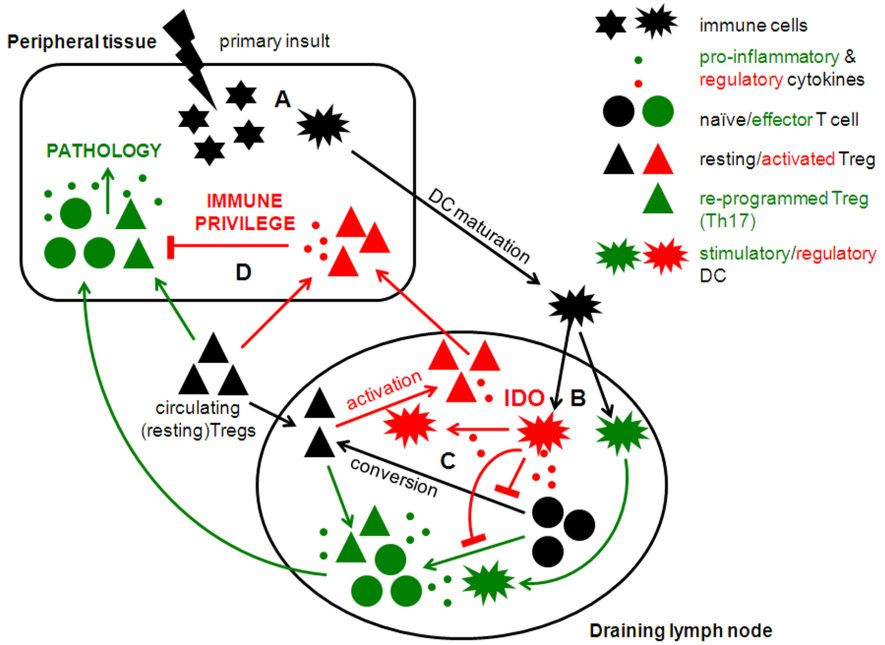

FIGURE 1 | Potential regulatory checkpoints involved in induced immune privilege in local tissue microenvironments.

and transforming growth factor (TGFβ) that suppress local release of pro-inflammatory cytokines and activate (or attract) immune regulatory cells. Thus initial local responses to primary insults exert profound influences on the subsequent course of immune responses, and elucidating how cells resident in primary tissue lesions respond to inflammatory cues is pivotal to understand the source of immune privilege. For example, pre-malignancies arise frequently in many tissues, but their ability to progress to form tumors is acquired over long periods, and overt tumors develop only when immune surveillance mechanisms are evaded successfully. Similar considerations may apply to pathogens that cause some persistent infections, though pathogens have probably evolved numerous mechanisms to evade – and even actively suppress – innate immune surveillance mechanisms to induce immune privilege.

In light of the considerations above it is probably no coincidence that tumors and chronic infections are often associated with induced local IDO expression because IDO has potent regulatory effects on immune and non-immune cell types (Uyttenhove et al., 2003; Boasso, 2011;Makala et al., 2011). Ablating the IDO1 gene in mice also enhanced their resistance to tumor formation in the standard of inflammation-dependent, carcinogen-induced (DMBA/TPA) cancer model (Banerjee et al., 2008). The unrelated enzyme tryptophan dioxygenase (TDO) is also expressed by some tumors and, like IDO catalyzes oxidative tryptophan catabolism to produce kynurenines (Opitz et al., 2011). Thus pre-malignant cells expressing IDO or TDO may create immune privilege via (a) tryptophan depletion to activate the general control non-derepressible-2 (GCN2) dependent integrated stress response (ISR) to amino acid withdrawal, and (b) kynurenine that binds to the aryl hydrocarbon receptor (AhR). Early induction of these (and perhaps other) metabolic processes may be critical steps in disease progression leading to the hypo-immune syndromes that characterize tumor growth and chronic infections. In this regard it may be significant that UV-irradiation (UVB) creates skin edema and associated inflammatory responses that include local IDO induction (unpublished data). Interestingly, skin tanning in response to UVB is mediated by the AhR (Jux et al., 2011), suggesting that exposure to UVB irradiation has both desirable *and* detrimental effects due to altered tryptophan metabolism. If these notions are correct early treatments to block immune regulatory processes may be effective in reducing the risk of contracting cancer and chronic infections. However, it is important to stress that IDO may serve dual functions as (a) an immune regulatory pathway that benefits developing tumors and pathogens, or (b) a host defense mechanism that impedes tumor growth and microbial infections to the benefit of hosts. This is a key consideration as the dominant effects of IDO at sites of inflammation may not be clear from simple observation, and must be determined by appropriate manipulation of experimental systems. For example, IDO is a frequent component of chronic inflammation associated with autoimmune destruction of healthy tissues, a correlation that could be interpreted as evidence that IDO promotes autoimmune pathology. However, exposure to IDO inhibitors accelerates autoimmune progression and potentiates disease severity in many autoimmune disease models, revealing that IDO regulates destructive autoimmunity in these syndromes. A helpful analogy to bear in mind is that firefighters are always in attendance at fires, but their presence does not guarantee that fires will eventually be brought under control to prevent total destruction. In summary, pre-malignancies and pathogens may exploit innate "host" regulatory mechanisms such as IDO to create local conditions that permit these agents

of disease to evade immune surveillance during the early phases of tumor development and infections. This paradigm implies that IDO-mediated regulation that creates immune privilege may be a key factor in disease progression, and not simply a target for therapy to break immune privilege in patients with established tumors and chronic infections. If correct, treatments that target the IDO pathway may reduce the risk that pre-malignancies and persistent pathogens establish immune privilege before they can create pathologic disease. However, such interventions may also interfere with homeostatic control of immunity to self-antigens and innocuous antigens such as allergens, and it remains to be determined if appropriate balances between hyper-immunity and prevention of diseases due to hypo-immunity can be engineered through early therapeutic interventions in individuals with high risk profiles.

CHECKPOINT B

Antigen presentation and lymphocyte activation in local draining lymph nodes

Presenting antigens to lymphocytes is necessary, but may not be sufficient to provoke adaptive immunity. Antigens in the local inflammatory lesion, usually skin or a mucosal surface, are captured by resident tissue macrophages (Mϕs) and dendritic cells (DCs) via phagocytosis or pinocytosis. DCs are specialized to undergo rapid maturation in response to innate inflammatory cues (generated at Checkpoint A), and migrate to local draining lymph nodes (dLNs) where they present processed antigens, in the form of peptides bound to surface MHC molecules, to T cells. Hence mature DCs are "professional antigen presenting cells" (APCs) that present antigens from (a) external sources such as pathogens or innocuous substances (food, allergens commensal microbes), and (b) tissue (self) antigens to T cells. T cells that recognize MHC/peptides in dLNs then undergo rapid activation and differentiation to generate helper T cells that promote optimal cellular and humoral responses, and effector (cytolytic) T cells. The processes of acquiring, transporting, and presenting antigens to T cells in dLNs may be subject to regulation in some settings of inflammation. Inhibiting DC maturation or migration would impede the process of generating helper/effector T cells. Moreover, immature DCs that enter dLNs may present antigens (signal 1) in the context of sub-optimal B7-CD28 co-stimulation (signal 2) leading to weak and ineffective T cell responses (Hackstein and Thomson, 2004). Even fully mature DCs may suppress effector responses if they acquire regulatory attributes that promote anergy or apoptosis in T cells that respond to antigens they present, and regulatory DCs may also induce naïve T cells to convert into regulatory T cells (Tregs; Reis e Sousa, 2006). Negative co-stimulatory pathways (e.g., PD-1/PD-L, ICO/ICOS-L) and certain metabolic processes (see below) may also attenuate effector responses and promote tolerance in dLNs. For example, DCs expressing IDO possess mature phenotypes but block effector T cell responses, promote CD4 T cell conversion into Foxp3-lineage Tregs, and activate pre-formed Tregs (Mellor et al., 2004, 2005; Munn et al., 2004;Baban et al., 2005, 2009, 2011;Sharma et al., 2007; Chen et al., 2008; Brenk et al., 2009;Chung et al., 2009). Thus DCs competent to express IDO or other regulatory pathways in response to appropriate cues may be pivotal in shaping adaptive immune responses to primary insults contingent on whether they are – or are not – induced to acquire regulatory phenotypes in particular settings of inflammation.

CHECKPOINT C

Prevailing regulatory conditions in draining LNs

Lymphoid tissues exhibit differential capacities to promote and support adaptive immunity. Thus skin dLNs typically support robust adaptive immune responses to a range of topical insults (though not all, see below), while LNs draining mucosal surfaces generally support weak adaptive immune responses due to prevailing physiologic conditions at these sites (Kraal et al., 2006). Thus, even if tissue DCs mature efficiently, and migrate in large numbers from primary lesions to dLNs their ability to promote effective adaptive immune responses to antigens they present is critically dependent on the prevailing physiologic status of dLNs. Some factors and pathways discussed in Checkpoint B may also help establish and sustain regulatory conditions that prevail in some lymphoid tissues, and regulatory cytokines such as TGFβ may be critical to maintain such conditions during homeostasis. The constitutive presence of certain cytokines may dictate the local "immunologic tone" in homeostatic tissues, but they may also reflect local cellular states pre-determined during homeostasis. For example, immunologic tone may be pre-determined by the relative proportions of pre-formed Tregs in a given lymphoid tissue, which in turn affects the ability of APCs to present antigens in a regulatory or stimulatory context through differential "licensing" of APCs by Tregs or helper T cells. As discussed below, the presence or absence of DCs expressing IDO may be a pivotal determinant of prevailing immunologic tone in lymphoid tissues.

CHECKPOINT D

Induced immune regulation in inflamed target tissues

Responses to primary insults elaborated via local dLNs may include regulatory components that impact what happens in target tissues affected by the primary insult. Over time, certain cell types with immune regulatory attributes may accumulate in primary lesions to dampen down initial pro-inflammatory responses. Indeed, delayed suppression of overt immunity is considered essential to attenuate induced immune responses as local infections are brought under control and pathogens are cleared from primary lesions. Several cell types possessing a variety of regulatory pathways may help down-regulate immune responses, including (but probably not limited to) lymphocytes, myeloid cells (Mϕs, DCs), natural killer (NK) cells, mast cells, and enigmatic myeloid-derived suppressor cells (MDSCs) and mesenchymal stem cells found in many settings of inflammation where hypo-immunity manifests (Ding et al., 2010; De Vries et al., 2011; Mantovani et al., 2011; Murphy et al., 2011;Ostrand-Rosenberg et al., 2012). In transplant settings tolerogenic factors that protect allografted tissues are complex, involving immunologic and metabolic processes required on a continuous basis, as disrupting these mechanisms usually leads to graft rejection even after prolonged periods of allograft acceptance (Cobbold, 2010). Thus regulatory cells and factors such as cytokines and IDO that promote immune regulation may be essential to maintain hypo-immunity following adaptive immune responses as well as mediating early responses

that promote hypo-immunity (Checkpoint A). It remains to be seen if similar processes promote and maintain immune privilege in inflamed tissues.

