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Marginal zone B cells: virtues of innatelike antibody-producing lymphocytes

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Abstract

Protective responses to microorganisms involve the nonspecific but rapid defence mechanisms of the innate immune system, followed by the specific but slow defence mechanisms of the adaptive immune system. Located as sentinels at the interface between the circulation and lymphoid tissue, splenic marginal zone B cells rapidly respond to blood-borne antigens by adopting 'crossover' defensive strategies that blur the conventional boundaries of innate and adaptive immunity. This Review discusses how marginal zone B cells function as innate-like lymphocytes that mount rapid antibody responses to both T cell-dependent and T cell-independent antigens. These responses require the integration of activation signals from germline-encoded and somatically recombined receptors for microorganisms with helper signals from effector cells of the innate and adaptive immune systems.

The innate and adaptive immune systems are functionally interconnected by mechanisms that were originally predicted by Charles Janeway Jr[1]. In his unified model of the immune response, dendritic cells (DCs) and macrophages of the innate immune system instruct specific lymphocytes of the adaptive immune system to initiate protective responses after sensing conserved microbial molecular signatures via germline-encoded pattern-recognition receptors (PRRs), including Tolllike receptors (TLRs)[1]. Unlike DCs and macrophages, lymphocytes recognize discrete antigenic epitopes in a specific but temporally delayed manner through somatically recombined T cell receptors (TCRs) or B cell receptors (BCRs)[2].

Most lymphocytes express specific antigen receptors encoded by highly diversified V(D)J genes. However, some subsets of B and T cells express less specific BCRs and TCRs

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encoded by semi-invariant or poorly diversified V(D)J genes that recognize multiple highly conserved microbial determinants[3]. These 'innate-like' lymphocytes are strategically positioned in 'sensitive' front-line areas that are continually exposed to microbial antigens, including the skin and mucosal surfaces[3]. A major population of innate-like lymphocytes comprises B cells from the marginal zone (MZ) of the spleen, a unique lymphoid area located at the interface between the circulation and the immune system[4].

Unlike follicular B cells, which primarily express monoreactive BCRs, many MZ B cells express polyreactive BCRs that bind to multiple microbial molecular patterns[1,3,5]. In some cases, the recognition profile of these polyreactive BCRs is broadly similar to that of TLRs. In addition, MZ B cells express high levels of TLRs (similarly to DCs, macrophages and granulocytes), which allows them to cross over the conventional boundaries between the innate and adaptive immune systems[6,7]. Indeed, dual engagement of BCR and TLR molecules by conserved microbial molecules such as lipopolysaccharide (LPS) or peptidoglycan stimulates MZ B cells to initiate low-affinity antibody responses that bridge the temporal gap required for the induction of high-affinity antibody production by conventional follicular B cells[3,4,8]. B-1 cells from the spleen and coelomic cavities also have very pronounced innate functional features and indeed often cooperate with MZ B cells in the response to bloodborne microorganisms[3,4], but these cells are not discussed in detail here.

This Review summarizes recent advances on the complex interplay of MZ B cells with different components of the innate and adaptive immune systems that lead to the initiation of rapid antibody responses. We describe the nature of the cellular and signalling pathways required for the diversification and production of antibodies by MZ B cells, and the species-specific differences in these pathways. In addition, we discuss evidence suggesting that MZ B cells take advantage of their unique innate properties not only to 'repel' invading pathogens, but also to communicate with mucosal commensal bacteria. This communication may be important for maintaining viable MZ B cells over time and for the generation of an innate layer of humoral protection against common microbial determinants.

Antigen capture in the MZ

The spleen has an important role in host defence against blood-borne pathogens[9]. In humans, the spleen receives about 5% of the cardiac output, which constitutes a large blood supply for an organ that does not have a high oxygen consumption under steady-state conditions[9]. The elevated perfusion of the spleen permits this organ, through the MZ, to provide efficient immune surveillance of the circulatory system. Strategically interposed between the lymphoid tissue of the white pulp and the circulation, the splenic MZ contains B cells enmeshed with macrophages, DCs and granulocytes in a stromal reticular cell network[9]. All of these cells readily interact with circulating antigens as a result of the low flow rate of the blood passing through the MZ.

In mice, the blood flowing in splenic central arterioles encounters an area of decreased resistance after entering the wider spaces of the marginal sinus (BOX 1). The fenestrated nature of the marginal sinus facilitates the entry of blood-borne antigens into the MZ[9]. At this site, metallophilic macrophages and MZ macrophages capture antigens through various PRRs (FIG. 1), including scavenger receptors and C-type lectin receptors (CLRs)[9–11]. These and other uptake receptors may convey antigens to non-degradative intracellular vesicular compartments that subsequently recycle to the cell surface, potentially permitting macrophages to expose native antigens for the activation of MZ B cells[4,12,13]. In an alternative pathway, blood-borne antigens are captured by DCs and granulocytes in the circulation and then transported to the MZ via the marginal sinus[14,15]. At least some of the

circulating DCs involved in this process express low levels of CD11c and therefore may correspond to plasmacytoid DCs rather than conventional DCs[14]. In humans, there is no marginal sinus and the MZ lacks specific macrophages[16,17]. One possibility is that antigens come into contact with human MZ B cells through the perifollicular zone[16], an area with open circulation that contains neutrophils with powerful B cell helper functions (see below)[18].

Box 1

MZ circulation

The marginal zone (MZ) is a highly transited area that receives large amounts of blood from the general circulation. Remarkably, the splenic microvasculature shows striking differences in mice and humans.

Mouse microvasculature

In mice, the spleen is connected with the general circulation through the splenic artery[9,17]. This vessel branches into central arterioles that are surrounded by the periarteriolar lymphoid sheath (PALS). Central arterioles further branch into smaller follicular arterioles that traverse the PALS and follicles of the white pulp to end as capillaries in the marginal sinus and red pulp[9,17]. The marginal sinus is a low-resistance vessel with a fenestrated structure that separates the MZ from the PALS and follicles of the white pulp[9,17]. The blood contained in the marginal sinus slowly flows through the MZ and subsequently drains into the splenic cords and venous sinuses of the red pulp to enter the general circulation[9,17]. As a result of this microcirculation, the mouse MZ can be considered to be an open compartment.

