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REVIEW
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Memory B Cells in Pregnancy Sensitization

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Memory B cells play an important role in immunity to pathogens as these cells are poised to rapidly differentiate into antibody-secreting cells upon antigen re-encounter. Memory B cells also develop over the course of HLA-sensitization during pregnancy and transplantation. In this review, we discuss the potential contribution of memory B cells to pregnancy sensitization as well as the impact of these cells on transplant candidacy and outcomes. We start by summarizing how B cell subsets are altered in pregnancy and discuss what is known about HLA-specific B cell responses given our current understanding of fetal antigen availability in maternal secondary lymphoid tissues. We then review the molecular mechanisms governing the generation and maintenance of memory B cells during infection – including the role of T follicular helper cells - and discuss the experimental evidence for the development of these cells during pregnancy. Finally, we discuss how memory B cells impact access to transplantation and transplant outcomes for a range of transplant recipients.

Keywords: memory B cell, pregnancy, sensitization, HLA, antibody

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INTRODUCTION

Pregnancy represents the most common alloimmune exposure in humans. Exposure to non-self antigens of paternal origin can prime maternal B cells to generate antibodies, and maternal production of antibody against fetal antigens can occur after immunization with either minor (i.e. blood group antigens) or major antigens [i.e. human leukocyte antigen (HLA)]. Rh alloimmunization occurs when anti-D antibodies are produced in response to immunization with fetal blood (1). While this maternal anti-D antibody can cross the placenta and cause hemolytic disease of the newborn, this review will focus on the generation and consequences of antibody against the major alloantigen - HLA. As HLA alloantigens expressed by the semi-allogeneic fetus can be re-encountered on a transplanted organ from either a living or deceased donor, alloimmunization from pregnancy has particular impact for female transplant candidates and recipients. The maternal immune response to fetal alloantigens thus sets the stage for what is to come later in life and influences both access to transplantation as well as post-transplantation outcome. Despite the prevalence of pregnancy alloimmunization, the immunologic consequences of this event are very poorly understood, as pregnancy represents a unique "immunologic paradox" (2) that differs significantly from other types of immunization contexts. In this review, we discuss our

current understanding of pregnancy alloimmunization with a particular focus on the generation of anti-HLA antibody and B cell memory. Herein, we use the term *pregnancy alloimmunization* to describe the response of any maternal adaptive immune subset to fetal antigen during pregnancy, whereas the term *pregnancy sensitization* refers specifically to the generation of alloantibody. In this regard, a parous woman has been alloimmunized by prior pregnancy even if she has not been sensitized (i.e. has detectable alloantibody).

CLINICAL IMPACT OF PREGNANCY SENSITIZATION

The clinical significance of pregnancy sensitization was first appreciated in the 1950s when JJ van Rood and colleagues were studying transfusion reactions in peripartum women (3). Although these investigators did not understand the structure or the etiology of the soluble factor(s) mediating cellular agglutination in their assays, this "factor" was later identified as anti-HLA antibody. This critical discovery by van Rood and colleagues impacted not only the emerging fields of transfusion medicine and organ transplantation but also allowed the development of the first reagents that were used to HLA type human tissue as well as the methodology (i.e. cytotoxicity assays). Subsequent studies which relied on cytotoxic assays later determined the prevalence and timing of pregnancy-induced anti-HLA antibody (4–6). The advent of single-antigen bead technology has greatly improved detection methods and revealed that pregnancy elicits a paternal HLA-reactive antibody response in 50-84% of mothers in the first year after pregnancy that may have HLA epitope bias (6–12). As in other types of immune exposures, the number of times that a woman is exposed to fetal alloantigen matters, as multiparous females have a higher incidence of paternal HLA-reactive antibodies (7) with strong binding to HLA epitopes (13). These anti-HLA antibodies make it more difficult for multiparous women with end organ disease to find appropriate organ donors and therefore contribute to sex-based disparities in organ transplantation (14–16). Even when transplantation in women with high levels of pre-existing anti-HLA antibody is avoided, the existence of low-level alloantibody or memory T and B cells generated by pregnancy alloimmunization may negatively impact transplant outcomes, although the specific impact of this alloimmunization event has been difficult to enumerate among other factors that influence graft outcomes (17–22). In particular, it is difficult to attribute post-transplant outcome to changes in anti-HLA antibody quantity over time as longitudinal investigation of pregnancy-induced anti-HLA antibody titers has not been performed in post-transplant recipients. Although the current available literature does not suggest that pregnancy alloimmunization promotes poor transplant outcomes *per se*, the available data sets are highly confounded. As discussion of this important topic is beyond the scope of this review, interested readers are referred to a review of pregnancy alloimmunization that discusses these data sets in detail (15). Altogether, these clinical observations underscore the importance of understanding how alloreactive B cells and antibody-secreting cells form and function during pregnancy.