IDO BIOCHEMISTRY AND METABOLIC EFFECTS ON IMMUNE CELLS

IDO is a highly conserved, heme-containing intracellular enzyme that catabolizes compounds containing indole rings such as the essential amino acid tryptophan. Two closely linked, homologous IDO genes (IDO1, IDO2) are located in syntenic regions of chromosome 8 in humans and mice. IDO1 and IDO2 may respond to distinct signals since their patterns of gene expression are not identical. IDO1 encodes functional IDO protein that regulates T cell responses when expressed in some DCs while IDO2 is expressed in other cell types. Unlike IDO genes, TDO gene expression is controlled by stress factors such as glucocorticoids, not inflammatory IFNs. Here we focus on the role of IDO1 gene expression immune regulation. Though IDO is expressed by few cell types during homeostasis a number of cell types mediate IDO enzyme activity in response to inflammatory insults including stromal, hematopoietic, and tumor cells. Interferons type I (IFNαβ) and II (IFNγ) are potent IDO inducers due to the presence of interferon stimulated response element (ISRE) and interferon-gamma activated sequence (GAS) elements in the proximal gene promoters of mammalian IDO genes (Dai and Gupta, 1990; Chon et al., 1995). Other molecular signals from cytokines (TGFβ, IL-10), Toll-like receptor (TLRs), B7, CD200, GITR, OX40, PD-1, and AhR ligands may also promote IDO expression, though in some cases IDO induction may be an indirect response to IFNs elicited by primary signals (see below). Mϕs expressing IDO in response to certain infections such as *Toxoplasma* also mediate innate host defense. Though IDO does not affect sterile clearance of *Toxoplasma* infections in mice, IDO ablation led to 100% mortality showing that IDO plays a crucial regulatory role in host–pathogen interactions in this chronic infection (Divanovic et al., 2012). IDO was first shown to regulate T cell immunity to fetal tissues during pregnancy (essentially a tolerated allograft) since treatment with the IDO inhibitor 1-methyl-tryptophan (1MT) caused pregnancy failure in mice due to maternal T cell mediated destruction of allogeneic but not syngeneic fetal tissues (Munn et al., 1998; Mellor et al., 2001). Since then IDO activity has been linked to tumor-induced tolerance, persistence of chronic infections, and attenuation of autoimmune, and allergic disease syndromes.

As mentioned above DCs expressing IDO suppress antigen-specific T cell responses. IDO activity in DCs does not attenuate antigen presentation and T cell activation but causes activated T cells to undergo cell cycle arrest and apoptosis, or induce anergy and promote Tregs conversion. IDO activity may suppress T cell responses in three ways: (a) tryptophan withdrawal, (b) production of kynurenines, and (c) altered redox potentials due to consumption of superoxide radicals (**Figure 2**). Reduced access to tryptophan during T cell activation triggers cell cycle arrest by activating GCN2 kinase, which senses reduced amino acid levels when uncharged tRNA molecules bind to ribosomes. GCN2 kinase phosphorylates eIF2α at serine 51 to inhibit general protein synthesis, and activate downstream stress response genes

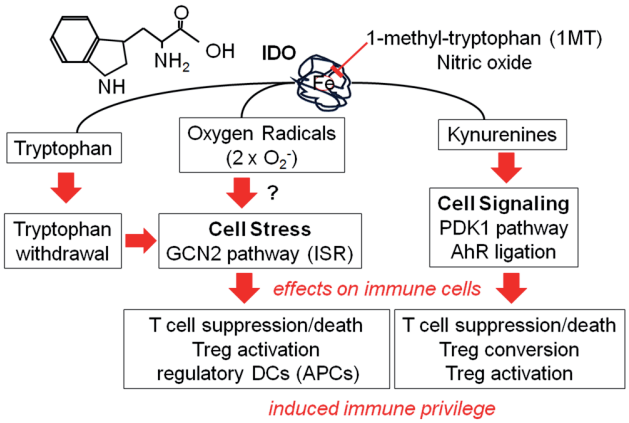

FIGURE 2 | Metabolic effects of IDO activity on immune cells.

such as CCAAT/enhancer-binding protein homologous protein (CHOP) which has pro-apoptotic functions. T cells from GCN2-deficient mice were resistant to the regulation by DCs expressing IDO (Munn et al., 2005), and resting Tregs from GCN2-deficient mice did not acquire regulatory phenotypes in response to DCs expressing IDO (Sharma et al., 2007; Baban et al., 2009). Arginase activity in Mϕs had similar effects on T cells and Tregs indicating that GCN2 kinase activation is a common downstream pathway by which amino acid catabolism promotes immune regulation (Bronte and Zanovello, 2005; Rodriguez et al., 2007). Some tryptophan catabolites made by IDO-expressing DCs also possess regulatory attributes by inhibiting T cell proliferation, inducing T cell apoptosis, and blocking differentiation of T_h1 helper cells (Frumento et al., 2002; Terness et al., 2002;Fallarino et al., 2003; Chen et al., 2008). Consistent with this notion 3-hydroxyanthranilic acid (3-HAA) blocked T cell responses and promoted T cell apoptosis by depletion of intracellular glutathione (GSH) and by inhibition of PDK1, which is an essential mediator of CD28-induced NFκB activation (Lee et al., 2010). Recent studies showing that kynurenine binds to AhR and that AhR signaling regulates T cell responses and activates Tregs also provide mechanistic explanations for IDO-mediated T cell regulation (Quintana et al., 2008; Vogel et al., 2008;Bankoti et al., 2010; Mezrich et al., 2010; Opitz et al., 2011). T cells may also be sensitive to changes in redox potential mediated by IDO activity in DCs. Reactive oxygen species (ROS) produced by phagocytes and other activated immune cells such as neutrophils have potent effects on cell metabolism and biological functions. Thus, mice with a genetic defect (pHox49-deficient) in NADPH oxidase-dependent ROS production exhibited defective IDO activity that contributed to T cell hyperactivity in a model of pulmonary aspergillosis, analogous to patients susceptible to chronic granulomatous disease (CGD; Romani et al., 2008). *In vitro* studies also showed that treatment of human NK cell line with IDO metabolite, L-kynurenine, significantly reduced cell viability through generation of ROS (Song et al., 2011).

IDO activity also blocks functional re-programming of preformed, resting Tregs into polyfunctional helper/effector (T_h17) T

cells in certain settings of inflammation. Thus, IDO activity in DCs activated GCN2 kinase in Tregs, and inhibited IL-6 production in tumor dLNs and in mice treated with TLR9 ligands to induce IDO (Baban et al., 2009; Sharma et al., 2009). Moreover, IDO-mediated Treg activation in response to systemic TLR9 ligands was dependent on regulatory cytokines (TGFβ, IL-10) and negative co-stimulation via CTLA4/B7 and PD-1/PD-L interactions between Tregs and DCs that maintained competence of DCs to express IDO in response to TLR9-induced IFN type I signaling (Baban et al., 2011). These data suggest that multiple pathways maintain default responsiveness via IDO to create regulatory outcomes during homeostasis, and that inflammatory signals must overcome these default regulatory pathways to promote pro-inflammatory, immune stimulatory outcomes. For example, vaccination with antigen plus (low dose) TLR9 ligands promoted Treg conversion into effector T cells, which were essential for initial priming of effector CD8 T cells by cross-presented antigens (Sharma et al., 2010). However IDO expression induced by established tumors prevented Treg re-programming, indicating that IDO was critical in regulating early effector T cell responses during inflammation and that vaccines were rendered ineffective, unless IDO activity was ablated. In human immunodeficiency virus (HIV) infected patients, elevated IDO activity by myeloid DCs was associated with loss of T_h17 cells and increase of Tregs (Favre et al., 2010). Thus IDO has a critical role in maintaining the balance of Tregs and helper/effector T cells, and may be critical for pathogens to persist in immunocompetent hosts despite host defense functions of IDO in some infections.

IDO-COMPETENT DCs

Dendritic cells are the only APC type capable of activating naïve T cells, underscoring their critical role in initiating T cell immunity. Few DCs in humans and mice are competent to express functional IDO. Though murine $CD8^-$ and $CD8^+$ DC subsets expressed IDO protein in response to IFNγ treatment, only $CD8^+$ DCs produced kynurenine, indicating IDO enzyme activity (Fallarino et al., 2002). Systemic treatments with soluble CTLA4 (CTLA4-Ig) and relatively high doses of TLR9 ligands (CpGs) were also able to induce $CD8^+$ DCs to express functional IDO by ligating B7 or TLR9 molecules and inducing IDO via IFNα signaling in a rare, but distinctive subset of splenic $CD19^+$ DCs co-expressing CD8, the plasmacytoid DC (pDC) marker B220, as well as CD19 and other B cell markers (Grohmann et al., 2002; Mellor et al., 2003, 2005;Baban et al., 2005;Johnson et al., 2010). In humans, monocyte-derived DCs competent to express IDO in response to IFNγ were a discrete subset of DCs expressing the chemokine receptor CCR6 and the pDC marker CD123 (Munn et al., 2002), and human DCs expressing IDO in response to CpGs also exhibited pDC attributes (Chen et al., 2008).

The relative paucity of IDO-competent DCs in mice (∼10% of total splenic DCs) and humans is a technical problem. To help counter this problem, we developed robust (MLR-based) assays to detect T cell suppression mediated by IDO^+ DCs from mice bearing tumors or treated with IDO inducers (**Table 1**). Using these DC suppression assays, and analogous assays to detect suppression mediated by small numbers of Tregs from lymphoid tissues, we determined that IDO^+ DCs mediate T cell suppression by blocking

Table 1 | IDO pathway inhibitors and inducers.

Effect	Compound	Mechanism (pathway)	Reference
IDO inhibitor	d-1MT	IDO pathway	Hou et al. (2007)
	MTH-Trp	IDO enzyme	Muller et al. (2005a)
	Brassinin	IDO enzyme	Gaspari et al. (2006)
	5I	IDO enzyme	Yue et al. (2009)
	Imatinib	PK inhibitor	Balachandran et al. (2011)
IDO inducer	Type I and Type II IFNs*	JAK/STAT (IDO promoter)	Taylor and Feng (1991), Mellor et al. (2005)
	CTLA4-Ig	B7 ligand (FOXO3a)	Baban et al. (2005), Fallarino et al. (2004b)
	CD40-Ig	IFNγ induction in CD8 T cells	Guillonneau et al. (2007)
	LPS*	TLR4 ligand (IFN type I)	Fujigaki et al. (2006)
	CpG*	TLR9 ligand (IFN type I)	Mellor et al. (2005)
	Resiquimod*	TLR7/8 ligand (IFN type I)	Manches et al. (2008)
	poly(I:C)*	TLR3 ligand (IFN type I)	Wang et al. (2011)
	Dexamethasone*	GITR ligand	Grohmann et al. (2007)
	CD200	CD200R ligand	Fallarino et al. (2004a)
	4-1BB	IFN γ induction in CD8 T cells	Seo et al. (2004)
	PGE2	Unknown	Von Bergwelt-Baildon et al. (2006)
	sCD83	Unknown	Lan et al. (2010)
	IgE/anti-IgE	FceRI aggregation	Von Bubnoff et al. (2002)
	HDAC inhibitor*	HDAC inhibition	Reddy et al. (2008)
	Dioxin*	AhR ligand	Vogel et al. (2008)

d-1MT, 1-Methyl-d-tryptophan; MTH-Trp, methyl-thiohydantoin-tryptophan; CTLA4, Cytotoxic T-Lymphocyte Antigen 4; LPS, Lipopolysaccharide; GITR, glucocorticoid-induced TNFR-related protein; HADC, histone deacetylase; AhR, aryl hydrocarbon receptor.