Human microvasculature

In humans, the spleen receives blood from the splenic artery, which branches into central arterioles and penicillary arterioles[9,17]. These vessels are usually covered by the PALS, but not as often as in rodents. Owing to the absence of a histologically defined marginal sinus, the blood flowing in penicillary arterioles directly drains into capillaries of the red pulp and perifollicular zone[9,17]. The perifollicular zone is a well-defined area of decreased resistance that separates the MZ from the red pulp. Both the perifollicular zone and the red pulp consist of an open circulatory system of blood-filled spaces known as splenic cords, which have no defined endothelial delimitation and are in close contact with the venous sinusoidal vessels of the red pulp[9,17].

In mice, MZ B cells are confined to the MZ of the spleen[5]. In humans, MZ B cells are also located in the inner wall of the subcapsular sinus of lymph nodes, the epithelium of tonsillar crypts and the subepithelial area of mucosa-associated lymphoid tissues, including the subepithelial dome of intestinal Peyer's patches[18–21]. These MZ-equivalent areas are poorly understood, but they have a cellular composition similar to that of the splenic MZ and may therefore provide alternative functional niches for MZ B cells, particularly in splenectomized individuals[22]. B cells identical to splenic MZ B cells are also present in human peripheral blood, which suggests that human MZ B cells recirculate[23,24].

After interacting with antigens exposed on macrophages, DCs or neutrophils, MZ B cells rapidly differentiate into plasmablasts that produce large amounts of IgM[4,14,18,25]. In addition, MZ B cells produce IgG and some IgA via class-switch recombination (CSR)[18,26]. In humans, MZ B cells also undergo somatic hypermutation (SHM)[22], and both CSR and SHM contribute to MZ B cell responses to pathogens and commensal antigens.

Role of MZ B cells in infections

Individuals that lack the spleen as a result of surgery or congenital asplenia are at a higher risk of pneumonitis, meningitis and fulminant septic syndrome caused by encapsulated bacteria such as Streptococcus pneumoniae, Haemophilus influenzae and Neisseria meningitidis[27–29]. These infections are generally attributed to an impaired production by MZ B cells of IgM and IgG specific for capsular polysaccharides[27–29]. However, these B cell defects seem to be less severe in children, suggesting that the pathogenesis of post-splenectomy infections may actually be more complex[30].

Infections by encapsulated bacteria are also more frequent in individuals with inflammatory bowel disease and correlate with a loss of MZ B cells that is possibly secondary to poorly understood inflammatory alterations of the spleen[31]. One possibility is that pro-inflammatory cytokines such as tumour necrosis factor (TNF) impair the generation of bone marrow precursors of MZ B cells by increasing myelopoiesis at the expense of lymphopoiesis[32]. An alternative but not mutually exclusive possibility is that the splenic MZ undergoes functional exhaustion and eventually atrophy as a result of an abnormally high influx of microbial antigens from the inflamed intestine to the general circulation.

Primary and secondary immunodeficiencies that involve the impaired survival or defective function of MZ B cells are also associated with an elevated risk of invasive pneumococcal disease and impaired antibody responses to capsular polysaccharides[33–35]. Similarly to splenectomized or immunodeficient humans, rodents that lack the spleen or have genetic manipulations that alter the survival, maintenance and/or activation of MZ B cells are more susceptible to infections by encapsulated bacteria[25,36]. In addition to combating blood-borne bacteria, MZ B cells may provide protection against some blood-borne viruses, at least in mice[37,38].

Role of MZ B cells in homeostasis

In mice, MZ B cells produce antibodies not only after infection, but also under homeostatic conditions[3,5]. Some of these natural antibodies recognize molecular signatures shared by both foreign and autologous cells (including certain nucleic acids and membrane phospholipids) and may therefore facilitate the clearance of both intruding microorganisms and host apoptotic cells[3,5]. In addition to interacting with antigenic epitopes expressed by pathogenic bacteria, natural antibodies produced by mouse MZ B cells bind to antigenic determinants associated with commensal bacteria inhabiting the intestinal tract (including microbial α-1,3-galactosyl residues) and may therefore provide a secondary line of systemic defence against microorganisms that breach the mucosal barrier[3,5,39,40]. But how do MZ B cells come into contact with intestinal antigens? Despite being segregated from the immune system by physical and biological barriers, mucosal commensal bacteria can influence the development and function of the immune system[41–45]. Although not essential for splenic MZ B cell development, commensal bacteria may enhance the function of MZ B cells. Indeed, germ-free mice have impaired natural and post-immune antibody responses to artificial and microbial carbohydrates that usually activate MZ B cells, including levan, dextran, peptidoglycan-associated polysaccharides and 3-fucosyllactosamine[45–47].

The mechanism by which the intestinal microbiota modulates the function of MZ B cells remains unclear, but studies in rodents show that commensal microbial products (such as LPS and peptidoglycan) can translocate from the intestinal lumen to the circulation and eventually reach systemic lymphoid organs, including the spleen and bone marrow[44,48]. This process could account for the presence of LPS and peptidoglycan in the blood of healthy individuals and probably leads to the capture of these and other microbial molecules by organs actively engaged in blood filtration (such as the spleen)[18,48–50]. The trapping of

these commensal molecules in the spleen MZ would cause innate 'priming' of resident immune cells via TLRs. MZ B cells may thus build a ready-to-use natural antibody repertoire while acquiring a state of active readiness against pathogens (see below)[18,39,40,51].

Basic features of MZ B cells

Considerable progress has been made regarding the mechanisms that underpin the ontogeny and homing of mouse MZ B cells. It is now clear that innate signals from stromal cells, endothelial cells and macrophages are crucial for the development of MZ B cells and their retention in the MZ.

Innate signals involved in MZ B cell ontogeny

In mice, MZ and follicular B cells belong to the B-2 cell lineage and arise from bone marrow precursors via transitional B cells. $IgM^{hi}IgD^{low}CD21^{low}CD23^{-}CD1d^{low}$ transitional stage 1 (T1) B cells colonize a T cell-rich area of the spleen known as the periarteriolar lymphoid sheath (PALS)[36,52]. After this population has undergone negative selection to remove strongly self-reactive cells, the remaining T1 B cells differentiate into $IgM^{hi}IgD^{hi}CD21^{med}CD23^{+}CD1d^{low}$ T2 B cells that colonize the follicle[36]. The survival of T2 B cells requires signals from the BCR, as well as signals from a receptor that binds the TNF family member B cell-activating factor (BAFF; also known as BLYS)[36,53]. This BAFF receptor (BAFFR; also known as BR3) activates an alternative nuclear factor-κB (NF-κB)-dependent pathway that cooperates with the classical NF-κB-dependent pathway downstream of the BCR to promote the survival of T2 B cells and their differentiation into $IgM^{hi}IgD^{low}CD21^{hi}CD23^{-}CD1d^{hi}$ MZ B cells or $IgM^{low}IgD^{hi}CD21^{med}CD23^{+}CD1d^{low}$ follicular B cells[36,53].

