



Marginal zone B-cells, a gatekeeper of innate immunity

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To maintain the integrity of an organism constantly challenged by pathogens, the immune system is endowed with a variety of cell types. B lymphocytes were initially thought to only play a role in the adaptive branch of immunity. However, a number of converging observations revealed that two B-cell subsets, marginal zone (MZ) and B1 cells, exhibit unique developmental and functional characteristics, and can contribute to innate immune responses. In addition to their capacity to mount a local antibody response against type-2 T-cell-independent (TI-2) antigens, MZ B-cells can participate to T-cell-dependent (TD) immune responses through the capture and import of blood-borne antigens to follicular areas of the spleen. Here, we discuss the multiple roles of MZ B-cells in humans, non-human primates, and rodents. We also summarize studies – performed in transgenic mice expressing fully human antibodies on their B-cells and in macaques whose infection with Simian immunodeficiency virus (SIV) represents a suitable model for HIV-1 infection in humans – showing that infectious agents have developed strategies to subvert MZ B-cell functions. In these two experimental models, we observed that two microbial superantigens for B-cells (protein A from *Staphylococcus aureus* and protein L from *Peptostreptococcus magnus*) as well as inactivated AT-2 virions of HIV-1 and infectious SIV preferentially deplete innate-like B-cells – MZ B-cells and/or B1 B-cells – with different consequences on TI and TD antibody responses. These data revealed that viruses and bacteria have developed strategies to deplete innate-like B-cells during the acute phase of infection and to impair the antibody response. Unraveling the intimate mechanisms responsible for targeting MZ B-cells in humans will be important for understanding disease pathogenesis and for designing novel vaccine strategies.

Keywords: *Staphylococcus aureus*, *Peptostreptococcus magnus*, HIV-1, Simian immunodeficiency virus, cynomolgus macaque, superantigen, protein A

To maintain the integrity of an organism constantly challenged by pathogens, the innate immune system is endowed with a variety of cell types. Initially, B lymphocytes were thought to play solely a role in the adaptive branch of immunity, with little impact on innate immunity. With further investigation, however, it became evident that B-cells can exert a number of antibody (Ab)-independent functions, capturing, and concentrating antigen for presentation, producing cytokines, influencing T-cell, and dendritic cell (DC) responses, contributing distinct functions during the immune response *in vivo*, affecting lymphoid tissue structures, and, even, participating in tissue repair (Zouali, 2008). A number of recent observations also indicate that B-cells engage in the innate arm of immune defense (Viau and Zouali, 2005;

Kearney, 2008). Central to this function are marginal zone (MZ) B-cells and B1 B-cells, two subsets of B lymphocytes that are classified as “innate B lymphocytes” due to unique developmental and functional characteristics. In rodents, these B-cell subsets exhibit an activated phenotype that allows their rapid proliferation and differentiation into Ab-secreting cells upon stimulation with thymus independent (TI) antigens (Ags). They seem to preferentially secrete antibodies of the IgM and IgG3 isotypes. Phenotypically, innate-like murine B lymphocytes express the cell surface molecule CD9, which distinguishes them from conventional follicular (FO) B-cells (Won and Kearney, 2002). Whereas murine B1 lymphocytes locate essentially in peritoneal and pleural cavities, MZ B lymphocytes are found principally in the MZ of the spleen, where they represent 5% of total splenic B-cells. The MZ is recognized as a microanatomical site that allows the transit of cells leaving the bloodstream through the erythrocyte-rich red pulp and entering the lymphocyte-rich white pulp. Its specialized organization facilitates filtering of blood-borne pathogens, and initiation of innate and adaptive immune responses. However, the MZ shows prominent structural variation in different mammals – well developed in rodents, intermediately in humans and non-human primates,

Abbreviations: Ab, antibody (dies); Ag, antigen; APC, antigen presenting cell; APRIL, a proliferation-inducing ligand; BAFF, B-cell activating factor of the TNF family; DC, dendritic cell; FO, follicular; HIV, human immunodeficiency virus; iNKT, invariant natural killer T-cell; MGZ, marginal zone; MMM, metallophilic macrophage; MZM, marginal zone macrophage; SAg, superantigen; SpA, *Staphylococcus aureus* protein A; SIV, Simian immunodeficiency virus; TD, T-cell-dependent, TI, T-cell-independent; TLR, Toll-like receptor.

and poorly developed in the canine and feline species (Koike et al., 1996). Whereas the white pulp is specialized in the adaptive response including humoral responses to thymus-dependent (TD) Ags, the MZ is more specialized in the response to blood-borne pathogens (bacteria, viruses) and to TI Ags (Kearney, 2008). This functional distinction between TD and TI responses correlates with the presence of FO and MZ B-cells, two specialized B-cell populations. To be fully efficient in mounting Ab responses, these B-cells must establish, in a well-controlled spatiotemporal manner, interactions with specialized subsets of macrophages, DCs, and fibroblast-like cells and respond to environmental cytokines.

Here, we focus on the role of the MZ B cell subset in immune defense and discuss how infectious agents can deploy strategies to subvert this important arm of immunity in mice, and in human and non-human-primates.