**Reagents with known toxic and/or pro-inflammatory (immune stimulatory) effects in vivo.*

clonal expansion of activated T cell, and by activating pre-formed, functionally quiescent (resting) Tregs to mediate bystander suppression. A distinctive feature of IDO-activated Tregs from tumor-bearing mice and mice treated with systemic TLR9 ligands was that suppression depended on interactions between PD-1 and its ligands PD-L1 and PD-L2. Intact GCN2 genes (in Tregs) were essential for Tregs to activate in response to IDO activity in DCs, emphasizing the critical role of tryptophan depletion in driving regulatory outcomes (Sharma et al., 2007; Baban et al., 2009). Increasing evidence also points to roles for tryptophan catabolites in promoting regulatory outcomes since kynurenine is an AhR ligand and that drives Treg conversion from naïve CD4 T cells (Mezrich et al., 2010; Nguyen et al., 2010). Moreover, requirements for signals from CTLA4, PD-1, TGFβ, and IL-10 for $CD19^+$ DCs to up-regulate IDO in response to TLR9 ligands suggest that IDO-competence is maintained by constitutive interactions between $CD19^+$ DCs and resting Tregs during homeostasis (Baban et al., 2011). Loss of any one of these molecular pathways caused Foxp3-lineage Tregs to undergo rapid functional re-programming into helper/effector T cells expressing TNFα, IL-17 IL-2 and IFNγ, probably due to IL-6 production which is suppressed by IDO activity in DCs. Thus, the relative numbers of $CD19^+$ DCs and Tregs and several non-redundant molecular pathways may be key factors that determine "immunologic tone" in particular lymphoid tissues (Checkpoint C) by regulating the ability of Tregs to become potent suppressor cells or polyfunctional helper/effector T cells in response to inflammatory cues.

MANIPULATING IDO TO CREATE OR DESTROY IMMUNE PRIVILEGE

Immune privilege helps protect tissues from damage mediated by excess immune responses generated when inflammation lowers thresholds that keep innate autoimmunity in check during homeostasis. In transplant settings artificial creation of immune privilege to mimic local tolerance induced by tumors and some pathogens that cause chronic infections is a key strategy to enhance allograft survival. Hence, manipulating the IDO pathway to create or destroy immune privilege offers potential therapeutic strategies to treat a range of chronic inflammatory disease syndromes.

USING IDO PATHWAY INHIBITORS TO ATTENUATE ESTABLISHED IMMUNE PRIVILEGE

Inhibiting the IDO pathway is a potential strategy to treat patients with hypo-immune syndromes (cancer and chronic infections) caused by induced immune privilege that permits persistence and progression of tumors and pathogen infections in immunocompetent individuals. In this section we discuss recent progress in developing new methods to inhibit IDO that may be effective in treating patients with cancer and chronic infections. We focus on the use of pharmacologic IDO pathway inhibitors to achieve this goal, though strategies using si/shRNA to knockdown IDO gene expression in specific cell or tissue types may be an alternative approach to achieve similar goals (Zheng et al., 2006; Flatekval and Sioud, 2009;Yen et al., 2009; Huang et al., 2011).

Cancer

At sites of tumor growth tryptophan catabolism mediated by IDO in host APCs, or IDO, or TDO in tumor cells can promote and sustain immune privilege. In humans, IDO1 expression in numerous tumor cell types is a significant predictor of poor prognosis (Sakurai et al., 2004; Astigiano et al., 2005;Brandacher et al., 2006; Ino et al., 2006;Riesenberg et al., 2007; Takao et al., 2007;Pan et al., 2008). In murine models, transfection of immunogenic tumor cell lines with recombinant IDO1 or TDO rendered them immunosuppressive and lethally progressive *in vivo* (Uyttenhove et al., 2003; Pilotte et al., 2012). The role of IDO in tumor immunosuppression was initially investigated using the racemic mixture of the IDO inhibitor 1MT (D,L-1MT). Thus D,L-1MT administration to mice bearing tumors that over-expressed IDO due to transfection or genetic deficiency of the tumor-suppressor Bin1 (leading to IDO1 over-expression) enhanced anti-tumor immunity and slowed tumor growth (Friberg et al., 2002; Uyttenhove et al., 2003;Muller et al., 2005b). Moreover D,L-1MT treatment synergized with several chemo-immunotherapy regimens to enhance anti-tumor effects in mice, as well as in regimens involving combination with radiotherapy (Muller et al., 2005b; Hou et al., 2007). A key question regarding D,L-1MT was whether both D and L stereoisomers of 1MT were biologically active. Based on *in vitro* assays using purified rabbit intestinal IDO L-1MT it was assumed that L-1MT would be more effective than D-1MT as an IDO inhibitor to promote T cell mediated anti-tumor immunity *in vivo* (Peterson et al., 1994). Unexpectedly, D-1MT was as effective, or more potent than L-1MT than the racemic mixture in restoring proliferation of T cells suppressed by physiologic IDO^+ pDCs (Munn et al., 2002). In addition, D-1MT was more effective than L-1MT in reversing suppression of T cell proliferation created by IDO^+ human monocyte-derived DCs and murine DCs from tumor-dLNs, and restored proliferation of primary $CD4^+$ and $CD8^+$ T cells blocked by IDO^+ fibroblast cells (Hou et al., 2007; Forouzandeh et al., 2008). D-1MT was also more effective than L-1MT as an anti-cancer agent in chemo-immunotherapy regimens in mouse models of transplantable melanoma and transplantable and autochthonous breast cancer (Hou et al., 2007). Despite these indications of superior D-1MT efficacy as an anti-tumor drug the molecular target of D-1MT has not been defined as D-1MT did not inhibit enzyme activity of recombinant human and murine IDO1 protein in cell-free assays, and IDO enzyme activity in human tumor cell lines and cell lines transfected with IDO1 genes (Lob et al., 2009). Nevertheless the anti-tumor and T cell enhancing effects of D-1MT were abrogated in the absence of functional IDO1 genes, and IDO1-deficient mice developed tumors that were not sensitized to chemo-immunotherapy involving D-1MT (Hou et al., 2007). Thus, the pharmacologic effects of D-1MT manifested only when the IDO1 pathway was intact, and D-1MT targets DCs that attenuate T cell responses. Several mechanisms could explain how D-1MT inhibits the IDO pathway in DCs while not inhibiting IDO enzyme activity directly as follows; (a) inhibition of high affinity tryptophan transporters in DCs to block substrate supply to IDO; (b) inhibition of uncharacterized IDO1 enzyme isoforms produced by RNA editing, post-translational modifications or association with other proteins into supramolecular complexes in DCs; (c) D-1MT conversion into the active enzymatic inhibitor L-1MT; (d) mimicking signals of tryptophan sufficiency, or blocking signals of tryptophan deficiency or downstream signals elicited by tryptophan metabolites in DCs. Regarding the last

mechanism, D-1MT may block downstream GCN2 activation or AhR signaling mediated by tryptophan withdrawal and kynurenine production. Thus D-1MT may target the "IDO pathway" but not IDO enzyme. Interestingly, the PK inhibitor Imatinib mesylate (Gleevec), an effective treatment for patients with gastrointestinal stromal tumor (GIST), blocked PK-mediated IDO induction in a mouse model of GIST (Balachandran et al., 2011). In addition halofuginone (Hf), a derivative of the bioactive compound Febrifugine found in a traditional Chinese herbal medicine, bypasses IDO by activating GCN2 kinase to block effector T_h17 responses (Sundrud et al., 2009). Recently, the molecular target of Hf was identified as glutamyl-prolyl-tRNA synthetase (EPRS), and Hf was shown to inhibit prolyl-tRNA synthetase activity (Keller et al., 2012). Thus Imatinib and Hf act, at least in part, by targeting components of the IDO pathway but not IDO directly to phenocopy IDO-mediated effects, and D-1MT may have a similar mode of action. It remains to be seen if D-1MT will be effective in cancer patients. Early indications from Phase I clinical trials hinted at efficacy that manifested as hypophysitis (in which the immune system attacks the pituitary gland) in some patients who had received prior antibody immunotherapy treatment (Garber, 2012). Anti-tumor efficacy is more likely to manifest when D-1MT is used in combination with other anti-cancer treatments because blocking IDO does not incite anti-tumor immunity *per se*. The anti-tumor and immune enhancing effects of D-1MT treatment were also replicated by administering other compounds that inhibit IDO1-encoded IDO enzyme activity *in vitro* and in IDO1-transfected cells such as 5-Br-brassinin, menadione, methyl-thiohydantoin-tryptophan, and analogs of phenylimidazole (Muller and Scherle, 2006; Banerjee et al., 2008;Kumar et al., 2008). Recently, hydroxyamidine inhibitors were also reported to suppress tryptophan catabolism and possess anti-tumor activity (Yue et al., 2009; Koblish et al., 2010;Liu et al., 2010), and TDO inhibitors may also be effective anti-tumor drugs based on a recent report that Kyn from tumor cells expressing TDO promoted tumor-induced immune privilege via AhR signaling (Opitz et al., 2011).

Chronic infections

The use of IDO inhibitors to enhance host immunity to infectious pathogens may seem paradoxical based on findings that Mϕs expressing IDO can mediate innate host defense in some infectious disease settings such as *Toxoplasma*, *Trypanosoma*, *Chlamydia*, and (Vincendeau et al., 1999; Njau et al., 2009;Knubel et al., 2010). Moreover IDO inhibition failed to restrict herpes simplex virus type 1 (HSV-1) replication or latency, and reactivated latent toxoplasma infections in mice leading to mortality (Divanovic et al., 2012). Nevertheless from the perspective of some pathogens, the disadvantages of needing to survive against IDO-mediated innate host defense may be outweighed by the potential advantages of IDO-mediated regulation of host adaptive immunity. For example HIV-1, which causes AIDS, is a potent IDO inducer leading to altered T_h17/Treg balance that may favor HIV-1 persistence (Boasso et al., 2007, 2009;Favre et al., 2010) In effect, HIV-1 may be an example of a pathogen that has evolved to exploit the IDO pathway as a means to prevent host-mediated viral clearance. If correct, this notion suggests that IDO inhibitors may destroy HIV-1-induced immune privilege that suppresses natural and vaccine-induced anti-HIV-1 immunity. Consistent with this idea, Boasso et al. (2009) reported that combined D-1MT, and antiretroviral therapy (ART) lowered virus titers in simian immunodeficiency virus (SIV)-infected rhesus macaques. Similar effects of D-1MT on lowering parasite burdens were observed in mice infected with *Leishmania major* (Makala et al., 2011; Divanovic et al., 2012). *In vitro* data showed 1MT treatment restored *M. tuberculosis*-specific CD4 T cell effector functions by increasing IFNγ production (Li et al., 2011). These observations support the use of IDO pathway inhibitors as a possible method to destroy pathogen-induced immune privilege; however, opposing effects of IDO pathway inhibitors on host defense mechanisms that maintain stable host–pathogen relationships developed over evolutionary time may complicate efforts to apply this basic approach in clinical settings.