OVERVIEW OF THE ANATOMY OF PREGNANCY SENSITIZATION AND THE AVAILABILITY OF FETAL ALLOANTIGEN

To understand the mechanisms of pregnancy sensitization, it is useful to first consider the locations where maternal immune cells may encounter fetal antigen. Given our present understanding of the anatomy of pregnancy and immunity, there are several potential locations. First, immune cells in the maternal blood contact the embryo-derived trophoblast of the placenta. However, the encounter of maternal blood with placental trophoblast is not thought to significantly prime maternal B cells to produce anti-HLA antibody because human trophoblast does not express HLA-A, HLA-B, HLA-DR, HLA-DP or HLA-DQ (23–26). Nevertheless, pregnancy sensitization might still occur locally at the fetomaternal interface as maternal T and B cells encounter conceptus-derived antigens in the uterine tissue that are presented by decidual macrophages or dendritic cells. Indeed, unsupervised high dimensional flow cytometric analyses identify B cell phenotypes in uterine tissue. While immunohistochemistry demonstrates that these decidual B cells are positioned next to T cells, ectopic lymphoid follicular structures in the gravid uterus have not been formally identified. Given the importance of follicular structures in the genesis of antibody-secreting cells (discussed further below), these data suggest that anti-HLA antibody from antibody-secreting cells is unlikely to be generated in the uterus. Furthermore, *in vitro* analyses of decidual B cells *versus* peripheral B cells demonstrate an augmented ability to produce the immunoregulatory cytokine, IL-10, in the presence or absence of co-stimulatory signals (27). These data therefore suggest that the uterus may comprise a specialized tissue-resident B cell population with an as yet undetermined interaction with uterine T cells. These studies also imply IL-10 producing decidual B cell subsets arise from naïve B cell precursors locally and are maintained in an antigen-selected manner as memory after interaction with fetal trophoblast cells. In support of the latter hypothesis, *in vitro* co-culture experiments demonstrate that fetal trophoblast cells induce IL-10 production in B cells (28). Altogether, these data suggest that B cell populations in the uterus are unique and may not participate in the generation of alloantibody. This conclusion is additionally supported by emerging data suggesting that B cells play an important role in determining pregnancy outcome. For example, in murine models of pregnancy, mice that inherently lack B cells (i.e. μMT), give birth to fetuses that were smaller than wild type and with fewer regulatory T cells (29). Moreover, recurrent miscarriage has been associated with phenotypic abnormalities in the B cell compartment (30). Altogether, these data suggest that B cells in the uterus are unlikely to contribute to a large degree to the total pool of HLA antibody that is produced during pregnancy, as B cells are much more frequent in locations

outside the uterus that promote the differentiation of antibody-secreting cells. We further discuss antigen availability outside the uterus below as well as our current understanding of B cell differentiation into both memory B cells and antibody-secreting cells.

In light of the findings discussed above, it is likely that the majority of pregnancy alloimmunization occurs at sites distant from the fetomaternal interface (**Figure 1**). It is indeed now well established in both mice and humans that conceptus-derived cells, proteins, exosomes, RNA, and DNA disseminate broadly in the maternal circulation and can deposit in maternal tissues (31–36). Importantly, disseminated protein antigens are detectable in the maternal spleen and other secondary lymphoid organs in mouse models of pregnancy (37) where these antigens can prime both maternal T cells (37–45) and B cells (46). Although fetal microchimerism clearly occurs, resulting in seeding of maternal tissues that may persist for decades, its impact on humoral immunity remains undefined (32, 36). In mothers, the development of regulatory T cells may be influenced by the presence of fetal microchimerism, and this T cell literature is excellently reviewed by Kinder et al. (47) However, the persistence of circulating anti-HLA antibodies with specificity for fetal HLA alleles and their vigorous recall response following transplant suggest that the B cell arm of the adaptive response is not similarly skewed towards a regulatory phenotype (48).