THE MARGINAL ZONE OF THE SPLEEN, FROM STRUCTURE TO FUNCTION

Several observations indicate that the human spleen plays an important role in defense against infectious agents. For example, the low Ab response found in young and aging individuals is associated with immaturity and compromised functions of the spleen MZ, respectively (Timens et al., 1989). Second, individuals with splenic dysfunctions or splenectomy are highly susceptible to infection with *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*, or other encapsulated bacteria, and this susceptibility correlates with a deficit in circulating MZ B-cells and a lack of Ab responses against these pathogens. Third, patients with common variable immunodeficiency presenting with recurrent infection of the respiratory tract and chronic lung disease have an extremely low frequency of IgM memory B-cells and do not produce anti-polysaccharide IgM (Carsetti et al., 2004). Fourth, splenic dysfunctions may also occur in patients with sickle-cell anemia known to manifest increased susceptibility to infection (Wong et al., 1992) or inflammatory bowel disease (Di Sabatino et al., 2007), in whom both impaired IgM production by MZ B-cells and reduced phagocytosis of opsonized particles prevent the clearance of encapsulated bacteria. Taken together, these observations indicate that the spleen exerts specific protective functions that cannot be totally replaced by other lymphoid organs during adult life. Yet, a normal blood MZ B-cell compartment is present in young children with congenital asplenia, suggesting that MZ B cell precursors can colonize alternative sites to develop (Weller et al., 2004). Similarly, cells with morphology and phenotype of MZ B-cells have been observed in the subcapsular sinus of lymph nodes, under the dome epithelium of Peyer's patches in the gut and in the crypt epithelium of tonsils (Spencer et al., 1998; Dono et al., 2000). It remains to be established whether these cells play a role in the defense against encapsulated bacteria.

MARGINAL ZONE B-CELLS

In the mouse, MZ B-cells differ in many respects from the predominating subset of mature re-circulating FO B-cells. Whereas the latter are small surface (s)IgM^{low}sIgD^{high} B-cells, the former are intermediate-sized sIgM^{high}sIgD^{low} cells located in a distinct area surrounding the B cell follicles and the periarteriolar lymphoid sheath. MZ B-cells exhibit a low re-circulating potential

and are involved in type-2 TI responses to lipopolysaccharide Ags that form the major constituent of cell walls of encapsulated bacteria, such as *S. pneumoniae*, *N. meningitidis*, and *H. influenzae*. *Ex vivo*, purified MZ B-cells respond generally more vigorously to mitogens like LPS, dextran-conjugated anti-IgM or IgD Abs, or CD40 ligation, than FO B-cells. Phenotypically, they exhibit a pre-activated state (pyroninophilic cytoplasm, less condensed nuclear chromatin, complement receptor CD21^{high}, CD23^{low}, CD35^{high}, and higher basal levels of CD80 and CD86). Reduced levels of CD23, a negative regulator of B-cells, on MZ B-cells may allow them to produce a rapid Ab response in comparison to FO B-cells. These remarkable features, together with their unique anatomical location in the spleen account for the fact that MZ B-cells are endowed with powerful immunological functions.

In rodents, splenic MZ B-cells are a heterogeneous population of cells comprising ≈80% of naive B-cells and a minor fraction (20%) with signs of Ag-selection (Dammers et al., 2000). Naive MZ B-cells harbor germline-encoded variable region genes and produce polyreactive Abs that play a crucial role in the first-line of defense against blood-borne infections with encapsulated bacteria. Such polyreactive naturally occurring Abs are essential in the primary immune response against pathogens (Zouali, 2009). Thus, by providing a strategically located buffer of polyreactive B-cell receptors (BCRs) in the spleen, naive MZ B-cells form an integral part of innate immunity.

THE FUNCTIONS OF MZ B-CELLS

In addition to the involvement of MZ B-cells in the initial response to TI Ag, other studies demonstrate that activated MZ B-cells are potent protein Ag presenters to CD4⁺ T-cells, and have the ability to induce Ag-specific T-cell clonal expansion both *in vitro* and *in vivo* (Attanavanich and Kearney, 2004). Specifically, MZ B-cells can mount rapid and efficient primary responses to soluble protein Ag and have an extraordinary ability to promote T-cell proliferation and cytokine production after immunization with protein Ag. Within a few hours of *in vivo* Ag priming, MZ B-cells are far superior to FO B-cells in the activation of naive CD4⁺ T-cells *in vitro*, and higher levels of B7.2 are expressed on the cell surface of MZ compared with FO B-cells after Ag stimulation.

As discussed above, MZ B-cells comprise an innate subset localized to the marginal sinuses of the spleen. Because they have the capacity to respond to specific foreign Ag more rapidly than FO B-cells, they provide early immune responses to blood-borne particulate Ags. In addition to being an early source of pathogen-specific T-cell-independent IgM, MZ B-cells serve to bridge the innate and adaptive immune systems. For example, studies of the spirochete *Borrelia hermsii* showed that MZ B-cells directly associate with blood-borne *Borrelia* spirochetes and are activated *in vivo* to produce pathogen-specific IgM within 72 h of infection (Belperron et al., 2005). When mice were depleted from MZ B-cells to determine their contribution to host defense against *B. burgdorferi*, the deficient animals showed a reduced *B. burgdorferi*-specific IgM, elevated pathogen burden, and reduced levels of *B. burgdorferi*-specific IgG that correlated with diminished splenic CD4⁺ T-cell responses (Belperron et al., 2007). These results demonstrate a role for MZ B-cells not only in *Borrelia*-specific IgM production, but also in promoting the early adaptive T- and B-cell responses

to this spirochete. They reveal an important role for this B-cell subset in priming splenic T-cells and the subsequent development of T-cell-dependent IgG responses during infection.

Recent studies suggest that in addition to TLR signaling in DCs, direct TLR-mediated activation of B-cells is also required for eliciting humoral immune responses. Thus, murine B-cells can be stimulated *in vitro* by TLR4 and TLR9 ligands to proliferate and secrete Ab (Whitlock and Watson, 1979). *In vivo*, chimeric mice in which only B-cells are deficient in TLR signaling fail to mount Ab responses to protein Ags given with adjuvants (Pasare and Medzhitov, 2005). Consistent with these observations, agonists to intracellular and extracellular TLRs activate MZ B-cells *in vivo* and promote their release from the MZ, thus potentially accelerating the kinetics of the Ag-specific IgM response (Rubtsov et al., 2008). In response to TLR agonists, MZ B-cells proliferate and exhibit a phenotypic maturation process characterized by an increased expression of MHC class II, CD40, and CD86 molecules. According to the TLR agonist used, they also display a different cytokine profile (Bialecki et al., 2009). The fact that MZ B-cells are well equipped in TLRs and that their stimulation leads to cell proliferation, maturation (i.e., cytokine production) and Ab production (Rubtsov et al., 2008) further supports the conclusion that they play a role as innate sensors in MZ B cell-mediated immune responses.