Vaccines

Regulatory pathways that create and maintain immune privilege may block or attenuate responses to vaccines designed to induce protective and therapeutic immunity in settings of cancer and chronic infections. Established tumors and infections are notoriously resistant to vaccine-induced immunity due to the intensity of local suppressive mechanisms. However, a common assumption is that regulatory pathways do not impede responses to prophylactic vaccines. This assumption may not be entirely correct in some cases. For example, the vaccine adjuvant effects of the NK cell inducer and CD1d ligand alpha-galactosylceramide (αgalcer) was masked by rapid induction of IDO in mice vaccinated with inactivated influenza A virus, and co-treatment with IDO inhibitor (1MT) augmented primary T cell responses to vaccination, though did not enhance memory T cell generation (Guillonneau et al., 2009). It is unclear why IDO was induced rapidly in this model; one potential explanation is that IFNγ from activated NK cells stimulated local IDO expression, though a subset of DCs expressing CD1d may respond directly to αgalcer by expressing IDO. In a recent report SIV infected macaques treated with a SIV-based vaccine and ART simultaneously succumbed to lethal acute pancreatitis and hyperglycemic coma when vaccination and ART were combined with D-1MT and CTLA4 mAb blockade to inhibit immune regulatory pathways (Vaccari et al., 2012). This study revealed that IDO and/or CTLA4 regulatory pathways impede catastrophic host responses to ART-related toxicities, and sounds a precautionary note that interfering with regulatory pathways during vaccination can provoke undesirable toxicities. Nevertheless, optimal vaccine design should take into account the effects of host regulatory pathways, especially in therapeutic settings when established immune privilege may completely eliminate any beneficial effects of vaccine administration where it matters, namely at local sites of tumor growth and infection.

USING IDO INDUCERS AND TRYPTOPHAN METABOLITES TO CREATE IMMUNE PRIVILEGE

Autoimmunity, allergy, and transplantation are all hyper-immune syndromes in which excessive immunity damages healthy tissues. Regulatory pathways are often chronically activated in such settings. Though enhanced regulation may delay disease progression and onset and reduce the severity of disease pathology enhanced

regulation may not prevent disease progression completely; a pertinent analogy is driving a car with the parking brake on. Thus, IDO enzyme activity is often elevated in hyper-immune syndromes (increased Kyn in blood and tissues) and IDO slows but does not prevent disease progression. Nevertheless, enhancing IDO expression and activity may be a potential strategy to ameliorate hyper-immune syndromes and protect healthy tissues in the same way that IDO-mediated dominant regulation protects allogeneic fetal tissues from destruction by maternal T cell cells. Three broad strategies may help achieve this goal; (a) IDO gene transfer into vulnerable tissues, (b) treatment with reagents that induce IDO, and (c) treatment with reagents that mediate downstream regulatory effects of IDO. In this section we discuss progress in developing these complementary strategies to treat hyper-immune syndromes.

IDO gene transfer

IDO gene transfer to drive increased IDO expression in local tissues is the most direct strategy to attenuate hyper-immunity. IDO gene transfer into donor tissues prolonged allograft survival in corneal (Beutelspacher et al., 2006), cardiac (Li et al., 2007), and lung transplantation (Swanson et al., 2004; Liu et al., 2006a,b, 2007, 2009). The mechanism of enhanced allograft survival is not fully understood, but correlated with reduced clonal expansion of donor-specific effector T cells and attenuated effector functions of CD8 T cells that were generated and migrated to donor tissues expressing increased IDO (Liu et al., 2009). Reduced cytokine levels, T cell proliferative capacity, and increased Tregs were associated with protection of cardiac allografts transfected with IDO (Yu et al., 2008). Regardless of the exact mechanisms of transplant protection in these models, these findings support the use of IDO gene transfer as a potential means to protect healthy tissues in clinical settings. However, the creation of more robust immune privilege by over-expressing IDO may have unintended and undesirable consequences that may preclude clinical application such as increased risks of infection and tumor formation in tissues where IDO activity is artificially and constitutively elevated.

IDO inducing reagents

Table 1 lists some biologic and pharmacologic reagents that have been reported to induce IDO activity in mice or cell lines. A key consideration from a clinical perspective is that reagents to induce IDO must have minimal toxicities, as it is likely that extended (chronic) treatments will be needed to attenuate established hyper-immune disease syndromes. This consideration precludes the use of many reagents listed in **Table 1** due to known toxicities. For example, type I and II IFNs that induce IDO via receptors distributed on many cell types via JAK–STAT signaling pathways have documented toxicities. However, IFN type I is the current standard-of-care for some hyper-immune syndromes, despite the undesirable side-effects of chronic exposure to exogenous IFN type I. Thus, IFNβ is used to treat patients with multiple sclerosis (MS), and pegylated-IFNα is used to treat patients with hepatitis C virus (HCV) infections in combination with Ribovirin, a broad range anti-viral drug (Masarone and Persico, 2011). The mode of action of type I IFNs in these settings is not fully understood. However signaling via IFN type I receptors on myeloid cells attenuated experimental autoimmune encephalitis (EAE) disease progression and severity in mice, a widely accepted model of MS (Prinz et al., 2008). Type I IFNs may be pivotal regulators of host immunity in a range of autoimmune and chronic infections diseases such as HIV and HCV. For this reason pDCs may be critical mediators of hyper-immune syndromes since they are major (but not exclusive) sources of type I IFNs, especially in response to pathogen-associated molecular patterns (PAMPs) such as TLRs. It may be pertinent that IDO-competent DCs in humans and mice exhibit attributes that overlap with those of conventional pDCs, as well as other lymphoid and myeloid subsets (Munn et al., 2002; Chen et al., 2008;Johnson et al., 2010). Nevertheless, the role of IFNs, and the cells that produce IFNs, in hyper-immune syndromes is far from clear as IFNs mediate multiple downstream effects that encompass immune stimulatory and immune regulatory pathways.

The B7 (CD80/86) ligand CTLA4-Ig was designed as reagents to block co-stimulation required to generate effector T cells. CTLA4-Ig induces some DC subsets to express IDO and acquire tolerogenic phenotypes due to "reverse signaling" via B7 molecules. Thus B7 ligands may promote immune privilege by co-stimulatory blockade and by inducing DCs to express IDO. B7 ligands approved for clinical treatment of hyper-immune syndromes may not induce IDO in humans as poorly defined structural features may be critical for reverse signaling via B7 to induce IDO. Hence, it is not clear to what extent the potential IDO inducing attributes of B7 ligands may account for their clinical efficacy as immune modulators. A key issue is that reagents for clinical use are based on human proteins that may not interact with homologous human and mouse receptors in identical ways. In our experience CTLA4-Ig isoforms engineered with human sequences and with modified immunoglobulin constant (Fc) domains do not induce IDO in mice. Hence the co-stimulatory blockade attributes of such reagents may manifest in mice but IDO-mediated effects will not. It remains to be seen if B7 ligands engineered specifically to induce IDO in humans are effective immune modulators in clinical settings of hyper-immune syndromes. These points notwithstanding, a recent study in mice revealed that combined CTLA4-Ig (abatacept) and donor-specific transfusion (DST) therapy promoted heart allograft acceptance in an IDO and Treg dependent manner (Sucher et al., 2012). Thus IDO induction, particularly in DCs, is a pivotal checkpoint that can promote immune privilege, though factors that control IDO induction in DCs are not fully understood.

Several TLR ligands stimulate IDO expression and enzyme activity in mice. The TLR4 ligand LPS induced IDO in human peripheral blood mononuclear cells, THP-1, and U937 cell lines, and mouse bone marrow derived DCs (Fujigaki et al., 2006; Jung et al., 2007). LPS also induced human monocyte-derived DCs to express IDO, which promoted DC maturation and Treg differentiation (Hill et al., 2007). TLR9 ligands (CpGs) also stimulated mouse and human DCs to express IDO and acquire tolerogenic phenotypes by autocrine/paracrine type I IFN signaling in mice (Baban et al., 2005; Mellor et al., 2005; Chen et al., 2008). As a consequence, TLR9 ligands promoted naïve CD4 T cells to differentiate (convert) into Tregs, and caused pre-formed (resting) Tregs to acquire potent regulatory phenotypes (activate) via

IDO (Baban et al., 2009, 2011). Despite these examples of TLR-mediated immune regulation, TLR ligands have well-documented pro-inflammatory and immune stimulatory attributes that preclude their use as immune modulators in clinical settings of hyper-immunity.

Other IDO inducing reagents include histone deacetylase (HDAC) inhibitors and dexamethasone that ligates glucocorticoid-induced TNF receptor (GITR; Grohmann et al., 2007; Reddy et al., 2008). Polyinosinic–polycytidylic acid [poly(I:C)], a synthetic double stranded RNA analog and TLR3 ligand, induced human trophoblast cells to express IDO (Wang et al., 2011). Surface ligand/receptors including CD200, 4-1BB, and the eicosanoid prostaglandin E2 (PGE2) were also reported to induce IDO (Fallarino et al., 2004a; Seo et al., 2004;Von Bergwelt-Baildon et al., 2006). Recently, the immunomodulatory reagent soluble CD83 (sCD83) was reported to be an IDO inducer, and the tolerizing effects of sCD83 in a murine renal transplant model were blocked by IDO inhibitor (Ge et al., 2010; Lan et al., 2010). Recently, we discovered that systemic treatment of mice with nanoparticles containing the cationic polymer polyethylenimine (PEI) complexed with DNA stimulated IDO expression in a range of mouse tissues including lymphoid tissues (manuscript submitted). This response was unexpected as DNA/PEI nanoparticles elicit well-documented pro-inflammatory and immune stimulatory responses that promote anti-tumor immunity (Rodrigo-Garzon et al., 2010). IDO activity was induced in splenic DCs, which activated resting Tregs via IDO and blocked antigen-specific T cell responses to vaccines within 24 h of treatment with DNA/PEI nanoparticles. These responses were dependent on type I, but not type II IFN signaling. Moreover, the presence of TLR9 ligands (CpG motifs) in bacterial (plasmid) DNA was not required to induce IDO or type I IFN. Removing TLR9 ligands abrogated rapid and uniform activation of NK cells, which released large amounts of IFNγ into serum in response to DNA/PEI nanoparticles containing immune stimulatory bacterial plasmid DNA containing TLR9 ligands. Treating mice with DNA/PEI nanoparticles also reduced the severity of antigen-induced arthritis, and prevented Type 1 Diabetes (T1D) progression and onset in non-obese diabetic (NOD) female mice prone to T1D onset. In ongoing studies, we are addressing the mechanism of IDO induction following DNA/PEI nanoparticle treatment, and engineering biodegradable isoforms of DNA/PEI nanoparticles as potential reagents to treat clinical hyper-immune syndromes that promote immune privilege by inducing IDO and activating Tregs.

In summary, IDO inducing reagents offer promising new approaches to slow, prevent, or reverse hyper-immune syndromes. Mechanisms of IDO induction and the cell types induced to express IDO by these reagents have are not well-documented for all IDO inducing reagents, and further studies will be necessary to evaluate if these reagents can create robust tolerogenic phenotypes in immune cells to create and sustain immune privilege that protects healthy tissue at risk from hyper-immunity.