Although it is possible that antigen presenting cells from the uterus may traffic to the uterine draining lymph node and prime maternal T and B cells as well, mouse studies suggest that such egress of maternal antigen presenting cells from the uterus is impaired during pregnancy (49). Collectively, these data suggest that pregnancy alloimmunization primarily occurs in secondary lymphoid organs such as the spleen and lymph nodes, perhaps after uptake of circulating antigen by antigen presenting cells within these sites. However, many knowledge gaps remain. It is important to note that the majority of these data have yet to be validated in humans owing to the extreme polymorphism of HLA and the lack of HLA-specific reagents. Moreover, it is unclear how effective B cell help is provided by maternal T cells given that pregnancy alloimmunization often propels the expansion of hypofunctional and/or suppressive regulatory T cell populations.

GENERATION OF MEMORY B CELLS AND ANTIBODIES

To understand B cells responses in pregnancy, it is first useful to review what is known about B cell activation and differentiation in the context of infection and immunization, where B cell biology has been better studied. In this section, we review the foundation of humoral responses by outlining the pathways of B cell activation and the subsequent production of memory B cells and antibody-secreting cells.

B Cell Activation Pathways

Naïve B cells circulate through the secondary lymphoid organs in search of their cognate antigen in either soluble or membrane-bound form (50). While naïve B cells may be activated outside of secondary lymphoid organs in ectopic lymphoid structures (51) or in mice lacking organized lymph nodes or spleens (52), secondary lymphoid organs represent the chief location for initial antigen encounter and B cell activation (53). Although B

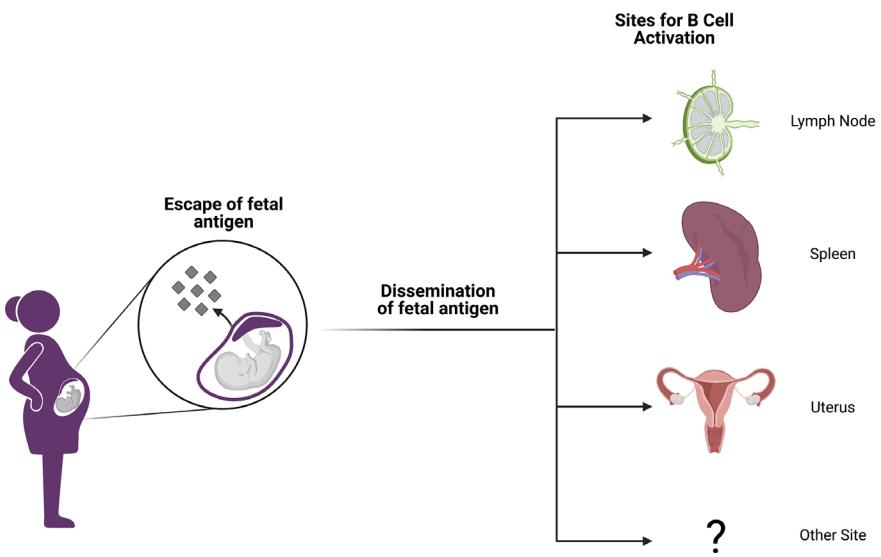

FIGURE 1 | Anatomic sites of alloimmunization in pregnancy. Although fetal antigen is known to disseminate widely, it is not well understood at which sites B and T cell priming take place in allosensitization. Candidate locations include secondary lymphoid organs (SLOs) like lymph nodes and spleen. However, it is also possible that immune structures within the gravid uterus may host alloantigen encounters or perhaps other anatomic sites are involved. Figure created with BioRender.com.

cell receptors are able to recognize intact, soluble antigen, multiple mechanisms exist to concentrate and present antigen for B cells. Afferent lymph enters a lymph node at the subcapsular sinus, where subcapsular sinus macrophages capture and present complement-opsonized antigen to B cells (54). Non-opsonized antigen may reach the medulla of the lymph node where it is bound by medullary macrophages or dendritic cells, phagocytosed, and presented or transported to the follicle (55). Within the follicle, follicular dendritic cells are specialized for the task of presenting antigen to B cells, capturing opsonized antigen and recycling it in a non-degradative endosomal compartment that facilitates long-term antigen presentation within a secondary follicle's germinal center (GC) response (56). How antigen is captured and presented in the context of pregnancy is unknown.