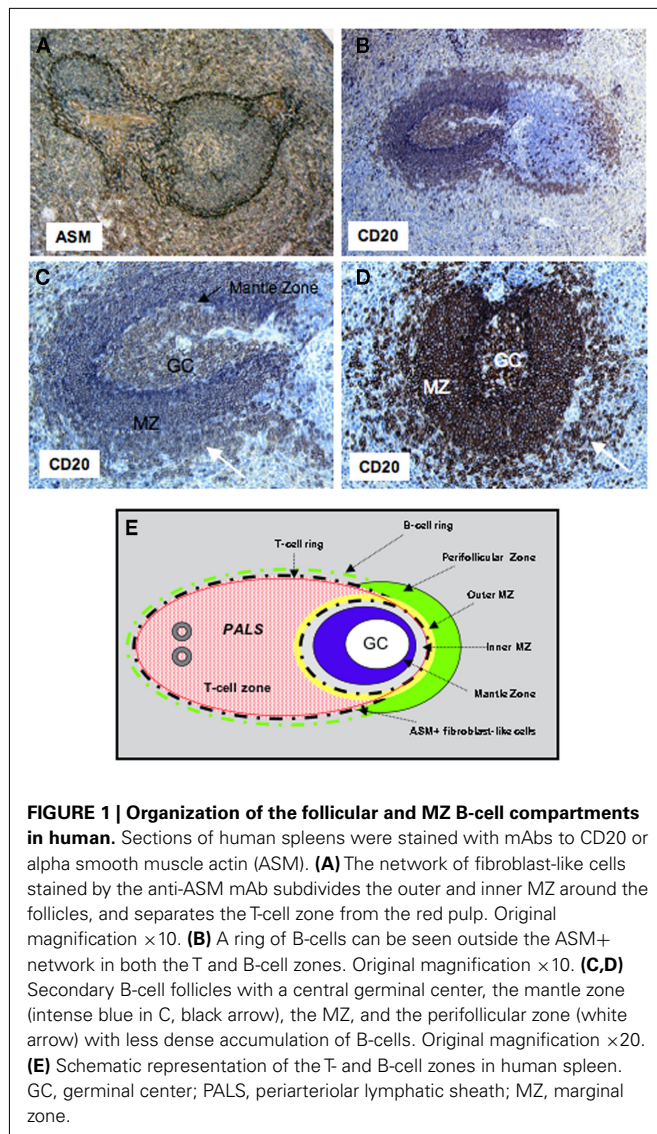
Strikingly, MZ B-cells are able to promote optimal immune responses by interacting with other cell types. For instance, they can transport immune complexes to follicular DCs and deposit them on their surface, or process Ag for direct presentation on MHC class II molecules to naïve CD4⁺ T-cells (Martin and Kearney, 2002; Attanavanich and Kearney, 2004). Further evidence comes from studies of invariant NKT (iNKT) cells, a subset of NKT cells that recognize exogenous and self glyco/lipid Ags presented by the non-classical class I molecule CD1d expressed on Ag presenting cells (APC; Bendelac et al., 2007). Upon primary stimulation, iNKT cells produce large amounts of immunoregulatory cytokines, including IFN- γ and IL-4 that lead to downstream activation of DC, NK cells, B-cells, and conventional T-cells (Bendelac et al., 2007). In the mouse, iNKT cells localize in the splenic B-cell area, including the MZ. In the presence of accessory cells (DC), MZ B-cells stimulate the release of both IFN- γ and IL-4 by iNKT cells, strongly suggesting that MZ B-cells have the potential to condition iNKT cell functions *in vivo* (Bialecki et al., 2009). The observation that MZ B-cells play a role in iNKT cell activation *in vivo*, together with the fact that the BCR facilitates the uptake of particulate Ags and their transport to CD1d-containing endocytic compartments, reinforce the view that the MZ B cell/iNKT cell interactions play important roles in the immune response.

It is remarkable that the MZ is a dynamic structure in which cell retention and trafficking require specific cell interactions. Histologically, the murine MZ comprises mucosal addressin cell adhesion molecule 1 (MAcCAM-1⁺) sinus-lining reticular endothelial cells, marginal metallophilic macrophages (MMM) that stain with Abs to the Monocyte/Macrophage Marker (MOMA-1), and SIGN-R1⁺ (specific intracellular adhesion molecule-grabbing non-integrin receptor 1) MZ macrophages (MZM). Apart from MZ B-cells, MZM represent the other predominant cell type of the MZ. They are distinct from MMM which express the marker