According to the signal-strength model, this fate decision may be dictated by the reactivity of the BCR to self antigens[36,54–56]. An intermediate BCR reactivity is thought to trigger the differentiation of mouse T2 B cells into follicular B cells through a pathway dependent on Bruton's tyrosine kinase (BTK)[36,54–56]. Indeed, robust signals from BTK may block the differentiation of mouse T2 B cells into MZ B cells by inhibiting inductive signals from the receptor NOTCH2 (REFS 36,54–57). Insufficient BTK-mediated inhibitory signals in mouse T2 B cells with poorer BCR reactivity would elicit the differentiation of these cells into $IgM^{hi}IgD^{hi}CD21^{hi}CD23^{+}CD1d^{hi}$ T2–MZ precursor cells, which become highly receptive to inductive signals from NOTCH2 (REF. 36). Indeed, engagement of NOTCH2 on T2–MZ precursor cells by the ligand Delta-like 1 (DLL1) on endothelial cells leads to the emergence of MZ B cells[57]. Although consistent with in vivo data, the signal-strength model is not supported by evidence indicating that NOTCH2 governs the differentiation of mouse MZ B cells independently of the BCR[36,58]. Of note, recent studies indicate that NOTCH2 also promotes the development of human MZ B cells[59].

Signals from TLRs may also contribute to the development of human MZ B cells. TLR ligands can promote the differentiation of transitional B cells into MZ-like B cells, and patients with defective TLR signalling have reduced numbers of MZ B cells[35,60]. However, this reduction may reflect a central role of TLRs in providing survival signals rather than differentiation signals to MZ B cells[35]. Patients with defective TLR signalling also have an alteration of the peripheral negative-selection checkpoints that remove autoreactive B cells, including autoreactive MZ B cells[61,62]. In general, the nature and origin of the TLR ligands involved in the development, survival and censoring of human MZ B cells remain poorly understood.

Innate signals involved in MZ B cell homing

In mice, the blood passing through the MZ contains sphingosine-1- phosphate (S1P), a lysophospholipid that binds to the S1P receptors $S1P_1$ (also known as S1PR1) and $S1P_3$ (also known as S1PR3), which are expressed by MZ B cells but not by follicular B cells[63]. Signals from these receptors interfere with the powerful attraction of B cells towards the follicles that is exerted through CXC-chemokine receptor 5 (CXCR5) in response to CXC-chemokine ligand 13 (CXCL13) produced by follicular dendritic cells (FDCs)[64]. Thus, mouse MZ B cells stall in the MZ, whereas follicular

B cells home to the follicle along the CXCL13 gradient[63,64]. The retention of mouse B cells in the MZ also requires the interaction of αLβ2 integrin (also known as LFA1) and α4β1 integrin (also known as VLA4) on MZ B cells with intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1), respectively[65], on stromal cells (BOX 2). The key role of integrins in the positioning of MZ B cells is exemplified by the phenotype of mice lacking the integrin signalling molecules PYK2, LSC (also known as ARHGEF1), DOCK2 or RAC2 (REFS 25,66–68). These mice have normal numbers of follicular B cells but low numbers of MZ B cells, and they have poor antibody responses to blood-borne antigens. Mouse MZ B cells receive further retention signals from MZ macrophages via the scavenger receptor MARCO (macrophage receptor with collagenous structure)[69]. It is unknown whether similar signals govern the homing and retention of human MZ B cells.

Box 2

Stromal cells of the MZ

The spleen requires various subsets of stromal cells for its correct development and function.

Mouse stromal cells

In the developing spleen, lymphoid tissue organizer (LTo) cells expressing intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1) and mucosal addressin cell adhesion molecule 1 (MADCAM1) promote the accumulation of lymphoid tissue inducer (LTi) cells around splenic blood vessels through a mechanism involving interleukin-7 (IL-7)[9,146]. LTi cells lack common lineage markers, but they express the transcription factor retinoic acid receptor-related orphan receptor-γt and secrete lymphotoxin[9,146]. Lymphotoxin stimulates LTo cells to secrete lymphocyte- and dendritic cell-attracting chemokines such as CC-chemokine ligand 19 (CCL19) and CCL21, which drive the development of the white pulp[9,146]. Moreover, lymphotoxin regulates the positioning of the MADCAM1-expressing endothelial cells that delimit the marginal sinus[147]. Additional splenic stromal cells include marginal reticular cells (MRCs), fibroblast reticular cells (FRCs) and follicular dendritic cells (FDCs)[9,146,148]. MRCs are located in the marginal zone (MZ) and express the ER-TR7 antigen, ICAM1, VCAM1 and the TNF family member RANKL (receptor activator of NF-κB ligand), whereas FRCs are located in the periarteriolar lymphoid sheath and red pulp and express the ER-TR7 antigen, ICAM1, VCAM1 and podoplanin together with typical fibroblast molecules, such as desmin, α-smooth muscle actin, fibrillin, laminin and fibronectin[148]. MRCs and FRCs regulate immune responses by producing collagen-rich reticular fibres and chemokines that guide the trafficking of antigens and lymphocytes via dynamic conduit networks[148]. Finally, FDCs provide a structural scaffold for splenic follicles and regulate the trafficking and survival of B cells by releasing CXC-chemokine ligand 13 (CXCL13) and B cell-activating factor (BAFF)[148]. All of these stromal cells may further

enhance immunity by releasing antiviral cytokines such as type I interferons in response to lymphotoxin produced by B cells[149].

Human stromal cells

In humans, stromal cells expressing ICAM1, VCAM1, MADCAM1, smooth muscle actin, CD90 and thrombomodulin divide the MZ into inner and outer regions[16,17]. These stromal cells may interact with LTi cells during splenic organogenesis and could regulate the trafficking of antigens to the MZ[16,17]. Another subset of stromal cells — known as sinus-lining cells (or littoral cells) — expresses unique molecules such as FH1/FH2 domain-containing protein 1 (FHOD1) and signal-regulatory protein-α (SIRPα; also known as CD172a), as well as CD8, the mannose receptor (also known as CD206), the adhesion molecules ICAM1 and VCAM1, and the endothelial molecules PECAM1 (platelet endothelial cell adhesion molecule 1), factor VIII and von Willebrand factor[16–18,98,99]. The function of sinus-lining cells is not clear, but they may mediate antigen capture, neutrophil reprogramming and B cell activation, including class switching and antibody production[18,98,99].