Following the naïve B cell's initial encounter with its cognate antigen, the B cell becomes activated and follows one of three major pathways, as indicated by the i, ii, iii numeric identifiers below (**Figure 2**). The B cell follows chemokine and oxysterol gradients to migrate to the interface of the B cell follicle and the T cell zone (T:B border), upregulating the receptors Epstein-Barr virus-induced molecule 2 (EBI2) (57) and C-C chemokine receptor 7 (CCR7) while maintaining C-X-C motif chemokine receptor 5 (CXCR5) expression (58). Some B cells then enter the germinal center (GC) while others remain in the extrafollicular (EF) region. In the germinal center (also known as the secondary follicle), somatic hypermutation and clonal selection produce high-affinity memory B cells and long-lived antibody-secreting cells in a T-dependent process (i). In the extrafollicular region, naïve B cells differentiate into memory B cells or antibody-secreting cells *via* T cell-dependent (ii) or T-independent processes (iii) (59). Although the spectrum of antigen that might prime B cell responses in pregnancy is unknown, fragments of HLA proteins are likely to be involved in the priming of anti-HLA antibody. We thus will focus on the first two pathways (i, ii) given that B cell priming by protein antigens is a T-dependent process (60, 61), and anti-HLA antibody is known to persist for decades in some women after pregnancy sensitization, thus implying the differentiation of long-lived antibody-secreting cells.

At the T:B border, B cells present their antigen and receive $CD4^+$ T cell help from developing T follicular helper cells (Tfhs). Tfhs, whose primary purpose is to help activated B cells become memory B cells or long-lived antibody-secreting cells, differentiate from naïve $CD4^+$ T cells after being primed by dendritic cells in the lymph node or spleen (62). They express the Tfh lineage-defining transcription factor *Bcl6* and the chemokine receptor CXCR5 and then migrate to the T:B border to interact with activated B cells (63). Here, B cells provide the key second step in Tfh differentiation by expressing ICOS ligand (ICOSL) and presenting the Tfh's cognate antigen *via* major histocompatibility complex class II (MHC class II) proteins (64). At the same time, the Tfh provides the B cell with critical support. Tfh expression of CD40 ligand (CD40L) delivers the second signal of B cell activation following ligation of the B cell receptor (65), and cytokines like IL-21 and IL-4 facilitate both T-dependent extrafollicular and germinal cell responses (66). From the T:B border, Tfhs then enter the germinal center, becoming germinal center Tfhs (GC-Tfhs) (67). Of note, others use the nomenclature *pre-Tfh* or *extrafollicular mantle Tfh* to refer to