MOMA-1 and are located at the border of the marginal and follicular zones. MZM are interspersed in several layers within the MZ, and express the markers MARCO, a scavenger receptor belonging structurally to the class A receptor family, and ER-TR9 which is identical to the C-type lectin SIGN-R1. Mouse MZM are highly phagocytic and responsible for clearance of blood-borne TI antigens and apoptotic material entering the spleen. Whereas MARCO binds a range of microbial Ags, including *Staphylococcus aureus* and *Escherichia coli*, SIGN-R1 is the predominant receptor for uptake of *S. pneumoniae* and polysaccharide dextran by MZMs (Lanoue et al., 2004; Birjandi et al., 2011). Importantly, the dialog between MZ B-cells and MZM is essential to maintain the MZ structure and to allow efficient immune responses (Karlsson et al., 2003). For example, once MZM bind pathogen, they establish direct cell-cell interactions with MZ B-cells that are required for potent responses (Chen et al., 2005), allowing the clearance of *Leishmania donovani* and *Listeria monocytogenes* (Odermatt et al., 1991; Aichele et al., 2003). In another model system, MZ B-cells were found to regulate the expression of molecules on macrophages that are important for trapping Ag, which, in turn, is required for Ag capture by the B-cells, thus modulating MZM functions that are important to protect against lethal infection (You et al., 2011). Consistently, the decreased frequency of MZM and MZ B-cells seen in old mice correlates with disruption in the marginal sinus and reduced capacity to bind and clear pathogen-like particles (Birjandi et al., 2011). Thus, MZ B-cells not only provide a link between the innate and adaptive immune responses, but they also function as regulatory cells that control the phenotype and function of other cells involved in the acute innate immune response.

HUMAN MZ B-CELLS

Studies of the Ig genes expressed by MZ B-cells of normal untreated, non-intentionally immunized rodents revealed that the majority of the splenic cells exhibit little mutations in their rearranged V_H genes and, thus, represent naïve B-cells, and that a minor fraction (20%) of the MZ B-cell pool express somatically mutated V_H genes (Dammers et al., 2000). These features contrast with the situation in the human spleen where the majority (>85%) of splenic MZ B-cells are somatically mutated (Dunn-Walters et al., 1995; Tierens et al., 1999), suggesting that memory B-cells constitute the majority of spleen MZ B-cells. These diversification characteristics could either reflect a heavy load of somatic hypermutation occurring during clonal expansion in the MZ, or a process of somatic hypermutations acquired in germinal centers (GCs) at distant sites before cell migration to the MZ. However, the microanatomical structure of the human spleen differs from that of rats and mice with respect to MZ organization and the presence of a so-called perifollicular zone surrounding the MZ (Steiniger et al., 2006). In contrast to rodents, the human spleen lacks a marginal sinus, and the MZ surrounds B-cell follicles, but not the PALS (periarteriolar lymphatic sheath) in the T-cell rich area. Yet, an additional compartment, the perifollicular zone, which surrounds B-cell follicles, makes a transition between the MZ and the red pulp (Figure 1). This zone has a dynamic composition with strong similarities with the red pulp in terms of terminal sinuses, blood-filled spaces, sheathed capillaries without endothelial lining,



and scattered B- and T-cells. Because terminal vessels directly open into the perifollicular zone, Ags and lymphocytes likely exit the circulation in this structure and traffic either to the MZ or to the red pulp (Timens, 1991). A meshwork of fibroblast-like cells, expressing alpha smooth muscle actin (ASM) and MadCAM-1 subdivides the MZ into large inner and small outer compartments, the latter being in close contact with the perifollicular zone. Along the network of ASM-positive cells, a small ring of B-cells delimits the T-cell zone from the red pulp, whereas a ring of T-cells is frequently present between the inner and outer MZ (Steiniger et al., 2006).

Interestingly, the MZM and MMM subsets found in rodents are lacking in humans. However, macrophages expressing CD68 (lysosome/macrosialin) and CD169 (sialoadhesin) preferentially form sheaths around capillaries in the perifollicular area, but can be also present as scattered cells expressing DC-SIGN (CD209; Steiniger et al., 2006). It remains unclear whether these macrophages can replace MZM in trapping pathogens and interacting with MZ B-cells. In humans, CD11c⁺CD205⁺ DCs are intertwined with

MadCAM-1⁺ cells at the inner border of the perifollicular zone whereas plasmacytoid DCs are absent from MZ at the steady state (Steiniger et al., 2006; Pack et al., 2008).

Mouse and human MZ B-cells can be distinguished from FO B-cells as being SIgM^{high}CD21^{high}SIgD^{low}CD23⁻. In humans, MZ B-cells are CD27⁺ memory B-cells and generally express somatically diversified V_H genes (Dunn-Walters et al., 1995; Tangye et al., 1998; Weller et al., 2004). Intriguingly, CDR3 spectratyping evidenced a higher clonal diversity in MZ B-cells than in other human memory B-cells (Weller et al., 2008). Starting from less than 1% in the cord blood, the frequency of MZ B-cells increases concomitantly with that of switched memory blood and spleen B-cells, and the frequency of Ig variable region gene mutation in blood MZ B-cells reaches adult value by 2–4 years of age. Overall, the available findings suggest that MZ B-cells constitute a pool of pre-diversified B-cells, with a slow rate of renewal, which can be rapidly mobilized by pathogens or polysaccharidic vaccines. Because MZ B-cells persist in CD40 or CD40L-deficient patients, it has been suggested that they develop independently of GCs and T-cells (Weill et al., 2009). The fact that BAFF and APRIL have been shown to support TI responses, activation-induced cytidine deaminase expression and hypermutation, and that BAFF is produced in the MZ may indicate that these cytokines contribute to diversification of MZ B-cells (Litinskiy et al., 2002).