Functional properties of MZ B cells

In mice, large blood-borne antigens activate MZ B cells after being captured by DCs and granulocytes circulating in the peripheral blood or by MZ macrophages in the spleen[10,14]. Smaller blood-borne antigens may directly interact with mouse MZ B cells with the help of complement opsonins[25,70,71]. In the presence of co-stimulatory signals from innate or adaptive immune cells, antigen-activated MZ B cells rapidly differentiate into antibody-secreting plasmablasts via either a T cell-independent pathway (TI pathway) or a T cell-dependent pathway (TD pathway)[4,72,73]. In addition to inducing IgM production, both mouse and human MZ B cells can undergo class switching to IgG or IgA[18,25,26], a process that requires the enzyme activation-induced cytidine deaminase (AID). In humans, MZ B cells also undergo SHM in the absence of immunization or infection through a poorly understood pathway that becomes active at a very early developmental phase[18,23,59,74,75]. Although SHM is only one of the several species-specific properties of MZ B cells, human and mouse MZ B cells also have several similarities (TABLE 1).

Mouse MZ B cells

In mice, MZ B cells are ontogenetically distinct from follicular B cells and B-1 cells, selectively occupy the MZ, have an $IgM^{hi}IgD^{low}CD21^{hi}CD23^{-}$ $CD1d^{hi}$ phenotype and primarily express non-mutated immunoglobulin variable (IgV) genes, some of which encode polyreactive BCRs[5,36]. These BCRs recognize conserved molecular signatures that are often shared by foreign and autologous antigens[3,5]. As a result of this promiscuous reactivity, antibodies from mouse MZ B cells can recognize and clear bacteria from the external environment and also damaged cells from the host[3,5].

Mouse MZ B cells also express high levels of TLRs, similarly to myeloid cells of the innate immune system[1,6,76,77]. This dual expression pattern permits mouse MZ B cells to uniquely integrate signals from both clonally distributed and germline-encoded microorganism-recognition receptors[8,78]. Signals generated via dual BCR and TLR engagement induce the extensive production of low-affinity antibodies by mouse MZ B cells, which bridges the temporal gap required for the production of high-affinity antibodies by follicular B cells[8]. In mice, similar signals regulate B cell tolerance; indeed, dysregulated co-engagement of BCR and TLR molecules by self antigens contributes to the onset of autoimmunity through the pathogenic activation of autoreactive B cells (including MZ B cells)[78].

Mouse MZ B cells are also specialized in the recognition of complement opsonins via the complement receptors CD21 and CD35 and in the recognition of microbial lipids via the MHC class I-like molecule CD1d[25,79,80]. After recognizing a microorganism, mouse MZ B cells rapidly give rise to non-mutated antibody-secreting plasmablasts via extrafollicular TI or TD pathways (see below)[4,14,79,81]. However, mouse MZ B cells can also generate long-lived plasma cells that secrete high-affinity antibodies via a canonical follicular TD pathway that involves the presentation of peptide–MHC class II complexes to $CD4^+$ T helper (T_H) cells, including T follicular helper cells (T_{FH} cells)[72]. Indeed, mouse MZ B cells have a high expression level of MHC class II, CD80 and CD86 molecules, which are required for the activation of T_{FH} cells[5,72]. Thus, mouse MZ B cells are functionally versatile, as they respond to multiple types of microbial challenge, including TI carbohydrate antigens and TD protein antigens.

Human MZ B cells

In humans, the splenic MZ contains B cells that have an $IgM^{hi}IgD^{low}CD1c^+CD21^{hi}CD23^-CD27^+$ phenotype[17,18,23,82,83]. As mentioned above, these B cells also inhabit the inner wall of the subcapsular sinus of lymph nodes, the epithelium of tonsillar crypts and the subepithelial dome of intestinal Peyer's patches[18–21]. Similarly to the splenic MZ, these MZ-like sites are highly exposed to antigens and contain antigen-capturing macrophages and DCs, which re inforces the notion that human MZ B cells serve as front-line sentinels involved in the induction of innate-like antibody responses at the interface between the host and the environment.

Similarly to mouse MZ B cells, human MZ B cells may be able to follow a TI pathway to produce antibodies specific for microbial polysaccharides[23,28,61]. Accordingly, MZ B cells and polysaccharide-specific antibodies are present in patients with hyper-IgM syndrome (HIGM syndrome), an immunodeficiency syndrome that impairs TD antibody responses[24,74].

However, there is also evidence pointing to the ability of human MZ B cells to participate in TD antibody responses. Indeed, $IgM^{hi}IgD^{low}CD1c^+CD21^{hi}CD23^-CD27^+$ B cells recirculate, express the TNF receptor family member CD27, and contain mutated V(D)J genes, and at least some of them show molecular footprints of a past germinal centre experience[84–86]. All of these traits are hallmarks of classical class-switched $IgM^-IgD^-CD1c^-CD21^{med}CD23^-CD27^+$ memory B cells, suggesting that human MZ B cells may be equivalent to memory B cells that have retained IgM expression by exiting the germinal centre before the induction of CSR[87]. However, unlike human class-switched (IgG- or IgA-expressing) or unswitched (IgM-expressing) memory B cells, human MZ B cells express IgD, and they also have a distinct mechanism of IgV gene repertoire diversification during ontogeny, a different pattern of IgV gene usage, fewer IgV gene mutations, a slower rate of accumulation of IgV gene mutations, a lesser dependence on germinal centres and CD40L-expressing $CD4^+$ T cells for the generation of IgV gene mutations, and fewer past cell divisions compared with memory B cells[24,74,75]. Additional studies indicate that human MZ B cells may develop during fetal life in the absence of germinal centres and undergo antigen-independent SHM by following a TI pathway[59,75,88].

Compared with canonical memory B cells, the human MZ B cell population also shows a slower recovery after B cell depletion therapy, a lesser dependence on CD40 signals and a stronger dependence on TLR signals[35,74,89]. In this regard, patients lacking TLR signalling molecules — such as myeloid differentiation primary-response protein 88 (MYD88), TIR domain-containing adaptor protein (TIRAP) or interleukin-1 receptor-associated kinase 4 (IRAK4) — have a selective loss of MZ B cells[35]. This finding implies that MZ and memory B cells have distinct requirements for their development and/or survival. In

particular, MZ B cells appear to be more dependent on TLR signals, which could be triggered by either self or commensal antigens.