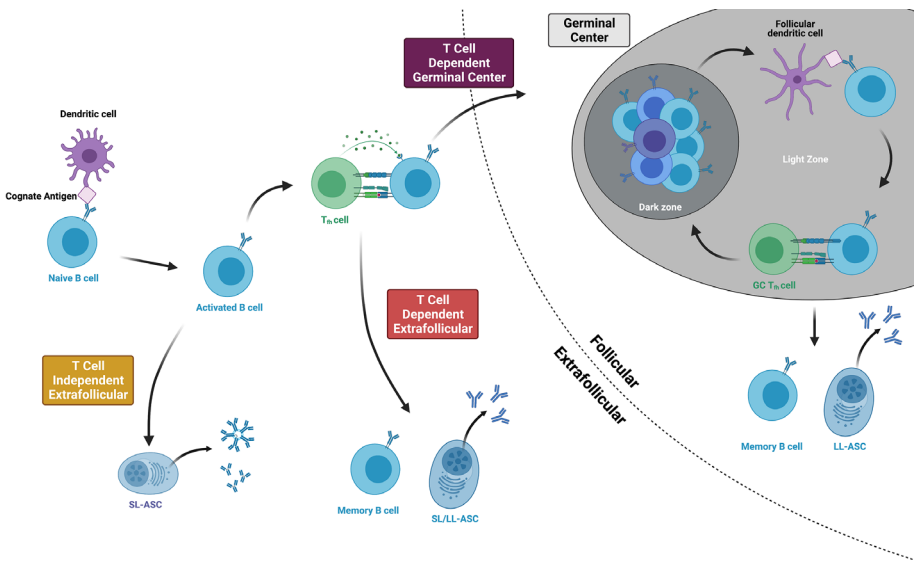

FIGURE 2 | Three main pathways of B cell activation and differentiation. Protein antigens promote T cell dependent responses, culminating in either germinal center or extrafollicular pathways that produce long-lived memory B cells and antibody-secreting cells (ASCs). Within the germinal center, B cells cycle between the dark and light zones, proliferating, mutating, and undergoing affinity maturation. T cell independent antigens include mitogens and highly repetitive polysaccharides that can stimulate B cell responses without T cell help. Figure created with BioRender.com.

BCL-6$^+$ CD4$^+$ T cells outside of the germinal center and simply *Tfh* for those within a germinal center structure (67, 68).

Following these events at the T:B border, B cells briefly proliferate in the outer follicle before making a fate decision to enter the germinal center or follow an extrafollicular pathway (69). The affinity of the B cell receptor, cytokine milieu, Tfh interactions, and innate sensors all contribute to this decision (70). High affinity B cell clones experiencing B cell receptor crosslinking may favor an extrafollicular response (71). Although B cells must meet a relative affinity threshold in order to effectively capture antigen and engage Tfhs at the T:B border (72), this threshold may be sufficiently low that it does not provide a considerable barrier to germinal center entrance among naïve antigen-specific clones (73). Tfhs assist activated B cells by indirectly assessing affinity and secreting IL-21 (66). and pathogen- or damage-associated patterns that signal through toll-like receptor 9 (TLR9) promote rapid antibody production by inducing the differentiation of antibody-secreting cells while decreasing B cell antigen presentation and thus, the effectiveness of B cell and T cell cooperation (74). Overall, these fate decisions correlate with EBI2 expression, whose loss guides the activated B cell into the germinal center and whose persistence corresponds with an extrafollicular response (75).

Germinal Center Responses

Activated B cells that enter the germinal center participate in the immunological equivalent of natural selection, resulting in a population of memory B cells and long-lived antibody-secreting cells that maintain long-lasting memory. Germinal centers feature two histologic compartments: a light zone where follicular dendritic cells present antigen for B cells (76) and Tfhs positively select higher-affinity B cell clones and a dark zone where B cells proliferate and acquire immunoglobulin gene somatic mutations (77). In the light zone, cognate B cells and Tfhs become "entangled" *via* multiple cell surface receptor interactions (78) with Tfhs providing B cell help *via* CD40L signaling and cytokines including IL-4, IL-21, and BAFF (79). B cells then migrate to the dark zone, where expression of the enzyme Activation-induced Cytidine Deaminase (AID) generates uracil bases which may be replaced with thymine bases leading to somatic hypermutation (80). These newly mutated germinal center B cells proliferate and return to the light zone to compete for antigen from follicular dendritic cells and re-engage Tfhs, with higher affinity B cells receiving continued Tfh help (79). It should be noted that several traditionally-held views about germinal centers have been challenged by new research. One such view, which holds that class-switch recombination occurs within the germinal center has been challenged by data showing that the majority of naïve B cells undergo class-switch recombination prior to germinal center entry and that class-switch recombination may in fact be repressed within the germinal center (81). Another view, which holds that somatic hypermutation and memory develop solely within the germinal center is addressed in the following paragraph, noting the existence of these processes in extrafollicular responses. Work continues to elucidate the factors that control the fate decision of a germinal center B cell to become a memory B cell or a long-lived antibody-secreting cell. Lower affinity B cell receptors may favor memory B cell development, as B cells receiving less Tfh help maintain high expression of the transcriptional repressor *Bach2* and subsequently become memory B cells (82). In addition to affinity, timing may also play a role in fate decisions, with the early germinal center response predominantly yielding memory B cells and the late germinal center response producing long-lived antibody-secreting cells (83). Overall, these fate decisions are consistent with observations that memory B cells may overall be more broadly reactive and of lower affinity than long-lived antibody-secreting cells (84, 85).