SUBVERSION OF INNATE-LIKE B CELL IMMUNITY BY SUPERANTIGENS

Studies of the confrontation of the mammalian host with infectious agents led to identification of subversion mechanisms that allow pathogens to persist in the organism (Hasler and Zouali, 2003). Such a strategy is used by *S. aureus* to impact MZ B-cells. This bacterium is a leading cause of human infections worldwide. It causes a variety of diseases, including impetigo, cellulitis, food poisoning, toxic shock syndrome, necrotizing pneumonia, endocarditis, and sepsis (Archer, 1998). Our understanding of the molecular mechanisms used by this pathogen to avoid destruction by host defenses remains limited. Even though *S. aureus* produces a variety of virulence factors, protein A (SpA) is the prototype staphylococcal surface protein. In addition to its Fc IgG binding capacity, which can be used for Ig purification, SpA also interacts with the V_H region of Igs, a property responsible for its superantigen (SAg) activity for B-cells (Zouali, 1995, 2007b). To test the effect of SpA *in vivo*, we used “XMG2L3” transgenic mice whose mouse Ig loci were inactivated and their humoral immune system restored by introduction of human V_H, V_K, and V_L loci in a germline configuration. As a result, their B lymphocytes express fully human surface Igs, and secrete IgM and IgG2 bearing κ or λ-human L-chains. A remarkable feature of these mice is that half of the V_H genes present on the translocus belong to the V_H3 gene family, a figure that closely mimics the complexity of the human Ig V_H locus. When immunized, they mount antigen-specific antibody responses with somatic mutation, isotype switching and affinity maturation.

Analyzing SpA effects in peripheral lymphoid organs, we found that a single injection of SpA to the transgenic humanized mice reduces B-1a cells in the peritoneal cavity and V_H3⁺ B-cells in the spleen. Importantly, even repeated administrations

of this SAg do not modify B-1b or B-2 cell numbers (Viau and Zouali, 2005). In the spleen of treated mice, we studied in detail the fate of various B cell subpopulations, including newly formed B-cells (B220⁺CD23⁻CD21⁻), transitional T1 (B220⁺CD21⁺IgM⁺), transitional T2 (B220⁺CD21⁻IgM⁺), re-circulating FO (B220⁺CD23⁺CD21⁺) and resident MZ B-cells (B220⁺CD23^{-/low}CD21⁺). Remarkably, we found that SpA induced a specific reduction of the MZ subpopulation (Viau and Zouali, 2005). The molecular basis of this specific deletion of MZ B-cells, as opposed to FO cells, remains unresolved. It is likely that the disparity in responses of MZ and FO B-cells to SpA reflects a functional dichotomy in these two splenic subpopulations.

In single cell PCR-based studies and sequencing of the expressed genes, we demonstrated that the reduction of MZ B-cells in SpA-treated mice reflects the decrease of the absolute number of V_H3-expressing B-cells in this subset (Viau and Zouali, 2005). Thus, the effect is a direct consequence of V_H3⁺ B-cell targeting capacity of SpA. To determine whether the SpA B-cell deleting potential had functional consequences, we immunized SpA-treated mice with either a type-2 TI Ag (DNP-Ficoll) or a TD Ag (tetanus toxoid). These studies disclosed a specific impact of SpA on the type-2 TI response (Viau and Zouali, 2005).

It is remarkable that, investigating the *in vivo* effects of another bacterial SAg, protein L from *Peptostreptococcus magnus*, we found a similar impact on B-cells with innate-like functions. In contrast to protein A, protein L from *P. magnus* binds predominantly to κ-light chains, regardless of the H-chain subclass, and it has affinity for all Ig classes. Since approximately two-thirds of human Igs have κ-type L-chains, protein L interacts with a significant proportion of Igs. *In vitro*, protein L acts as a SAg for human Igs. It also cross-links the V_L domains of IgE bound to Fcε receptors, and stimulates the release of histamine by basophils and mast cells (Zouali, 1995, 2007a). To study protein L effects *in vivo*, we used “5-feature” transgenic mice whose endogenous loci coding for the H- and L-chains have been inactivated, and human H-, κ, and λ chain transloci were “knocked-in.” Humanized mice injected intraperitoneally with 1 mg of recombinant protein L led to a loss of mature B-cells in the peripheral immune system and a reduction of protein L⁺ B-cells in the spleen. Further characterization of B-cell subsets in protein L-injected mice revealed a depression of MZ B-cells in the spleen and a reduction of CD5⁺ B-cells (B1) in the peritoneal cavity (Viau et al., 2004b).

IMPACT OF HIV-1 ON INNATE-LIKE B-CELLS IN A MODEL OF MICE EXPRESSING FULLY HUMAN IGS

Although there is no convincing evidence that HIV-1 infects B-cells, marked changes in B-cell responses have been described, including hyper-gammaglobulinemia, increased expression of markers of activation and terminal differentiation, augmented secretion of Igs *in vitro* and *in vivo*, and increased susceptibility to apoptosis (Viau and Zouali, 2001; Cagigi et al., 2008). Several longitudinal and cross-sectional studies have indicated that most of these B-cell abnormalities are directly associated with high levels of HIV-1 plasma viremia, and its reduction, by effective anti-retroviral therapy, diminishes polyclonal and HIV-1-specific Ig levels, partially restores APC function and responsiveness to T-cell help. Even efficient anti-retroviral therapy only partially

normalizes expression of B-cell activation markers, proportions of memory B-cells, and terminal differentiation (Viau and Zouali, 2001; Cagigi et al., 2008). Thus, B-cell dysfunctions represent a central feature of chronic HIV-1 infection, with potential pathogenic consequences.

Although the mechanisms of these abnormalities remain the focus of much investigation, direct and indirect effects of HIV-1 remain possible. One possibility is that the capacity of gp120 to act as SAg for human B-cells could account directly for the biased repertoire expression. *In vitro*, gp120 binds human V_H3⁺ Igs, and activates proliferation and differentiation of V_H3⁺ B-cells (Berberian et al., 1993). As it is hard to assess the consequences of these *in vitro* and *ex vivo* observations in patients, we used mice into whose genome human Ig loci have been introduced, along with functional inactivation of the endogenous mouse IgH and Igκ loci (Viau et al., 2007). In these transgenic animals, B-cells expressing a virtually full human Ig repertoire develop, allowing investigation of the *in vivo* consequences of confronting B-cells expressing human Igs with soluble gp120 or inactivated virions. We found that soluble gp120 induced an inversion in the B-1a/B-1b cell ratios, without impacting B-2 cells or affecting substantially the T-cell compartment. Treatment with inactivated AT-2 virions specifically and dramatically depressed B-1a cells, which represent the majority of B1 cells in normal mice. The observed B-cell changes were associated with a functional alteration of the humoral response to tetanus toxoid. Thus, the results reveal a capacity of HIV-1 to specifically impact a highly specialized B cell subpopulation. Because there is evidence that human IgM memory B-cells are functionally equivalent to murine B-1a cells, our findings suggest that gp120 may have a direct deleting activity on B-cell memory.