One way to reconcile all of the divergent phenotypical, molecular and functional findings in humans is to consider $IgM^{hi}IgD^{low}CD1c^{+}CD21^{hi}CD23^{-}CD27^{+}$ B cells as a heterogeneous population that is composed of at least two distinct subsets, including MZ B cells that develop outside the germinal centre and unswitched memory B cells that develop inside the germinal centre. These subsets probably reflect the versatile nature of human MZ B cells, which are now viewed as a population capable of proficiently mounting antibody responses to both TI and TD antigens.

Regulation of MZ B cell responses

Although they are generally thought to mount innatelike TI responses against microbial carbohydrates, MZ B cells can also initiate adaptive TD responses to microbial proteins[25,72]. In mice, this functional plasticity could reflect the presence in the spleen of multiple MZ B cell precursors that give rise to functionally heterogeneous subsets of MZ B cells[55,56,90]. Remarkably, mouse follicular B cells show a similar functional flexibility, as they can accelerate the kinetics and enhance the magnitude and quality of TI responses to microbial carbohydrates in addition to mediating conventional TD responses to protein antigens[91]. Thus, despite their divergent ontogeny, MZ and follicular B cells can establish a protective alliance to combat blood-borne pathogens.

Innate signals involved in MZ B cell responses to TI antigens

TD IgG and IgA responses by follicular B cells promote immune protection and memory, but they usually require 5–7 days to develop, a delay that can prove fatal in the presence of fast-replicating blood-borne pathogens, including encapsulated bacteria. In mice, MZ B cells compensate for this limitation by entering a faster TI pathway (FIG. 1), which generates short-lived plasmablasts that secrete low-affinity IgM (as well as IgG and some IgA) as early as 1–3 days after exposure to blood-borne microorganisms[4,15,92]. These plasmablasts are located in the red pulp of the spleen, and their rapid formation is facilitated by an elevated baseline expression in MZ B cells of transcripts encoding the plasma cell-inducing trans criptional regulator B lymphocyte-induced maturation protein 1 (BLIMP1; also known as PRDM1)[4].

The mechanism underlying the state of active readiness of MZ B cells remains poorly understood, but exposure to small amounts of complement-decorated commensal antigens may play a part. Consistent with this possibility, mouse MZ B cells respond more robustly to complement proteins than follicular B cells do, partly because the NOTCH2-mediated signalling programme required for the differentiation of MZ B cells induces the robust expression of complement receptors in these cells[6,93]. Owing to their constitutive partial activation, MZ B cells rapidly produce antibodies in response to either type 1 TI antigens (such as LPS), which bind to the BCR and often co-engage TLRs, or type 2 TI antigens (such as polysaccharides), which induce extensive BCR crosslinking and co-engage multiple PRRs[8,11,12,94].

In mice, MZ B cells become exposed to large TI antigens after interacting with metallophilic macrophages, which are strategically positioned along the marginal sinus, or with MZ macrophages, which are scattered throughout the MZ (BOX 3). These MZ-specific macrophages capture antigens through various PRRs of the scavenger receptor and CLR families[10,11]. Particulate TI antigens are also conveyed to mouse MZ B cells by circulating DCs that weakly express CD11c and therefore may correspond to plasmacytoid DCs[14]. Such DCs home to the MZ, together with neutrophils, in mice exposed to blood-borne

microorganisms and, similarly to macrophages, capture whole microorganisms or fragments thereof and make them available to MZ B cells as antigenic ligands[14,15]. In mice, DCs further sustain TI antibody responses by providing survival signals to extrafollicular plasmablasts via BAFF and its homologue APRIL (a proliferation-inducing ligand)[14,95,96].

Box 3

MZ macrophages

Marginal zone (MZ) B cells cooperate with distinct subsets of antigen-capturing macrophages to mount protective antibody responses to blood-borne pathogens.

Mouse macrophages

In mice, there are two specific subsets of MZ macrophages. Metallophilic macrophages express the sialoadhesin CD169 (also known as SIGLEC1) and lie as sentinels in proximity of mucosal addressin cell adhesion molecule 1 (MADCAM1)-positive endothelial cells lining the marginal sinus[5,9,147]. Metallophilic macrophages form a ring between the MZ and the white pulp and have an important role in the clearance of blood-borne particulate antigens[5,9,147]. Similarly to CD169-expressing subcapsular macrophages from lymph nodes, metallophilic macrophages might activate CD1d-restricted invariant natural killer T (iNKT) cells to promote rapid antibody responses via extrafollicular B cells[106,117,137]. MZ macrophages are found throughout the MZ and lack CD169, but express the scavenger receptor MARCO (macrophage receptor with collagenous structure) and the endocytic receptor SIGN R1 (DC-SIGN-related protein l)[9–11]. These pattern-recognition receptors bind to blood-borne antigens with the help of complement and enable MZ macrophages to interact with antigens and present them to MZ B cells[9–11].

Human macrophages

In humans, the MZ lacks metallophilic macrophages and MZ macrophages but contains dispersed nonspecific macrophages that express the scavenger receptor CD68 and the haemoglobin receptor CD163 (REFS 16,17). Similar nonspecific macrophages are present in the red pulp[16,17]. Macrophages expressing CD169 form pericapillary sheaths in the perifollicular zone, an area of low resistance adjacent to the MZ[16,17]. Together with neutrophils, sinus-lining cells and dendritic cells expressing CD11c and the endocytic receptor DEC205 (also known as CD205), perifollicular macrophages may capture antigens to activate MZ B cells[16,18,97–99].

In humans, the lack of metallophilic macrophages and MZ macrophages raises questions as to how MZ B cells interact with blood-borne antigens (FIG. 2). Neutrophils, some DCs and perhaps sinus-lining cells (known as littoral cells) may compensate for the lack of MZ-specific macrophages[18,97–99]. Similarly to macrophages and DCs, neutrophils and sinus-lining cells release BAFF and APRIL, which promote B cell and plasma cell survival, antibody production and CSR through a T_{FH} cell-independent pathway[7,18,96,99,100].

In both mice and humans, antigen-mediated engagement of BCR and TLR molecules activates MZ B cells by cooperating with transmembrane activator and CAML interactor (TACI), which binds to both BAFF and APRIL[7,8,14,18,96,101]. TACI is highly expressed by MZ B cells and is further upregulated in response to microbial TLR ligands[6,7,102]. TACI also cooperates with TLRs to induce CSR and antibody production via a TLR-like pathway involving MYD88, IRAK1, IRAK4 and TNF receptor-associated factor (TRAF) adaptor proteins[7,102]. Moreover, TACI cooperates with the BCR and CD40 (REFS 7,103), suggesting that MZ B cells integrate classical innate and adaptive signalling pathways to mount rapid antibody responses.