Tryptophan metabolites

Some tryptophan catabolites generated and released by cells expressing IDO or TDO possess immunomodulatory attributes that may promote regulation in inflamed tissues and associated dLNs to create and maintain local immune privilege. Combined treatment with Kyn and 3-hydroxy-anthranilic acid (3-HAA) prolonged allograft survival in a rat allogeneic skin transplant model (Bauer et al., 2005), and 3-HAA treatment inhibited PDK1 activation and promoted T cell apoptosis to suppress T cell-mediated lung pathology in a murine model of asthma (Hayashi et al., 2007). IDO-mediated elevation of Treg:T_h17 ratios characteristic of the progressive chronic inflammatory state that manifests during active HIV disease may also be mediated in part by 3-HAA, based on the finding that 3-HAA replicated this phenotype *in vitro* (Favre et al., 2010). Metabolic interplay between IDO and AhR signaling is mediated by tryptophan metabolites as Kyn is an AhR ligand, and AhR ligands such as Kyn, 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) a natural AhR ligand found in lungs, and the artificial AhR ligand iVAG539 induce IDO expression in DCs; in addition, Kyn promotes Treg conversion, possibly by direct AhR-mediated effects on Tregs or indirectly by inducing DCs to express IDO (Song et al., 2002; Hauben et al., 2008;Vogel et al., 2008; Mezrich et al., 2010;Nguyen et al., 2010; Opitz et al., 2011). These observations suggest that the IDO and AhR pathways reinforce regulatory responses via positive feedback loops involving tryptophan catabolites that are AhR ligands and other AhR ligands that induce IDO. The metabolic effects of AhR ligands on inflammatory responses by DCs were the subject of a recent review (Bankoti et al., 2010).

In summary, cells expressing functional IDO or TDO may modulate local immune effector functions by stimulating AhR in innate and adaptive immune cells, such as T cells, Th17, Tregs, and DCs, which reinforces IDO-mediated regulatory phenotypes in these cells. AhR expressed on non-immune cells including lung epithelial cells, liver hepatocytes, and endothelial cells may also mediate cell responses in tissue development, physiological function, and immune responses (Walisser et al., 2005; Chiba et al., 2011, 2012), indicating ubiquitous influences of tryptophan metabolites on both hematopoietic and non-hematopoietic cells. The relative importance of the immune modulatory effects of IDO-mediated tryptophan withdrawal to trigger the ISR in immune cells and production of tryptophan catabolites is not known. However these novel insights identify several metabolic pathways that create and sustain immune privilege, thereby providing new therapeutic targets to disrupt (or reinforce) immune privilege according to clinical need. As described above, Imatinib and Hf are already examples of compounds that target the IDO pathway indirectly, though with diametric effects on T cell immunity. These findings reinforce the hypothesis that altered amino acid metabolism at sites of local inflammation is a pivotal immune control mechanism that helps create and sustain immune privilege.

SUMMARY AND PROSPECTS

In this review we summarize key recent developments relating to the concept that induced immune privilege explains differential immunogenicities observed at local sites of inflammation. Clearly interactions between small numbers of innate and adaptive immune cells in microenvironmental niches exert profound influences on immune outcomes and disease progression that have not been fully appreciated. This knowledge deficiency arose because

key initiating events are far removed in time, and perhaps in space too from their measurable consequences in terms of disease progression and onset. The application of new imaging tools to examine interactions between small numbers of cells that lead to measurable metabolic changes has potential to improve understanding of the fundamental biochemical and metabolic changes that explain immunologic outcomes relevant to chronic inflammatory diseases. It is also clear that we are only just beginning to understand how initial responses to inflammatory insults in tissues, and the prevailing immunologic tone of lymphoid tissues draining such tissues integrate information about the primary insult that shapes subsequent responses in the innate and adaptive immune systems. Some immune modulatory drugs that interfere with processes that create local immune privilege are already approved for clinical use, and many others are in the pipeline, with more potential targets still to be considered. It will be interesting to see how these new immunotherapies fare in clinical settings, and how this field develops based on data from clinical trials and basic research.

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REFERENCES

Astigiano, S., Morandi, B., Costa, R., Mastracci, L., D'Agostino, A., Ratto, G. B., Melioli, G., and Frumento, G. (2005). Eosinophil granulocytes account for indoleamine 2,3-dioxygenase-mediated immune escape in human non-small cell lung cancer. *Neoplasia* 7, 390–396.

Baban, B., Chandler, P. R., Johnson, B. A.III, Huang, L., Li, M., Sharpe, M. L., Francisco, L. M., Sharpe, A. H., Blazar, B. R., Munn, D. H., and Mellor, A. L. (2011). Physiologic control of IDO competence in splenic dendritic cells. *J. Immunol.* 187, 2329–2335.

Baban, B., Chandler, P. R., Sharma, M. D., Pihkala, J., Koni, P. A., Munn, D. H., and Mellor, A. L. (2009). IDO activates regulatory T cells and blocks their conversion into Th17-like T cells. *J. Immunol.* 183, 2475–2483.

Baban, B., Hansen, A. M., Chandler, P. R., Manlapat, A., Bingaman, A., Kahler, D. J., Munn, D. H., and Mellor, A. L. (2005). A minor population of splenic dendritic cells expressing CD19 mediates IDO-dependent T cell suppression via type I IFN signaling following B7 ligation. *Int. Immunol.* 17, 909–919.

Balachandran, V. P., Cavnar, M. J., Zeng, S., Bamboat, Z. M., Ocuin, L. M., Obaid, H., Sorenson, E. C., Popow, R., Ariyan, C., Rossi, F., Besmer, P., Guo, T., Antonescu, C. R., Taguchi, T., Yuan, J., Wolchok, J. D., Allison, J. P., and Dematteo, R. P. (2011). Imatinib potentiates antitumor T cell responses in gastrointestinal stromal tumor through the inhibition of Ido. *Nat. Med.* 17, 1094–1100.

Banerjee, T., Duhadaway, J. B., Gaspari, P., Sutanto-Ward, E., Munn, D. H., Mellor, A. L., Malachowski, W. P., Prendergast, G. C., and Muller, A. J. (2008). A key in vivo antitumor mechanism of action of natural product-based brassinins is inhibition of indoleamine 2,3-dioxygenase. *Oncogene* 27, 2851–2857.

Bankoti, J., Rase, B., Simones, T., and Shepherd, D. M. (2010). Functional and phenotypic effects of AhR activation in inflammatory dendritic cells. *Toxicol. Appl. Pharmacol.* 246, 18–28.

Bauer, T. M., Jiga, L. P., Chuang, J. J., Randazzo, M., Opelz, G., and Terness, P. (2005). Studying the immunosuppressive role of indoleamine 2,3-dioxygenase: tryptophan metabolites suppress rat allogeneic T-cell responses in vitro and in vivo. *Transpl. Int.* 18, 95–100.

Beutelspacher, S. C., Pillai, R., Watson, M. P., Tan, P. H., Tsang, J., Mcclure, M. O., George, A. J., and Larkin, D. F. (2006). Function of indoleamine 2,3-dioxygenase in corneal allograft rejection and prolongation of allograft survival by over-expression. *Eur. J. Immunol.* 36, 690–700.

Boasso, A. (2011). Wounding the immune system with its own blade: HIV-induced tryptophan catabolism and pathogenesis. *Curr. Med. Chem.* 18, 2247–2256.

Boasso, A., Herbeuval, J. P., Hardy, A. W., Anderson, S. A., Dolan, M. J., Fuchs, D., and Shearer, G. M. (2007). HIV inhibits CD4+ T-cell proliferation by inducing indoleamine 2,3-dioxygenase in plasmacytoid dendritic cells. *Blood* 109, 3351–3359.

Boasso, A., Vaccari, M., Fuchs, D., Hardy, A. W., Tsai, W. P., Tryniszewska, E., Shearer, G. M., and Franchini, G. (2009). Combined effect of antiretroviral therapy and blockade of IDO in SIV-infected rhesus macaques. *J. Immunol.* 182, 4313–4320.

Brandacher, G., Perathoner, A., Ladurner, R., Schneeberger, S., Obrist, P., Winkler, C., Werner, E. R., Werner-Felmayer, G., Weiss, H. G., Gobel, G., Margreiter, R., Konigsrainer, A., Fuchs, D., and Amberger, A. (2006). Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: effect on tumor-infiltrating T cells. *Clin. Cancer Res.* 12, 1144–1151.

Brenk, M., Scheler, M., Koch, S., Neumann, J., Takikawa, O., Hacker, G., Bieber, T., and Von Bubnoff, D. (2009). Tryptophan deprivation induces inhibitory receptors ILT3 and ILT4 on dendritic cells favoring the induction of human CD4+CD25+Foxp3+ T regulatory cells. *J. Immunol.* 183, 145–154.

Bronte, V., and Zanovello, P. (2005). Regulation of immune responses by L-arginine metabolism. *Nat. Rev. Immunol.* 5, 641–654.

Chen, W., Liang, X., Peterson, A. J., Munn, D. H., and Blazar, B. R. (2008). The indoleamine 2,3-dioxygenase pathway is essential for human plasmacytoid dendritic cell-induced adaptive T regulatory cell generation. *J. Immunol.* 181, 5396–5404.

Chiba, T., Chihara, J., and Furue, M. (2012). Role of the arylhydrocarbon receptor (AhR) in the pathology of asthma and COPD. *J. Allergy* 2012, 8.

Chiba, T., Uchi, H., Tsuji, G., Gondo, H., Moroi, Y., and Furue, M. (2011). Arylhydrocarbon receptor (AhR) activation in airway epithelial cells induces MUC5AC via reactive oxygen species (ROS) production. *Pulm. Pharmacol. Ther.* 24, 133–140.

Chon, S. Y., Hassanain, H. H., Pine, R., and Gupta, S. L. (1995). Involvement of two regulatory elements in interferon-gamma-regulated expression of human indoleamine 2,3-dioxygenase gene. *J. Interferon Cytokine Res.* 15, 517–526.

Chung, D. J., Rossi, M., Romano, E., Ghith, J., Yuan, J., Munn, D. H., and Young, J. W. (2009). Indoleamine 2,3-dioxygenase-expressing mature human monocyte-derived dendritic cells expand potent autologous regulatory T cells. *Blood* 114, 555–563.

Cobbold, S. P. (2010). Combining regulation with suppression and aiming for tolerance. *Transplantation* 89, 909–910.

Dai, W., and Gupta, S. L. (1990). Regulation of indoleamine 2,3-dioxygenase gene expression in human fibroblasts by interferon-gamma. Upstream control region discriminates between interferon-gamma and interferon-alpha. *J. Biol. Chem.* 265, 19871–19877.

De Vries, V. C., Pino-Lagos, K., Nowak, E. C., Bennett, K. A., Oliva, C., and Noelle, R. J. (2011). Mast cells condition dendritic cells to mediate allograft tolerance. *Immunity* 35, 550–561.

Ding, Y., Bushell, A., and Wood, K. J. (2010). Mesenchymal stem-cell immunosuppressive capabilities: therapeutic implications in islet transplantation. *Transplantation* 89, 270–273.