IMPACT OF SIMIAN IMMUNODEFICIENCY VIRUS ON MZ B-CELLS IN THE CYNOMOLGUS MACAQUE

In addition to the presence of immature/transitional CD10⁺ or exhausted CD27 negative B-cells in the peripheral blood (Ho et al., 2006), alterations in the architecture of GC and splenic MZ were observed in HIV-infected patients with advanced disease (Legendre et al., 1998; Wilkins et al., 2003). Despite the presence of polyclonal activation, HIV-1-specific Ab responses are strongly impaired, resulting in a decreased response to natural or vaccine TI and TD antigens (Scamurra et al., 2000; Hart et al., 2007), and a loss of peripheral memory B-cells (De Milito et al., 2001; Chong et al., 2004; Titanji et al., 2006). The view that spleen dysfunctions occur during HIV infection is also supported by observations showing that an increased frequency and severity of infection by encapsulated bacteria is a sign of AIDS progression in HIV-1-infected children that has been correlated with histological immaturity or dysfunction of the spleen MZ in HIV-negative children (Timens et al., 1989).

Because recent studies in humans suggest that virus-induced early activation has a major role in shaping the repertoire of memory B-cells and plasma cells, and in trafficking (Badr et al., 2005; Titanji et al., 2005; He et al., 2006; Hart et al., 2007), it became important to examine changes in B-cell subsets in different lymphoid organs during the acute phase of infection. As the study of primary HIV infection in humans is limited by the availability of