In mice, complement opsonins decorating TI antigens deliver additional activating signals to MZ B cells via CD21 and CD35, two complement receptors that enhance BCR signalling by interfering with the expression of the inhibitory receptor programmed cell death protein 1 (PD1)[11,25,80]. Complement opsonins may further activate mouse MZ B cells via CD40 (REF. 104). CD40 may also be engaged by CD40L on neutrophils, natural killer (NK) cells or invariant natural killer T cells (iNKT cells)[18,105,106], which provides an additional layer of complexity in the interplay between the innate and adaptive immune systems during MZ B cell responses. In general, currently available mouse and human studies indicate that MZ B cells initiate rapid responses to TI antigens by integrating adaptive signals from antigenic BCR ligands with innate helper signals from microbial TLR ligands, opsonins, TNF-like factors, cytokines and chemokines.

Innate signals involved in MZ B cell responses to TD antigens

The composition of the immunizing antigen is likely to be crucial for determining whether MZ B cells produce antibodies via the TI or the TD pathway[107]. In general, blood-borne bacteria express not only TI antigens, such as TLR ligands and conventional poly saccharides, but also TD antigens, such as outer-membrane proteins and zwitterionic polysaccharides[4,8,15,25,42,108]. Protein antigens and zwitterionic polysaccharides are processed to peptides and low-molecular- weight carbohydrates, respectively, and both are presented to $CD4^+$ T cells through the MHC class II endocytic pathway[42,109]. Unlike follicular B cells, MZ B cells constitutively express elevated levels of surface MHC class II, CD80 and CD86 molecules and therefore display a robust antigen-presenting activity, at least in mice[110,111]. This antigen-presenting capability may partly relate to the continuous exposure of MZ B cells to microbial or self TLR ligands, which are powerful inducers of T cell co-stimulatory molecules[1].

After capturing an antigen, mouse MZ B cells migrate to the PALS of the spleen to establish cognate interactions with antigen-specific naive $CD4^+$ T_H cells[72,111] (FIG. 3). The ensuing germinal centre reaction probably involves the differentiation of MZ B cell-primed naive $CD4^+$ T_H cells into canonical T_{FH} cells, with or without the help of local DCs[72,112]. By expressing CD40L and cytokines such as interleukin-4 (IL-4), IL-21 and interferon-γ (IFNγ), T_{FH} cells stimulate antigen-specific B cells to undergo CSR and SHM through a CD40-dependent pathway that leads to the emergence of long-lived memory B cells and plasma cells expressing class-switched high-affinity IgG antibodies[72,112]. In mice, MZ B cells may further enhance follicular TD antibody responses by continuously depositing complement-decorated blood-borne antigens in the follicle. The interaction of antigens with CD21 and CD35 on MZ B cells causes the downregulation of S1P receptor expression, followed by the temporary interruption of MZ-retaining signals[63,64]. The resulting predominance of CXCR5 signals induces the displacement of antigen-transporting MZ B cells to the follicle[63,64]. After depositing antigens on FDCs, MZ B cells re-express S1P receptors and return to the MZ[63,64]. This shuttling involves a large proportion of mouse MZ B cells and occurs under homeostatic conditions independently of BCR engagement by antigens[63,64]. An important implication of these findings is that mouse MZ B cells are continuously exposed to complement-opsonized antigens, which may include commensal antigens from mucosal surfaces. By delivering microbial antigens to FDCs, MZ B cells may inform follicles as to the antigenic composition of the blood flowing through the marginal sinus, thereby facilitating the selection of germinal centre B cells that have a high affinity for those antigens.

In mice, MZ B cells also engage in extrafollicular TD responses that rapidly give rise to short-lived plasmablasts secreting low-affinity IgM and/or IgG antibodies[73,108] (FIG. 3). Recent findings suggest that the isotypic composition of this extrafollicular response is regulated by distinct subsets of DCs[73]. MZ-based DCs expressing the CLR DC inhibitory

receptor 2 (DCIR2; also known as CLEC4A4) selectively stimulate rapid IgG1 but not IgM production by mouse MZ B cells[73]. DCIR2$^+$ DCs make protein antigens available to MZ B cells as BCR ligands, which activate MZ B cells and induce them to become potent antigen-presenting cells. Such MZ B cells establish cognate interactions with CD4$^+$ T cells, which subsequently undergo robust proliferation in response to DCIR2$^+$ DCs. The T_H2 cells emerging from these interactions selectively induce IgG1 production by stimulating MZ B cells via CD40L and cytokines (including IL-4)[73]. Remarkably, DCIR2$^+$ DCs fail to induce affinity maturation through a T_{FH} cell-controlled germinal centre reaction, unless they are exposed to certain microbial TLR ligands[73]. Unlike MZ-localized DCIR2$^+$ DCs, DCs in the PALS express the CLR DEC205 (also known as LY75) and may stimulate low-affinity extrafollicular or high-affinity follicular IgM and IgG responses by activating MZ B cells through a pathway involving IL-12-induced T_{FH} cells[73,108,113–115].

In mice, rapid extrafollicular TD antibody responses may also involve the cognate interaction of lipid-specific MZ B cells with iNKT cells[106,116,117]. These cells express an invariant Vα14$^+$ TCR that binds to microbial glycolipids presented by CD1d on MZ B cells[106,117]. The ensuing activation of iNKT cells causes the upregulation of CD40L expression and the secretion of IFNγ, which elicit the production of low-affinity IgM and IgG antibodies by MZ B cells[106,116,117]. Activated iNKT cells have recently been shown to modulate the T cell-suppressive activity of IL-10-secreting neutrophils, at least in mice[118]. Perifollicular neutrophils from human spleens also have T cell-suppressive activity in addition to MZ B cell helper function (see below)[18], which raises the possibility that iNKT cells establish a dialogue with splenic neutrophils to modulate the differentiation of MZ B cells along TD or TI pathways. Overall, these findings indicate that MZ B cells interact with multiple cells of the innate immune system to mount TD antibody responses.

Innate signals involved in MZ B cell differentiation to plasmablasts

In mice, antigen-activated MZ B cells differentiate into plasmablasts that migrate to the red pulp in response to CXCL12, which is a CXCR4 ligand expressed by stromal cells, macrophages, neutrophils and DCs in the red pulp and perifollicular zone[18,119,120]. The homing of mouse MZ B cell-derived plasmablasts to the red pulp also requires the downregulation of their expression of integrins, S1P receptors, CXCR5 (a receptor for CXCL13) and CC-chemokine receptor 7 (CCR7; a receptor for CCL19 and CCL21) through a mechanism that involves the stimulation of these cells by microbial TLR ligands[63,119]. Once in the red pulp, plasma blasts receive trophic signals from multiple innate cells, including red-pulp DCs, macrophages, neutrophils, stromal cells and sinus-lining cells. In both mice and humans, these innate cells release BAFF and APRIL, which support the maturation and survival of plasmablasts by engaging TACI and B cell maturation antigen (BCMA)[7,14,18,95,99,121].