Divanovic, S., Sawtell, N. M., Trompette, A., Warning, J. I., Dias, A., Cooper, A. M., Yap, G. S., Arditi, M., Shimada, K., Duhadaway, J. B., Prendergast, G. C., Basaraba, R. J., Mellor, A. L., Munn, D. H., Aliberti, J., and Karp, C. L. (2012). Opposing biological functions of tryptophan catabolizing enzymes during intracellular infection. *J. Infect. Dis.* 205, 152–161.

Fallarino, F., Asselin-Paturel, C., Vacca, C., Bianchi, R., Gizzi, S., Fioretti, M. C., Trinchieri, G., Grohmann, U., and Puccetti, P. (2004a). Murine plasmacytoid dendritic cells initiate the immunosuppressive pathway of tryptophan catabolism in response to CD200 receptor engagement. *J. Immunol.* 173, 3748–3754.

Fallarino, F., Bianchi, R., Orabona, C., Vacca, C., Belladonna, M. L., Fioretti, M. C., Serreze, D. V., Grohmann, U., and Puccetti, P. (2004b). CTLA-4-Ig activates forkhead transcription factors and protects dendritic cells from oxidative stress in nonobese diabetic mice. *J. Exp. Med.* 200, 1051–1062.

Fallarino, F., Grohmann, U., Vacca, C., Orabona, C., Spreca, A., Fioretti, M. C., and Puccetti, P. (2003). T cell apoptosis by kynurenines. *Adv. Exp. Med. Biol.* 527, 183–190.

Fallarino, F., Vacca, C., Orabona, C., Belladonna, M. L., Bianchi, R., Marshall, B., Keskin, D. B., Mellor, A. L., Fioretti, M. C., Grohmann, U., and Puccetti, P. (2002). Functional expression of indoleamine 2,3-dioxygenase by murine CD8 alpha(+) dendritic cells. *Int. Immunol.* 14, 65–68.

Favre, D., Mold, J., Hunt, P. W., Kanwar, B., Loke, P., Seu, L., Barbour, J. D., Lowe, M. M., Jayawardene, A., Aweeka, F., Huang, Y., Douek, D. C., Brenchley, J. M., Martin, J. N., Hecht, F. M., Deeks, S. G., and Mccune, J. M. (2010). Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the balance of TH17 to regulatory T cells in HIV disease. *Sci. Transl. Med.* 2, 32ra36.

Flatekval, G. F., and Sioud, M. (2009). Modulation of dendritic cell maturation and function with mono- and bifunctional small interfering RNAs targeting indoleamine 2,3-dioxygenase. *Immunology* 128, e837–e848.

Forouzandeh, F., Jalili, R. B., Germain, M., Duronio, V., and Ghahary, A. (2008). Differential immunosuppressive effect of indoleamine 2,3-dioxygenase (IDO) on primary human CD4+ and CD8+ T cells. *Mol. Cell. Biochem.* 309, 1–7.

Friberg, M., Jennings, R., Alsarraj, M., Dessureault, S., Cantor, A., Extermann, M., Mellor, A. L., Munn, D. H., and Antonia, S. J. (2002). Indoleamine 2,3-dioxygenase contributes to tumor cell evasion of T cell-mediated rejection. *Int. J. Cancer* 101, 151–155.

Frumento, G., Rotondo, R., Tonetti, M., Damonte, G., Benatti, U., and Ferrara, G. B. (2002). Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. *J. Exp. Med.* 196, 459–468.

Fujigaki, H., Saito, K., Fujigaki, S., Takemura, M., Sudo, K., Ishiguro, H., and Seishima, M. (2006). The signal transducer and activator of transcription 1alpha and interferon regulatory factor 1 are not essential for the induction of indoleamine 2,3-dioxygenase by lipopolysaccharide: involvement of p38 mitogen-activated protein kinase and nuclear factor-kappaB pathways, and synergistic effect of several proinflammatory cytokines. *J. Biochem.* 139, 655–662.

Garber, K. (2012). Evading immunity: new enzyme implicated in cancer. *J. Natl. Cancer Inst.* 104, 349–352.

Gaspari, P., Banerjee, T., Malachowski, W. P., Muller, A. J., Prendergast, G. C., Duhadaway, J., Bennett, S., and Donovan, A. M. (2006). Structure-activity study of brassinin derivatives as indoleamine 2,3-dioxygenase inhibitors. *J. Med. Chem.* 49, 684–692.

Ge, W., Arp, J., Lian, D., Liu, W., Baroja, M. L., Jiang, J., Ramcharran, S., Eldeen, F. Z., Zinser, E., Steinkasserer, A., Chou, P., Brand, S., Nicolette, C., Garcia, B., and Wang, H. (2010). Immunosuppression involving soluble CD83 induces tolerogenic dendritic cells that prevent cardiac allograft rejection. *Transplantation* 90, 1145–1156.

Grohmann, U., Orabona, C., Fallarino, F., Vacca, C., Calcinaro, F., Falorni, A., Candeloro, P., Belladonna, M. L., Bianchi, R., Fioretti, M. C., and Puccetti, P. (2002). CTLA-4-Ig regulates tryptophan catabolism in vivo. *Nat. Immunol.* 3, 1097–1101.

Grohmann, U., Volpi, C., Fallarino, F., Bozza, S., Bianchi, R., Vacca, C., Orabona, C., Belladonna, M. L., Ayroldi, E., Nocentini, G., Boon, L., Bistoni, F., Fioretti, M. C., Romani, L., Riccardi, C., and Puccetti, P. (2007). Reverse signaling through GITR ligand enables dexamethasone to activate IDO in allergy. *Nat. Med.* 13, 579–586.

Guillonneau, C., Hill, M., Hubert, F. X., Chiffoleau, E., Herve, C., Li, X. L., Heslan, M., Usal, C., Tesson, L., Menoret, S., Saoudi, A., Le Mauff, B., Josien, R., Cuturi, M. C., and Anegon, I. (2007). CD40Ig treatment results in allograft acceptance mediated by CD8CD45RC T cells, IFN-gamma, and indoleamine 2,3-dioxygenase. *J. Clin. Invest.* 117, 1096–1106.

Guillonneau, C., Mintern, J. D., Hubert, F. X., Hurt, A. C., Besra, G. S., Porcelli, S., Barr, I. G., Doherty, P. C., Godfrey, D. I., and Turner, S. J. (2009). Combined NKT cell activation and influenza virus vaccination boosts memory CTL generation and protective immunity. *Proc. Natl. Acad. Sci. U.S.A.* 106, 3330–3335.

Hackstein, H., and Thomson, A. W. (2004). Dendritic cells: emerging pharmacological targets of immunosuppressive drugs. *Nat. Rev. Immunol.* 4, 24–34.

Hauben, E., Gregori, S., Draghici, E., Migliavacca, B., Olivieri, S., Woisetschlager, M., and Roncarolo, M. G. (2008). Activation of the aryl hydrocarbon receptor promotes allograft-specific tolerance through direct and dendritic cell-mediated effects on regulatory T cells. *Blood* 112, 1214–1222.

Hayashi, T., Mo, J. H., Gong, X., Rossetto, C., Jang, A., Beck, L., Elliott, G. I., Kufareva, I., Abagyan, R., Broide, D. H., Lee, J., and Raz, E. (2007). 3-Hydroxyanthranilic acid inhibits PDK1 activation and suppresses experimental asthma by inducing T cell apoptosis. *Proc. Natl. Acad. Sci. U.S.A.* 104, 18619–18624.

Hill, M., Tanguy-Royer, S., Royer, P., Chauveau, C., Asghar, K., Tesson, L., Lavainne, F., Remy, S., Brion, R., Hubert, F. X., Heslan, M., Rimbert, M., Berthelot, L., Moffett, J. R., Josien, R., Gregoire, M., and Anegon, I. (2007). IDO expands human CD4++CD25high regulatory T cells by promoting maturation of LPS-treated dendritic cells. *Eur. J. Immunol.* 37, 3054–3062.

Hou, D. Y., Muller, A. J., Sharma, M. D., Duhadaway, J., Banerjee, T., Johnson, M., Mellor, A. L., Prendergast, G. C., and Munn, D. H. (2007). Inhibition of indoleamine 2,3-dioxygenase in dendritic cells by stereoisomers of 1-methyl-tryptophan correlates with antitumor responses. *Cancer Res.* 67, 792–801.

Huang, T. T., Yen, M. C., Lin, C. C., Weng, T. Y., Chen, Y. L., Lin, C. M., and Lai, M. D. (2011). Skin delivery of short hairpin RNA of indoleamine 2,3 dioxygenase induces antitumor immunity against orthotopic and metastatic liver cancer. *Cancer Sci.* 102, 2214–2220.

Ino, K., Yoshida, N., Kajiyama, H., Shibata, K., Yamamoto, E., Kidokoro, K., Takahashi, N., Terauchi, M., Nawa, A., Nomura, S., Nagasaka, T., Takikawa, O., and Kikkawa, F. (2006). Indoleamine 2,3-dioxygenase is a novel prognostic indicator for endometrial cancer. *Br. J. Cancer* 95, 1555–1561.

Johnson, B. A. III, Kahler, D. J., Baban, B., Chandler, P. R., Kang, B., Shimoda, M., Koni, P. A., Pihkala, J., Vilagos, B., Busslinger, M., Munn, D. H., and Mellor, A. L. (2010). B-lymphoid cells with attributes of dendritic cells regulate T cells via indoleamine 2,3-dioxygenase. *Proc. Natl. Acad. Sci. U.S.A.* 107, 10644–10648.

Jung, I. D., Lee, C. M., Jeong, Y. I., Lee, J. S., Park, W. S., Han, J., and Park, Y. M. (2007). Differential regulation of indoleamine 2,3-dioxygenase by lipopolysaccharide and interferon gamma in murine bone marrow derived dendritic cells. *FEBS Lett.* 581, 1449–1456.

Jux, B., Kadow, S., Luecke, S., Rannug, A., Krutmann, J., and Esser, C. (2011). The aryl hydrocarbon receptor mediates UVB radiation-induced skin tanning. *J. Invest. Dermatol.* 131, 203–210.

Keller, T. L., Zocco, D., Sundrud, M. S., Hendrick, M., Edenius, M., Yum, J., Kim, Y. J., Lee, H. K., Cortese, J. F., Wirth, D. F., Dignam, J. D., Rao, A., Yeo, C. Y., Mazitschek, R., and Whitman, M. (2012). Halofuginone and other febrifugine derivatives inhibit prolyl-tRNA synthetase. *Nat. Chem. Biol.* 8, 311–317.

Knubel, C. P., Martinez, F. F., Fretes, R. E., Diaz Lujan, C., Theumer, M. G., Cervi, L., and Motran, C. C. (2010). Indoleamine 2,3-dioxigenase (IDO) is critical for host resistance against *Trypanosoma cruzi*. *FASEB J.* 24, 2689–2701.

Koblish, H. K., Hansbury, M. J., Bowman, K. J., Yang, G., Neilan, C. L., Haley, P. J., Burn, T. C., Waeltz, P., Sparks, R. B., Yue, E. W., Combs, A. P., Scherle, P. A., Vaddi, K., and Fridman, J. S. (2010). Hydroxyamidine inhibitors of indoleamine-2,3-dioxygenase potently suppress systemic tryptophan catabolism and the growth of IDO-expressing tumors. *Mol. Cancer Ther.* 9, 489–498.