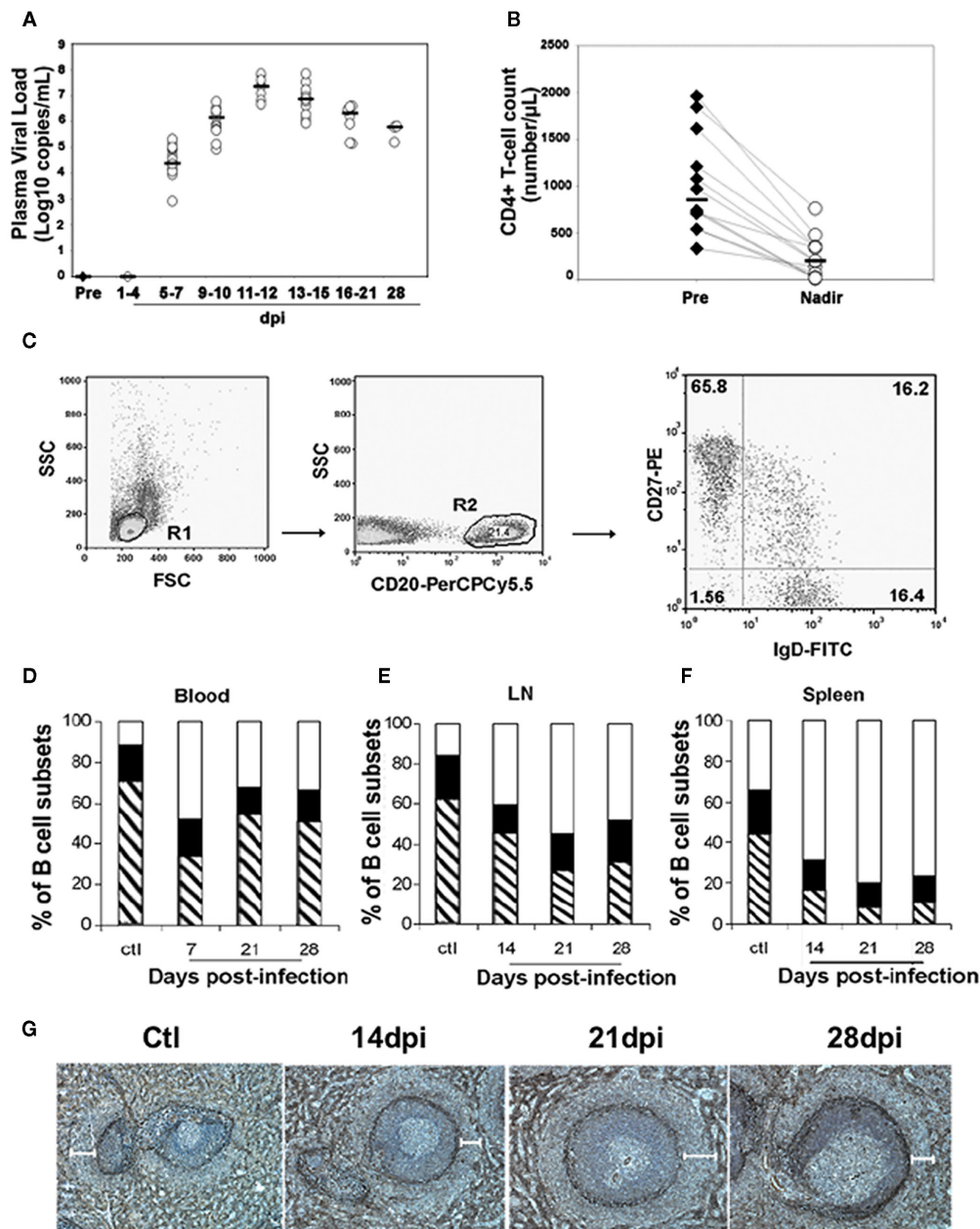


FIGURE 2 | Loss of MZ B-cells and switched memory B-cells during the acute phase of SIV infection. Macaques were infected intravenously with the pathogenic SIVmac251 strain. For each macaque, blood samples were taken before infection and every 3 days post-infection (dpi). Three groups of five macaques were sacrificed 14, 21, and 28 dpi. At euthanasia, spleen, peripheral blood, and mesenteric lymph nodes (LN) were collected from SIV-Infected animals and from four non-infected control animals. **(A)** Plasma viral load was determined by real-time PCR as described (Peruchon et al., 2009). Horizontal bars indicate median values. **(B)** Absolute numbers of blood CD4 T-cells were determined in each animal as described (Peruchon et al., 2009) before infection and 11–12 dpi when they reached their lowest values (nadir). Horizontal bars indicate median values. **(C)** CD27 and SIgD expression was studied by three-parameter immunofluorescence analysis on CD20-gated populations from various organs. Cells were gated on forward and side scatter (R1), and then on CD20 (R2) expression. CD27 and SIgD expression on CD20⁺ cells is shown on a representative dot plot of

peripheral blood mononuclear cells staining. The percentage of positive cells is indicated in each quadrant. Flow cytometry plots depict log₁₀ fluorescence. **(D–F)** Shown are the mean percentages of naive (SIgD⁺CD27⁻, open area), switched memory (SIgD⁻CD27⁺, hatched area), and MZ (SIgD⁺CD27⁺, black area) B-cells for each group of animals ($n = 3$). **(D)** Shows data in peripheral blood before and after exposure to SIV. **(E)** Shows data from the spleen of non-infected animals (ctl, $n = 4$) and SIV-infected animals ($n = 3$ for all groups). **(F)** shows data from LN of non-infected animals (ctl, $n = 4$) and SIV-infected animals ($n = 5$ for all groups). **(G)** Spleen sections from non-infected macaques (Ctl) and from SIV-infected animals collected 14, 21, and 28 dpi were stained with alpha smooth muscle actin (ASM) mAb for visualization of the MZ. Shown are staining for one representative animal from each group. White bars indicate the MZ region width as measured in 19–22 ASM-stained regions in each spleen. Mean values ± SEM are 132 ± 14 μm for Ctl, and 114 ± 23, 136 ± 27, 106 ± 17 μm for macaques infected for 14, 21, and 28 dpi, respectively.

lymphoid organs and by the difficulties associated with performing longitudinal investigations using tissues other than peripheral blood, we used a model of experimental pathogenic infection in cynomolgus macaques by Simian immunodeficiency virus (SIV), a suitable model that reproduces the long-lasting HIV-1 disease (Peruchon et al., 2009). We found a decrease in switched memory and MZ B-cells that differed in intensity and kinetics in the various organs (Figure 2). This decrease correlates with rapid homing of

peripheral B-cells into secondary lymphoid organs and their preferential retention in the spleen and the small intestine where SIV replication is particularly intense. Although the histological size of the MZ did not significantly vary, the frequency of MZ B-cells decreased steadily in the spleen and transiently before 21 dpi in peripheral and mesenteric lymph nodes. The MZ B-cell loss was concomitant with a transient peak of apoptosis on 14 dpi and their increased commitment to TI terminal differentiation (Figure 3).