In humans, DCs upregulate their release of BAFF and APRIL in response to microbial TLR ligands and cytokines such as type I IFNs and IFNγ[96,100,122]. In mice, IFNγ stimulates DCs to produce BAFF through a signal transducer and activator of transcription 1 (STAT1)-dependent pathway that is negatively regulated by autoimmune regulator (AIRE), a transcription factor mostly known for its role in thymic negative selection[123]. In humans, plasmablasts receive additional maturation and survival signals from IL-6, type I IFNs and CXCL10 (REFS 124,125). CXCL10 is a CXCR3 ligand that stimulates activated B cells to release IL-6, which in turn enhances CXCL10 production by a subset of macrophages expressing the haemoglobin receptor CD163 (REF. 125). Thus, MZ B cell-derived plasmablasts require a multicomponent survival niche to deliver their effector functions in the red pulp. Inhibitory signals restricting the lifespan of these plasmablasts must also be involved, but their nature remains obscure.

MZ B cell diversification via CSR

Although usually associated with potent IgM responses, MZ B cells also produce class-switched IgG and IgA under steady-state conditions and after immunization. Whereas IgM is mostly associated with the neutralization and complement-mediated killing of microorganisms, IgG and IgA amplify the phagocytosis and killing of opsonised microorganisms and thereby provide MZ B cells with additional effector functions. But how do MZ B cells undergo CSR?

Neutrophils trigger CSR in MZ B cells

A fraction of mouse and human MZ B cells produce class-switched antibodies under steady-state conditions, possibly in response to TI antigens from mucosal surfaces, including the intestine[18,44,48,126]. In humans, this process is associated with the induction of CSR by a unique subset of neutrophils that interact with MZ B cells through a non-inflammatory pathway that begins during fetal life and accelerates after birth, a time that coincides with the colonization of mucosal surfaces by commensal bacteria[18]. Crosstalk between neutrophils and MZ B cells may seem surprising, but it is consistent with studies showing that neutrophils release BAFF and APRIL[127]. Unlike circulating conventional neutrophils, splenic neutrophils deliver powerful antibody-inducing signals to human MZ B cells, and therefore we have termed them B cell-helper neutrophils (N_{BH} cells)[18].

Compared with conventional neutrophils, N_{BH} cells have similar morphological and ultrastructural features, but they have a distinct phenotype, gene expression profile and function. In particular, human N_{BH} cells express higher levels of BAFF, APRIL, CD40L, IL-6 and IL-21, produce more plasmablast-attracting factors (such as CXCL12) and spontaneously activate MZ B cells[18]. These unique features probably reflect the activation and reprogramming of N_{BH} cells by local microenvironmental signals[18]. Consistent with this possibility, the accumulation of N_{BH} cells in peri-MZ areas coincides with postnatal deposition of discrete amounts of microbial products of probable mucosal origin (such as LPS) in the peri-MZ[18,48]. In addition to activating N_{BH} cells, LPS and other microbial TLR ligands stimulate perifollicular sinus-lining cells to express neutrophil-attracting chemokines (such as CXCL1, CXCL2, CXCL3, CXCL6 and CXCL8), which may contribute to the recruitment of N_{BH} cells[18]. Accordingly, a lack of TLR signals leads to a dramatic reduction in the number of N_{BH} cells and MZ B cells[18,35].

Microbial TLR ligands may also stimulate the reprogramming of human conventional neutrophils into N_{BH} cells by eliciting the release of non-inflammatory STAT3-inducing cytokines such as IL-10 from perifollicular sinus-lining cells and macrophages[18]. Considering that IL-10 provides regulatory signals to neutrophils[128], this cytokine may be instrumental in generating antibody-inducing N_{BH} cells devoid of inflammatory activity[18]. The generation and/or maintenance of N_{BH} cells may also require granulocyte–macrophage colony-stimulating factor (GM-CSF), a neutrophil-activating STAT3- and STAT5-inducing cytokine produced by innate response activator B cells[129].

In humans, N_{BH} cells induce AID expression, class switching to IgG and IgA, and the generation of antibody-secreting plasmablasts by activating MZ B cells through a TI pathway involving BAFF, APRIL and IL-21 (REF. 18). In agreement with these in vitro findings, patients with neutropenia, defective TACI signalling or impaired STAT3 signalling have fewer MZ B cells and reduced steady-state production of IgG and IgA specific for various TI antigens, including LPS[18]. By contrast, neutropenic patients show normal steady-state production of IgG and IgA specific for TD antigens[18]; such antibodies are usually derived from long-lived plasma cells located in the bone marrow. In mice, these plasma cells require APRIL-mediated signals from eosinophils to survive in the bone marrow[130]. Thus,

there may be a division of labour among different granulocyte subsets for the maintenance of antibody-secreting cells in distinct lymphoid districts.

Optimal stimulation of human MZ B cells by N_{BH} cells may involve neutrophil extracellular trap (NET)-like structures[18]. By capturing commensal antigens, NETs could enhance the provision of BCR and TLR ligands to MZ B cells[18]. Moreover, NETs may further stimulate MZ B cells by delivering endogenous TLR ligands, including self DNA[131,132]. The stimulation of MZ B cells by N_{BH} cells could involve a largely TI pathway, because spleens from adults contain neither T_{FH} cells nor active germinal centres[75,114]. This possibility is further suggested by the observation that human N_{BH} cells strongly suppress $CD4^+$ T cell proliferation in a contact-independent manner, at least *in vitro*[18]. However, there is also the possibility that, in the presence of appropriate pro-inflammatory signals, N_{BH} cells acquire antigen-presenting cell function to enhance TD antibody production.

DCs, macrophages and iNKT cells trigger CSR in MZ B cells

In mice, MZ B cells are thought to receive TLR-mediated CSR-inducing signals from soluble microbial products or whole microorganisms captured by MZ macrophages or DCs. Consistent with this possibility, mouse MZ B cells produce IgG3 but also IgG2 in response to infection-associated LPS[8,93]. This type 1 TI antigen induces CD40-independent CSR through an extrafollicular pathway that involves dual BCR and TLR engagement[8]. Mouse MZ B cells also produce IgG3 and possibly IgA in response to polysaccharides[92]. In humans, these type 2 TI antigens may trigger CD40-independent CSR through an extrafollicular pathway involving co-engagement of the BCR and carbohydrate receptors such as CLRs[133]. In addition to BAFF and APRIL, TI CSR may require macrophage- and DC-derived cytokines such as type I IFNs and transforming growth factor-β1 (TGFβ1) in both mice and humans[96,134–136].