Extrafollicular Responses

Some activated B cells do not enter the germinal center reaction and instead proliferate and differentiate *via* an extrafollicular pathway. Recent work utilizing a semiallogeneic mouse model of pregnancy suggests that the maternal B cell response to fetal antigens may proceed in a fashion independent of the germinal center, which may implicate the extrafollicular pathway in the development of maternal sensitization (46). In an excellent review, Elsner et al. distinguish between canonical B cell responses, in which a brief extrafollicular phase precedes the germinal center response, and non-canonical ones, which have extended extrafollicular phases (86). Although the factors which skew a response towards the non-canonical extrafollicular pathway are an area of active investigation, it appears that both pathogen and host factors play important roles (**Figure 3**). *Salmonella Typhimurium, Borrelia Burgdorferi, and Ehrlichia Muris* promote the development of a non-canonical extrafollicular response *via* lipopolysaccharide (LPS)- and tumor necrosis factor-α (TNF-α)-mediated collapse of traditional lymph node and splenic architecture (87, 88). Innate sensors and cytokines play a role in extrafollicular responses as well, as studies of systemic lupus erythematosus have shown that B cell TLR7 hyperresponsiveness and IL-21 signaling synergize to generate autoreactive antibody-secreting cells outside of germinal centers (89). Although it appears that only germinal centers can generate IgG-secreting long-lived antibody-secreting cells (90, 91), mice lacking germinal centers are still capable of generating IgM-secreting long-lived antibody-secreting cells that home to the spleen as opposed to the bone marrow (92). Aside from these differences in the output of antibody-secreting cells, extrafollicular responses are still capable of inducing AID expression, yielding somatic hypermutation and class-switch recombination, and generating antigen-specific memory B cells (87, 93, 94). Consequently, it will be important in future work studying pregnancy sensitization in both humans and mice to determine the isotype of the fetal-specific or anti-HLA antibodies which are detected. This information will allow us to draw inferences about whether B cell responses originate within the follicle (i.e. germinal center) or outside the follicle (i.e. extrafollicular response). Discrimination between these two pathways has significant implications for multiple facets of pregnancy sensitization, including the durability of the antibody that is generated.

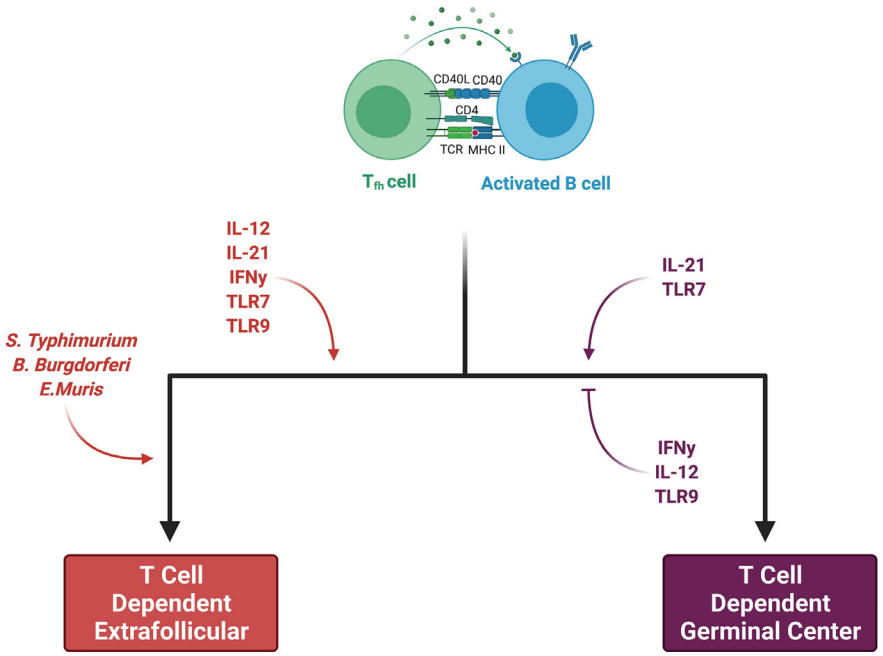

FIGURE 3 | Factors skewing responses toward either germinal center or extrafollicular pathways. Both pathogen and host factors contribute to a B cell's decision between an extrafollicular (EF) and germinal center (GC) response. Certain bacteria promote an EF response through collapse of secondary lymphoid organ (SLO) architecture. While IL-21 and TLR7 may promote both EF and GC responses, IL-12, IFN-y, and TLR9 all favor EF responses. IL-12 suppresses Tfh development while favoring short-lived ASC production. INF-y may have the same effects on Tfh suppression. TLR9 clearly stimulates EF responses, whereas it may inhibit GC responses, at least in autoimmune diseases. Figure created with BioRender.com.