Kraal, G., Samsom, J. N., and Mebius, R. E. (2006). The importance of regional lymph nodes for mucosal tolerance. *Immunol. Rev.* 213, 119–130.

Kumar, S., Jaller, D., Patel, B., Lalonde, J. M., Duhadaway, J. B., Malachowski, W. P., Prendergast, G. C., and Muller, A. J. (2008). Structure based development of phenylimidazole-derived inhibitors of indoleamine 2,3-dioxygenase. *J. Med. Chem.* 51, 4968–4977.

Lan, Z., Ge, W., Arp, J., Jiang, J., Liu, W., Gordon, D., Healey, D., Debenedette, M., Nicolette, C., Garcia, B., and Wang, H. (2010). Induction of kidney allograft tolerance by soluble CD83 associated with prevalence of tolerogenic dendritic cells and indoleamine 2,3-dioxygenase. *Transplantation* 90, 1286–1293.

Lee, S. M., Lee, Y. S., Choi, J. H., Park, S. G., Choi, I. W., Joo, Y. D., Lee, W. S., Lee, J. N., Choi, I., and Seo, S. K. (2010). Tryptophan metabolite 3-hydroxyanthranilic acid selectively induces activated T cell death

via intracellular GSH depletion. *Immunol. Lett.* 132, 53–60.

Li, J., Meinhardt, A., Roehrich, M. E., Golshayan, D., Dudler, J., Pagnotta, M., Trucco, M., and Vassalli, G. (2007). Indoleamine 2,3-dioxygenase gene transfer prolongs cardiac allograft survival. *Am. J. Physiol. Heart Circ. Physiol.* 293, H3415-3423.

Li, Q., Li, L., Liu, Y., Fu, X., Wang, H., Lao, S., Yang, B., and Wu, C. (2011). Biological functions of *Mycobacterium tuberculosis*-specific CD4+ T cells were impaired by tuberculosis pleural fluid. *Immunol. Lett.* 138, 113–121.

Liu, H., Liu, L., Fletcher, B. S., and Visner, G. A. (2006a). Novel action of indoleamine 2,3-dioxygenase attenuating acute lung allograft injury. *Am. J. Respir. Crit. Care Med.* 173, 566–572.

Liu, H., Liu, L., Fletcher, B. S., and Visner, G. A. (2006b). Sleeping Beauty-based gene therapy with indoleamine 2,3-dioxygenase inhibits lung allograft fibrosis. *FASEB J.* 20, 2384–2386.

Liu, H., Liu, L., Liu, K., Bizargity, P., Hancock, W. W., and Visner, G. A. (2009). Reduced cytotoxic function of effector CD8+ T cells is responsible for indoleamine 2,3-dioxygenase-dependent immune suppression. *J. Immunol.* 183, 1022–1031.

Liu, H., Liu, L., and Visner, G. A. (2007). Nonviral gene delivery with indoleamine 2,3-dioxygenase targeting pulmonary endothelium protects against ischemia-reperfusion injury. *Am. J. Transplant.* 7, 2291–2300.

Liu, X., Shin, N., Koblish, H. K., Yang, G., Wang, Q., Wang, K., Leffet, L., Hansbury, M. J., Thomas, B., Rupar, M., Waeltz, P., Bowman, K. J., Polam, P., Sparks, R. B., Yue, E. W., Li, Y., Wynn, R., Fridman, J. S., Burn, T. C., Combs, A. P., Newton, R. C., and Scherle, P. A. (2010). Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity. *Blood* 115, 3520–3530.

Lob, S., Konigsrainer, A., Zieker, D., Brucher, B. L., Rammensee, H. G., Opelz, G., and Terness, P. (2009). IDO1 and IDO2 are expressed in human tumors: levo- but not dextro-1-methyl tryptophan inhibits tryptophan catabolism. *Cancer Immunol. Immunother.* 58, 153–157.

Makala, L. H., Baban, B., Lemos, H., El-Awady, A. R., Chandler, P. R., Hou, D. Y., Munn, D. H., and Mellor, A. L. (2011). *Leishmania major* attenuates host immunity by stimulating local indoleamine 2,3-dioxygenase expression. *J. Infect. Dis.* 203, 715–725.

Manches, O., Munn, D., Fallahi, A., Lifson, J., Chaperot, L., Plumas, J., and Bhardwaj, N. (2008). HIV-activated human plasmacytoid DCs induce Tregs through an indoleamine 2,3-dioxygenase-dependent mechanism. *J. Clin. Invest.* 118, 3431–3439.

Mantovani, A., Germano, G., Marchesi, F., Locatelli, M., and Biswas, S. K. (2011). Cancer-promoting tumor-associated macrophages: new vistas and open questions. *Eur. J. Immunol.* 41, 2522–2525.

Masarone, M., and Persico, M. (2011). Antiviral therapy: why does it fail in HCV-related chronic hepatitis? *Expert Rev. Anti Infect. Ther.* 9, 535–543.

Mellor, A. L., Baban, B., Chandler, P., Marshall, B., Jhaver, K., Hansen, A., Koni, P. A., Iwashima, M., and Munn, D. H. (2003). Cutting edge: induced indoleamine 2,3 dioxygenase expression in dendritic cell subsets suppresses T cell clonal expansion. *J. Immunol.* 171, 1652–1655.

Mellor, A. L., Baban, B., Chandler, P. R., Manlapat, A., Kahler, D. J., and Munn, D. H. (2005). Cutting edge: CpG oligonucleotides induce splenic CD19+ dendritic cells to acquire potent indoleamine 2,3-dioxygenase-dependent T cell regulatory functions via IFN Type 1 signaling. *J. Immunol.* 175, 5601–5605.

Mellor, A. L., Chandler, P., Baban, B., Hansen, A. M., Marshall, B., Pihkala, J., Waldmann, H., Cobbold, S., Adams, E., and Munn, D. H. (2004). Specific subsets of murine dendritic cells acquire potent T cell regulatory functions following CTLA4-mediated induction of indoleamine 2,3 dioxygenase. *Int. Immunol.* 16, 1391–1401.

Mellor, A. L., and Munn, D. H. (2008). Creating immune privilege: active local suppression that benefits friends, but protects foes. *Nat. Rev. Immunol.* 8, 74–80.

Mellor, A. L., Sivakumar, J., Chandler, P., Smith, K., Molina, H., Mao, D., and Munn, D. H. (2001). Prevention of T cell-driven complement activation and inflammation by tryptophan catabolism during pregnancy. *Nat. Immunol.* 2, 64–68.

Mezrich, J. D., Fechner, J. H., Zhang, X., Johnson, B. P., Burlingham, W. J., and Bradfield, C. A. (2010). An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J. Immunol.* 185, 3190–3198.

Muller, A. J., Duhadaway, J. B., Donover, P. S., Sutanto-Ward, E., and Prendergast, G. C. (2005a). Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. *Nat. Med.* 11, 312–319.

Muller, A. J., Malachowski, W. P., and Prendergast, G. C. (2005b). Indoleamine 2,3-dioxygenase in cancer: targeting pathological immune tolerance with small-molecule inhibitors. *Expert Opin. Ther. Targets* 9, 831–849.

Muller, A. J., and Scherle, P. A. (2006). Targeting the mechanisms of tumoral immune tolerance with small-molecule inhibitors. *Nat. Rev. Cancer* 6, 613–625.

Munn, D. H., and Mellor, A. L. (2006). The tumor-draining lymph node as an immune-privileged site. *Immunol. Rev.* 213, 146–158.

Munn, D. H., Sharma, M. D., Baban, B., Harding, H. P., Zhang, Y., Ron, D., and Mellor, A. L. (2005). GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. *Immunity* 22, 633–642.

Munn, D. H., Sharma, M. D., Hou, D., Baban, B., Lee, J. R., Antonia, S. J., Messina, J. L., Chandler, P., Koni, P. A., and Mellor, A. L. (2004). Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes. *J. Clin. Invest.* 114, 280–290.

Munn, D. H., Sharma, M. D., Lee, J. R., Jhaver, K. G., Johnson, T. S., Keskin, D. B., Marshall, B., Chandler, P., Antonia, S. J., Burgess, R., Slingluff, C. L. Jr., and Mellor, A. L. (2002). Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. *Science* 297, 1867–1870.

Munn, D. H., Zhou, M., Attwood, J. T., Bondarev, I., Conway, S. J., Marshall, B., Brown, C., and Mellor, A. L. (1998). Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* 281, 1191–1193.

Murphy, S. P., Porrett, P. M., and Turka, L. A. (2011). Innate immunity in transplant tolerance and rejection. *Immunol. Rev.* 241, 39–48.

Nguyen, N. T., Kimura, A., Nakahama, T., Chinen, I., Masuda, K., Nohara, K., Fujii-Kuriyama, Y., and Kishimoto, T. (2010). Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. *Proc. Natl. Acad. Sci. U.S.A.* 107, 19961–19966.

Njau, F., Geffers, R., Thalmann, J., Haller, H., and Wagner, A. D. (2009). Restriction of *Chlamydia pneumoniae* replication in human dendritic cell by activation of indoleamine 2,3-dioxygenase. *Microbes Infect.* 11, 1002–1010.

Opitz, C. A., Litzenburger, U. M., Sahm, F., Ott, M., Tritschler, I., Trump, S., Schumacher, T., Jestaedt, L., Schrenk, D., Weller, M., Jugold, M., Guillemin, G. J., Miller, C. L., Lutz, C., Radlwimmer, B., Lehmann, I., Von Deimling, A., Wick, W., and Platten, M. (2011). An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature* 478, 197–203.

Ostrand-Rosenberg, S., Sinha, P., Beury, D. W., and Clements, V. K. (2012). Cross-talk between myeloid-derived suppressor cells (MDSC), macrophages, and dendritic cells enhances tumor-induced immune suppression. *Semin. Cancer Biol.* PMID: 22313874. [Epub ahead of print].

Pan, K., Wang, H., Chen, M. S., Zhang, H. K., Weng, D. S., Zhou, J., Huang, W., Li, J. J., Song, H. F., and Xia, J. C. (2008). Expression and prognosis role of indoleamine 2,3-dioxygenase in hepatocellular carcinoma. *J. Cancer Res. Clin. Oncol.* 134, 1247–1253.

Peterson, A. C., Migawa, M. T., Martin, M. J., Hamaker, L. K., Czerwinski, K. M., Zhang, W., Arend, R. A., Fisette, P. L., Ozaki, Y., Will, J. A., Brown, R. R., and Cook, J. M. (1994). Evaluation of functionalized tryptophan derivatives and related compounds as competitive inhibitors of indolamine 2,3-dioxygenase. *Med. Chem. Res.* 3, 531–544.

Pilotte, L., Larrieu, P., Stroobant, V., Colau, D., Dolusic, E., Frederick, R., De Plaen, E., Uyttenhove, C., Wouters, J., Masereel, B., and Van Den Eynde, B. J. (2012). Reversal of tumoral immune resistance by inhibition of tryptophan 2,3-dioxygenase. *Proc. Natl. Acad. Sci. U.S.A.* 109, 2497–2502.