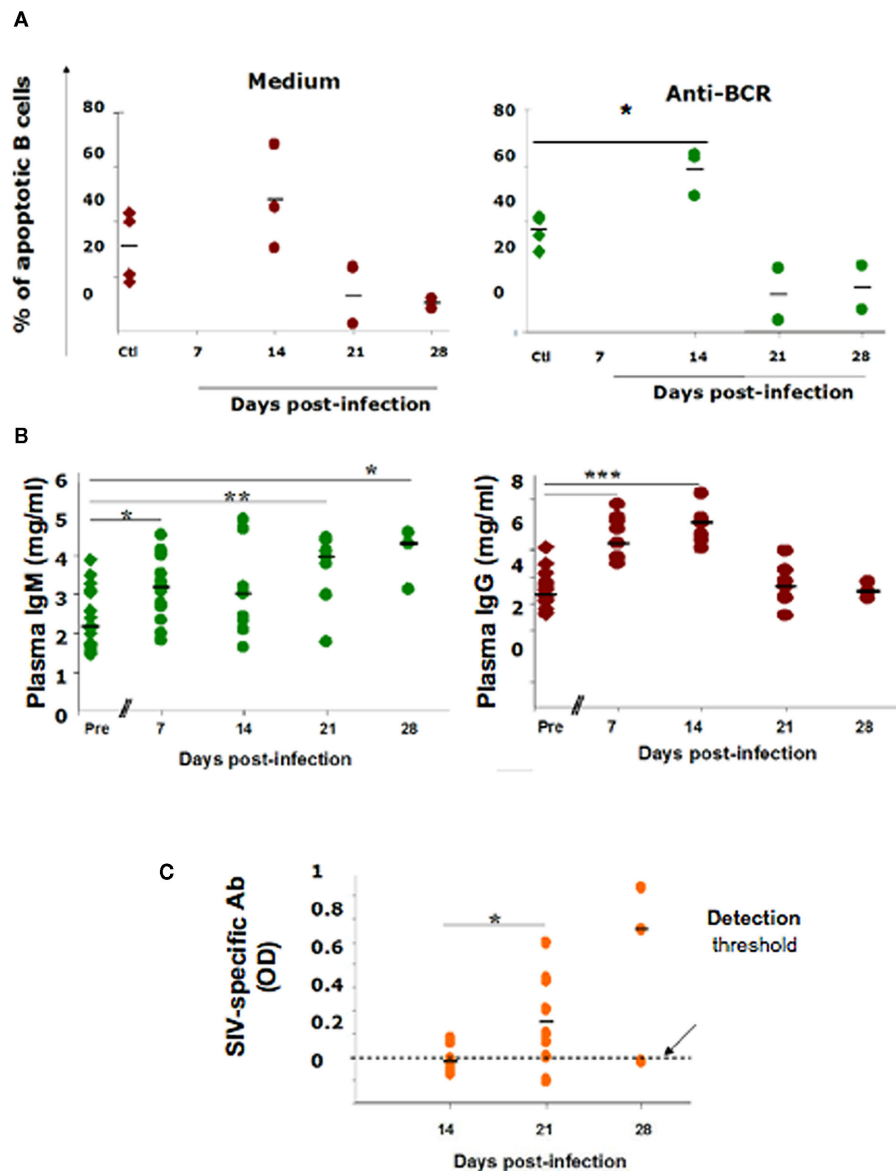


FIGURE 3 | B-cell apoptosis and antibody production in acutely SIV-infected macaques. (A) Spleen B-cells were purified from macaques using PE-conjugated anti-CD20 mAb and PE-conjugated magnetic beads. They were cultured for 24 h with medium or with 25 μ g/ml rabbit anti Ig(H + L) Ab before staining with DAPI. Apoptotic cell percentages were determined in splenocyte cultures from four non-infected animals (Ctl, diamond symbols) and from two SIV-infected macaques sacrificed on 14, 21, or 28 dpi (circle symbols). Each point represents the percentage of

hypodiploid B-cells from one animal. Bars represent the mean percentages. * p Value <0.05. (B) Plasma IgM and IgG concentrations were quantified before infection (Ctl, diamond symbols) and 7, 14, 21, and 28 dpi (filled circles). Horizontal bars indicate the median concentrations. Statistically significant differences are indicated (* p < 0.05; ** p < 0.01; *** p < 0.001). (C) IgG, IgM, and IgA SIV-specific Ab concentrations in the plasma of SIV-infected macaques 14, 21, and 28 dpi. Horizontal bars indicate the median OD values. * p Value <0.05.

Indeed, we observed an increased density of IgG plasma cells in the MZ and the red pulp, and augmented plasma IgG and IgM levels before 14 dpi. In contrast, new IgG and IgM plasma cells in GCs and plasma SIV-specific Ab were only detectable after 21 dpi. It is possible that SIV-induced soluble FasL and TRAIL, or a direct interaction between gp120 and V_H3-expressing BCRs contributed to this MZ B-cell apoptosis (Berberian et al., 1993; Karray and Zouali, 1997; Samuelsson et al., 1997; Herbeuval et al., 2006). Since gp120-induced production of BAFF by monocytes can enhance polyclonal IgG production by a subset of MZ B-cells (He et al., 2006), the Ig production we saw would rather be promoted by increased cytokine production, including BAFF and APRIL (Ettinger et al., 2007). Consistently, we observed a transient increase in plasma BAFF levels with a peak on 11–12 dpi. During the acute phase of infection, increased plasma levels correlated with plasma viral load, CD4 T-cell depletion and plasma IgG. In these SIV-infected macaques, no change in plasma APRIL was observed (Yolande Richard, unpublished data). As previous studies in the mouse suggest (Lesley et al., 2004; Thien et al., 2004), BAFF overexpression may also contribute to the preferential production of polyclonal or autoreactive Ab at the expense of SIV-specific Ab.

As MZ and switched memory B-cells home together in the spleen MZ, their concomitant depletion during the acute phase of infection is intriguing, and may suggest the existence of a common mechanism. In viremic HIV-infected patients, a sustained BAFF overexpression was postulated to impair the trafficking of B-cells between the MZ and B-cell follicles (Fontaine et al., 2011). In support to this conclusion, we had previously shown that *in vitro* short-term exposure of human B-cells to BAFF preferentially increased the chemotactic response of CD27⁺ B-cells to CXCL13 (Badr et al., 2008). It is possible that type I IFN and BAFF, produced early during the acute phase of infection, may increase the retention of MZ B-cells into B-cell follicles, decrease the import of virus particles, and delay the onset of virus-specific TD responses. This hypothesis is consistent with SIV-specific Ab

being detectable in the serum only after the decrease of plasma type I IFN and BAFF (e.g., after 16 dpi). A better understanding of the mechanisms and consequences of this death on the Ab response will be essential for vaccine development.

CONCLUSIONS

As discussed above, MZ B-cells are a first-line of defense against blood-borne pathogens entering the spleen freely through the blood or transported by circulating APCs. They represent the main B-cell population involved in the generation of rapid TI responses to blood-borne bacteria. They play an important role in the production of early Ab responses against infectious bacterial and virus particles. Splenectomized mice or mice lacking functional MZ B-cells produce delayed Ab responses to infectious agents that require T-cell collaboration. The easy accessibility of MZ B-cells to blood-borne Ags, their lower threshold of activation, and the enrichment in clones specific for TI antigens account for the critical role that this B-cell subset plays in the induction of rapid TI Ab responses.

Taking into account that infectious agents are complex and composed of both TD and TI epitopes, MZ B-cells are likely to be heterogeneous, multireactive, and react with a wide variety of pathogen-associated Ags. Yet, the observations summarized above and obtained in a model of mice expressing human Igs (Viau et al., 2004a,b, 2005, 2007; Viau and Zouali, 2005) and in a non-human primate (Peruchon et al., 2009) revealed that viruses and bacteria have developed strategies to deplete MZ B-cells during the acute phase of infection. Unraveling the intimate mechanisms responsible for targeting MZ B-cells in humans and non-human primates will be important for understanding disease pathogenesis and for designing novel vaccine formulations.

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