FUNCTION OF MEMORY B CELL AND ANTIBODY-SECRETING CELL RESPONSES

Memory B cells provide the host protection from subsequent pathogen challenge through mechanisms that largely complement long-lived antibody-secreting cells. Importantly, multiple studies have shown that the reactivities of memory B cells and antibody-secreting cells do not overlap perfectly (85, 95). For example, memory B cells produced in response to certain viral infections are more broadly reactive than the antibodies produced by antibody-secreting cells, and these memory B cells may cross-react with different epitopes on other viral strains, affording protection from variants that are not neutralized by the antibodies produced by antibody-secreting cells (84, 85, 96). Memory B cell recall responses are facilitated by B cell receptor reactivity that is broader than the reactivity of long-lived antibody-secreting cells but is still antigen-specific, and memory B cells can rapidly respond to a second infection by differentiating into antibody-secreting cells or by entering germinal centers where they may affinity-mature, switch isotypes, and emerge as new memory B cells or high-affinity antibody-secreting cells (97). Although a mouse model with homologous boosting showed that memory B cells infrequently reentered germinal centers (98), humans respond to heterologous boosting with broadly reactive memory B cells that rapidly differentiate into antibody-secreting cells and reenter germinal centers (99). In addition to B cell receptor affinity, multiple other factors regulate the memory B cell recall response. Circulating antibodies (generated by long-lived antibody-secreting cells) may bind epitopes that memory B cells would otherwise recognize, effectively inhibiting a memory B cell recall response against those bound, cognate epitopes while promoting a response against exposed epitopes (100). The isotype of the memory B cells also appears to indicate its fate preference during a recall response, as both mouse and human IgM+ memory B cells show a propensity to reenter germinal centers, whereas IgG + memory B cells are more likely to differentiate into antibody-secreting cells (101, 102). It is not clear whether the isotype of the B cell receptor mediates this effect through properties related to intracellular domains of the B cell receptor or as a result of cell-intrinsic properties generated at the time of memory B cell formation.

HLA-REACTIVE B CELLS

HLA-reactive B cells have been studied following both pregnancy and transplant, revealing insights into their frequency, persistence, and specificity. Three techniques have been used in humans to study HLA-reactive B cells: 1) ELISPOT, 2) polyclonal activation, and 3) fluorochrome-labeled HLA

tetramers. Following pregnancy or transplant, HLA-reactive memory B cells are detectable by ELISPOT at frequencies of approximately 1 in 1,000 to 1 in 40,000 B cells, and these cells are notably absent from unsensitized individuals (103–106). Studies that polyclonally activated circulating HLA-reactive memory B cells and studied their supernatants have shown a restricted number of specificities as compared to circulating anti-HLA antibodies. However, "hidden sensitization," which is the presence of memory B cells which target HLA specificities that are not represented among circulating anti-HLA antibodies, is estimated to exist in perhaps 40% of sensitized patients (107, 108). Like ELISPOT, HLA tetramers have been used to estimate frequencies of HLA-reactive B cells following a sensitizing event. However, in light of data showing that up to 6% of B cells in sensitized humans and 1% of B cells in unsensitized humans bind such tetramers, the assay may lack specificity (109, 110). Notably, other investigators who have studied women sensitized through pregnancy have shown significantly lower frequencies, estimating that roughly 1 in 10,000 memory B cells is HLA-reactive (13). Concerns about the specificity of tetramer-binding B cells are likely justified, given that fewer than 30% of antibodies expressed from these tetramer-positive B cells bind the HLA molecule of interest, a finding consistent for both HLA class I and class II tetramers (13, 111). While these data therefore showcase how HLA-reactive memory B cells in humans can be captured and characterized, it is clear that available assays provide a limited view of prevalence and specificity. Given that the detection of HLA-reactive B cells in transplant patients carries prognostic significance (112), it is critical that we better understand the biology of these cells and close the significant gaps that remain in our understanding of how and where these cells differentiate, the signals governing their differentiation, and the molecular and epigenetic programs that guide this differentiation in humans. In summary, we conclude that although HLA-reactive B cells can be detected using current assays, their prevalence and the breadth of their anti-HLA repertoire may be underestimated given the use of small volume peripheral blood samples. Future work that pairs the above techniques with more advanced sampling, phenotyping, and antibody cloning approaches from vaccine (99, 113) and infectious disease studies (114) will enhance our understanding of HLA-reactive memory B cells and may provide insights that allow for better diagnosis and treatment of anti-HLA responses in transplant.