Prinz, M., Schmidt, H., Mildner, A., Knobeloch, K. P., Hanisch, U. K., Raasch, J., Merkler, D., Detje, C., Gutcher, I., Mages, J., Lang, R., Martin, R., Gold, R., Becher, B.,

Bruck, W., and Kalinke, U. (2008). Distinct and nonredundant in vivo functions of IFNAR on myeloid cells limit autoimmunity in the central nervous system. *Immunity* 28, 675–686.

Quintana, F. J., Basso, A. S., Iglesias, A. H., Korn, T., Farez, M. F., Bettelli, E., Caccamo, M., Oukka, M., and Weiner, H. L. (2008). Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. *Nature* 453, 65–71.

Reddy, P., Sun, Y., Toubai, T., Duran-Struuck, R., Clouthier, S. G., Weisiger, E., Maeda, Y., Tawara, I., Krijanovski, O., Gatza, E., Liu, C., Malter, C., Mascagni, P., Dinarello, C. A., and Ferrara, J. L. (2008). Histone deacetylase inhibition modulates indoleamine 2,3-dioxygenase-dependent DC functions and regulates experimental graft-versus-host disease in mice. *J. Clin. Invest.* 118, 2562–2573.

Reis e Sousa, C. (2006). Dendritic cells in a mature age. *Nat. Rev. Immunol.* 6, 476–483.

Riesenberg, R., Weiler, C., Spring, O., Eder, M., Buchner, A., Popp, T., Castro, M., Kammerer, R., Takikawa, O., Hatz, R. A., Stief, C. G., Hofstetter, A., and Zimmermann, W. (2007). Expression of indoleamine 2,3-dioxygenase in tumor endothelial cells correlates with long-term survival of patients with renal cell carcinoma. *Clin. Cancer Res.* 13, 6993–7002.

Rodrigo-Garzon, M., Berraondo, P., Ochoa, L., Zulueta, J. J., and Gonzalez-Aseguinolaza, G. (2010). Antitumoral efficacy of DNA nanoparticles in murine models of lung cancer and pulmonary metastasis. *Cancer Gene Ther.* 17, 20–27.

Rodriguez, P. C., Quiceno, D. G., and Ochoa, A. C. (2007). L-arginine availability regulates T-lymphocyte cell-cycle progression. *Blood* 109, 1568–1573.

Romani, L., Fallarino, F., De Luca, A., Montagnoli, C., D'Angelo, C., Zelante, T., Vacca, C., Bistoni, F., Fioretti, M. C., Grohmann, U., Segal, B. H., and Puccetti, P. (2008). Defective tryptophan catabolism underlies inflammation in mouse chronic granulomatous disease. *Nature* 451, 211–215.

Sakurai, K., Enomoto, K., Amano, S., Kimura, T., Sugito, K., Kimizuka, K., Ikeda, T., Shibata, M., and Negishi, N. (2004). Study of indoleamine 2,3-dioxygenase expression in patients of esophageal squamous cell carcinoma. *Gan To Kagaku Ryoho* 31, 1780–1782.

Seo, S. K., Choi, J. H., Kim, Y. H., Kang, W. J., Park, H. Y., Suh, J. H., Choi, B. K., Vinay, D. S., and Kwon, B. S. (2004). 4-1BB-mediated immunotherapy of rheumatoid arthritis. *Nat. Med.* 10, 1088–1094.

Sharma, M. D., Baban, B., Chandler, P., Hou, D. Y., Singh, N., Yagita, H., Azuma, M., Blazar, B. R., Mellor, A. L., and Munn, D. H. (2007). Plasmacytoid dendritic cells from mouse tumor-draining lymph nodes directly activate mature Tregs via indoleamine 2,3-dioxygenase. *J. Clin. Invest.* 117, 2570–2582.

Sharma, M. D., Hou, D. Y., Baban, B., Koni, P. A., He, Y., Chandler, P. R., Blazar, B. R., Mellor, A. L., and Munn, D. H. (2010). Reprogrammed foxp3(+) regulatory T cells provide essential help to support cross-presentation and CD8(+) T cell priming in naive mice. *Immunity* 33, 942–954.

Sharma, M. D., Hou, D. Y., Liu, Y., Koni, P. A., Metz, R., Chandler, P., Mellor, A. L., He, Y., and Munn, D. H. (2009). Indoleamine 2,3-dioxygenase controls conversion of Foxp3+ Tregs to TH17-like cells in tumor-draining lymph nodes. *Blood* 113, 6102–6111.

Song, H., Park, H., Kim, Y. S., Kim, K. D., Lee, H. K., Cho, D. H., Yang, J. W., and Hur, D. Y. (2011). L-kynurenine-induced apoptosis in human NK cells is mediated by reactive oxygen species. *Int. Immunopharmacol.* 11, 932–938.

Song, J., Clagett-Dame, M., Peterson, R. E., Hahn, M. E., Westler, W. M., Sicinski, R. R., and Deluca, H. F. (2002). A ligand for the aryl hydrocarbon receptor isolated from lung. *Proc. Natl. Acad. Sci. U.S.A.* 99, 14694–14699.

Sucher, R., Fischler, K., Oberhuber, R., Kronberger, I., Margreiter, C., Ollinger, R., Schneeberger, S., Fuchs, D., Werner, E. R., Watschinger, K., Zelger, B., Tellides, G., Pilat, N., Pratschke, J., Margreiter, R., Wekerle, T., and Brandacher, G. (2012). IDO and regulatory T cell support are critical for cytotoxic T lymphocyte-associated Ag-4 Ig-mediated long-term solid organ allograft survival. *J. Immunol.* 188, 37–46.

Sundrud, M. S., Koralov, S. B., Feuerer, M., Calado, D. P., Kozhaya, A. E., Rhule-Smith, A., Lefebvre, R. E., Unutmaz, D., Mazitschek, R., Waldner, H., Whitman, M., Keller, T., and Rao, A. (2009). Halofuginone inhibits TH17 cell differentiation by activating the amino acid starvation response. *Science* 324, 1334–1338.

Swanson, K. A., Zheng, Y., Heidler, K. M., Mizobuchi, T., and Wilkes, D. S. (2004). CDllc+ cells modulate pulmonary immune responses by production of indoleamine 2,3-dioxygenase. *Am. J. Respir. Cell Mol. Biol.* 30, 311–318.

Takao, M., Okamoto, A., Nikaido, T., Urashima, M., Takakura, S., Saito, M., Okamoto, S., Takikawa, O., Sasaki, H., Yasuda, M., Ochiai, K., and Tanaka, T. (2007). Increased synthesis of indoleamine-2,3-dioxygenase protein is positively associated with impaired survival in patients with serous-type, but not with other types of, ovarian cancer. *Oncol. Rep.* 17, 1333–1339.

Taylor, M. W., and Feng, G. S. (1991). Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. *FASEB J.* 5, 2516–2522.

Terness, P., Bauer, T. M., Rose, L., Dufter, C., Watzlik, A., Simon, H., and Opelz, G. (2002). Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. *J. Exp. Med.* 196, 447–457.

Uyttenhove, C., Pilotte, L., Theate, I., Stroobant, V., Colau, D., Parmentier, N., Boon, T., and Van Den Eynde, B. J. (2003). Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. *Nat. Med.* 9, 1269–1274.

Vaccari, M., Boasso, A., Fenizia, C., Fuchs, D., Hryniewicz, A., Morgan, T., Weiss, D., Doster, M. N., Heraud, J. M., Shearer, G. M., and Franchini, G. (2012). Fatal pancreatitis in simian immunodeficiency virus SIV(mac251)-infected macaques treated with 2',3'-dideoxyinosine and stavudine following cytotoxic-T-lymphocyte-associated antigen 4 and indoleamine 2,3-dioxygenase blockade. *J. Virol.* 86, 108–113.

Vincendeau, P., Lesthelle, S., Bertazzo, A., Okomo-Assoumou, M. C., Allegri, G., and Costa, C. V. (1999). Importance of L-tryptophan metabolism in trypanosomiasis. *Adv. Exp. Med. Biol.* 467, 525–531.

Vogel, C. F., Goth, S. R., Dong, B., Pessah, I. N., and Matsumura, F. (2008). Aryl hydrocarbon receptor signaling mediates expression of indoleamine 2,3-dioxygenase. *Biochem. Biophys. Res. Commun.* 375, 331–335.

Von Bergwelt-Baildon, M. S., Popov, A., Saric, T., Chemnitz, J., Classen, S., Stoffel, M. S., Fiore, F., Roth, U., Beyer, M., Debey, S., Wickenhauser, C., Hanisch, F. G., and Schultze, J. L. (2006). CD25 and indoleamine 2,3-dioxygenase are up-regulated by prostaglandin E2 and expressed by tumor-associated dendritic cells in vivo: additional mechanisms of T-cell inhibition. *Blood* 108, 228–237.

Von Bubnoff, D., Matz, H., Frahnert, C., Rao, M. L., Hanau, D., De La Salle, H., and Bieber, T. (2002). FcepsilonRI induces the tryptophan degradation pathway involved in regulating T cell responses. *J. Immunol.* 169, 1810–1816.

Walisser, J. A., Glover, E., Pande, K., Liss, A. L., and Bradfield, C. A. (2005). Aryl hydrocarbon receptor-dependent liver development and hepatotoxicity are mediated by different cell types. *Proc. Natl. Acad. Sci. U.S.A.* 102, 17858–17863.

Wang, B., Koga, K., Osuga, Y., Cardenas, I., Izumi, G., Takamura, M., Hirata, T., Yoshino, O., Hirota, Y., Harada, M., Mor, G., and Taketani, Y. (2011). Toll-like receptor-3 ligation-induced indoleamine 2, 3-dioxygenase expression in human trophoblasts. *Endocrinology* 152, 4984–4992.

Yen, M. C., Lin, C. C., Chen, Y. L., Huang, S. S., Yang, H. J., Chang, C. P., Lei, H. Y., and Lai, M. D. (2009). A novel cancer therapy by skin delivery of indoleamine 2,3-dioxygenase siRNA. *Clin. Cancer Res.* 15, 641–649.

Yu, G., Dai, H., Chen, J., Duan, L., Gong, M., Liu, L., Xiong, P., Wang, C. Y., Fang, M., and Gong, F. (2008). Gene delivery of indoleamine 2,3-dioxygenase prolongs cardiac allograft survival by shaping the types of T-cell responses. *J. Gene Med.* 10, 754–761.

Yue, E. W., Douty, B., Wayland, B., Bower, M., Liu, X., Leffet, L., Wang, Q., Bowman, K. J., Hansbury, M. J., Liu, C., Wei, M., Li, Y., Wynn, R., Burn, T. C., Koblish, H. K., Fridman, J. S., Metcalf, B., Scherle, P. A., and Combs, A. P. (2009). Discovery of potent competitive inhibitors of indoleamine 2,3-dioxygenase with in vivo pharmacodynamic activity and efficacy in a mouse melanoma model. *J. Med. Chem.* 52, 7364–7367.

Zheng, X., Koropatnick, J., Li, M., Zhang, X., Ling, F., Ren, X., Hao, X., Sun, H., Vladau, C., Franek, J. A., Feng, B., Urquhart, B. L., Zhong, R., Freeman, D. J., Garcia, B., and Min, W. P. (2006). Reinstalling antitumor immunity by inhibiting tumor-derived immunosuppressive molecule IDO through RNA interference. *J. Immunol.* 177, 5639–5646.

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