Murine models of pregnancy and allotransplantation have been used to overcome some of these limitations, and mouse investigations have thus revealed several insights into the origins and functions of alloreactive B cells (46, 115). Pertinent to this review, a recent study used peptide:MHC tetramers to examine alloreactive T and B cells concordantly in mice alloimmunized by either pregnancy or transplantation. The authors mated congenic mice and then performed allogeneic heart transplantation with and without co-stimulation blockade on the postpartum female partner to assess the role of pregnancy-induced alloimmunity on transplant outcome. The authors found that postpartum mice developed fetal alloreactive B and T cell responses and that the alloreactive T cell response remained tolerogenic after secondary heart allotransplantation. Next, the authors performed experiments on animals that lacked circulating immunoglobulins (sIgKO) or animals that lacked immunoglobulins and B cells (μMT) to dissect the relative roles of pregnancy-induced alloreactive memory B cells *versus* antibody-secreting cells in precipitating allograft rejection. Postpartum μMT animals were able to spontaneously accept allogenic heart transplants long-term without any immunosuppressive medication, suggesting that the alloreactive humoral immune arm that develops after pregnancy is critical to mediating subsequent organ transplant rejection. This spontaneous acceptance was lost when postpartum sIgKO were given allogeneic heart transplants, suggesting that even in the absence of circulating fetal specific antibody, a subset of alloreactive memory B cells elicited by pregnancy and recalled after transplant, was sufficient to abrogate tolerance. What subset of memory B cells mediates this break in tolerance and the mechanisms by which this occurs remain to be elucidated.

LESSONS FROM MEMORY B CELLS IN OTHER IMMUNE STATES

Although the authors do not perform molecular characterization of the alloreactive memory B cell compartment that develops in these postpartum mice and is recalled after subsequent allotransplantation, the authors perform limited immuno phenotyping. Despite the presence of circulating fetal-specific antibody, the alloreactive B cells of postpartum animals were found to lack classic germinal center markers in uterine-draining lymph nodes. Only after subsequent heart allotransplantation did the alloreactive B cells of postpartum animals express classical germinal center markers. Thus, the alloreactive B cells in postpartum animals after primary pregnancy appear to arise in extrafollicular reactions and have memory properties as they can be recalled after allotransplantation, present antigen to donor-specific T cells and form secondary antibody-secreting cells in germinal center reactions. These data are interesting in the context of recent insights into the functional diversity of antigen-specific memory B cells from the autoimmune (89) and immunization literature (116, 117) which have identified certain transcriptionally distinct antigen-specific memory B cells which are poised to directly form antibody-secreting cells in extrafollicular reactions (effectors) *versus* others that are recalled into germinal center reactions in lymphoid tissues to undergo affinity maturation and produce daughter antibody-secreting cells (effector memory) and daughter memory B cells (central memory). It will be important for future studies of the pregnancy-induced alloreactive immune response to clarify the transcriptional and functional diversity of this response in order to understand if particular alloreactive memory B cell subsets that develop after pregnancy sensitization are more or less important to mediating subsequent allograft rejection.

CONCLUSIONS

While our knowledge of B cell responses in pregnancy is underdeveloped, recent work suggests that fundamental mechanisms underlying B cell responses in infection are generalizable to the pregnancy setting. The further development of reagents that can track antigen-specific B cell responses in humans will be critical to improve our understanding of B cell differentiation and fate after pregnancy alloimmunization, including the generation of memory B cells, as well as short-lived and long-lived antibody-secreting cells. An improved understanding of these cell types and the mechanisms by which they arise will be critical to improve transplant access and outcomes among female transplant recipients and alleviate the significant sex disparity that exists in organ transplantation.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

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