Mouse MZ B cells also produce IgG1 and IgG2 in response to microbial proteins[72,73,108]. These TD antigens trigger CSR through follicular and extrafollicular pathways that involve CD40L-expressing T_{FH} or T_H2 cells[8,72,73,108]. Mouse MZ B cells also produce IgG2 and IgG3 in response to CD1d-binding glycolipids[106,116]. These molecules induce CD40-dependent CSR through an extrafollicular pathway involving CD1d-restricted iNKT cells[106,117]. In this pathway, splenic MZ B cells function as antigen-presenting cells in a similar manner to CD169-expressing subcapsular macrophages from lymph nodes[137]. In all of these TD pathways, the induction of CSR in MZ B cells requires the cooperation of CD40L with IL-4, IL-21 and IFNγ from T_{FH}, T_H2 or iNKT cells[73,106,114,116].

Although not formally proven, human MZ B cells may also undergo CSR following the immunization of the host with TI or TD antigens. In particular, type 2 TI antigens (such as polysaccharides) induce the production of IgG1, as well as that of IgG2 and IgA2, two subclasses preferentially produced by B cells in response to TI signals[18,24,122,138,139]. Remarkably, human MZ B cells secrete large amounts of IgA after undergoing CSR in response to BAFF, APRIL and IL-21 from N_{BH} cells[18]. The functional advantage of systemic IgA production by MZ B cells remains poorly understood, but this response could provide a secondary line of non-inflammatory IgA-mediated defence against commensal bacteria that breach the mucosal barrier[18].

MZ B cell diversification via SHM

Mouse MZ B cells seem to primarily express non-mutated immunoglobulin V(D)J genes[3,5], whereas some rat MZ B cells harbour small numbers of mutations[126,140]. Low-level SHM has also been reported in B cells from BCR-transgenic mice exposed to TI antigens such as (4-hydroxy-3-nitrophenyl)acetyl–Ficoll or to self antigens such as IgG–nucleic acid

complexes[141,142]. In humans, most MZ B cells contain mutations of the more limited extrafollicular variety, but the underlying mechanism is unclear[23,24]. One line of thought is that human MZ B cells correspond to memory B cells that exited the germinal centre after accumulating some mutations but before undergoing CSR[87]. This suggestion explains the follicular mutational pattern of some MZ B cells[86], but is inconsistent with the observation that patients with HIGM syndrome, who have impaired germinal centres, retain mutated MZ B cells[24,74]. The unconventional nature of SHM in human MZ B cells is also suggested by the finding that these cells have molecular footprints of past proliferation in an extrafollicular environment and accumulate mutations during fetal life in the absence of germinal centres and independently of antigenic stimulation[24,59,75].

Innate SHM in fetal MZ B cells

Human MZ B cells undergo SHM at a very early developmental stage through a mechanism that does not require the presence of an anatomical MZ[59]. Indeed, MZ B cells producing mutated antibodies can be detected in fetuses and infants that physiologically lack the MZ[59,75]. Studies in humanized mice and human fetal tissues indicate that MZ B cells undergo SHM in the fetal liver and mesenteric lymph nodes through a TI pathway involving AID but not antigens[59,88]. Remarkably, this innate pathway requires NOTCH2 (REF. 59), which is also necessary for the development of MZ B cells in mice[57]. The implication of these findings is that human MZ B cells may be generated through innate post-rearrangement immuno globulin gene diversification events similar to those present in birds and some mammals (including rabbits)[22]. In these species, the IgV genes of B cell precursors located in gut-associated lymphoid tissues undergo post-rearrangement SHM and/or gene conversion to generate a ready-to-use pre-immune antibody repertoire capable of rapidly recognizing microbial antigens[22,143]. The challenge ahead lies in the identification of the innate signals that diversify the human MZ B cell repertoire before birth.

Innate SHM in adult MZ B cells

The SHM machinery of human MZ B cells remains functional after birth, as MZ B cells accumulate more mutations through antigen-dependent and antigen-independent mechanisms in both children and adults[23,75]. The splenic MZ may be involved in the induction of these mutations, because it contains foci of proliferating and clonally related B cells, some of which express AID[18,20]. These MZ B cells may undergo limited SHM via a TI pathway involving the recognition of commensal antigens trapped on NETlike structures formed by N_{BH} cells[18]. Consistent with this possibility, SHM is decreased in MZ B cells from neutropenic patients, whereas SHM is normal in MZ B cells from patients with HIGM syndrome, who have impaired TD antibody responses[18,74]. In agreement with findings indicating that microbial TLR ligands control the number of splenic N_{BH} cells with SHM-inducing activity[18], TLR ligation induces SHM in MZ B cell precursors[60,144,145].

However, signals from non-TLR PRRs may have an even more important role, because MZ B cells from patients with defective TLR signalling have normal levels of mutations in their immunoglobulin genes[35]. In principle, a slow postnatal induction of SHM via an extrafollicular TI pathway involving MZ B cell helper signals from commensal microbial products and innate immune cells may be instrumental for increasing the diversity of the pre-immune antibody repertoire of MZ B cells after birth. Immunization or infection would introduce additional mutations via extrafollicular or follicular TD pathways that probably involve MZ B cell helper signals from T_{FH} cells[86]. The combination of these TI and TD pathways would ensure the development of a highly diversified repertoire of ready-to-use MZ B cells in both children and adults.

Concluding remarks

Follicular B cells generate protective responses and long-term immune memory by producing highly specific antibodies. The price for this specificity is that rousing follicular B cells takes time, as plasma cells emerging from the germinal centre reaction do not produce high-affinity antibodies until several days after infection. MZ B cells fill this gap through their ability to deploy numerous innate immune defensive strategies, including the rapid sensing of microorganisms via both germline-encoded and somatically recombined receptors and robust antigen-presenting cell activity. As a result of their prominent innate immune functions, MZ B cells rapidly generate low-affinity antibodies not only via TI pathways, but also via TD pathways that were previously ascribed solely to follicular B cells.

Despite recent advances, there are several questions that remain to be answered regarding MZ B cells. In humans, at least some MZ B cells develop during fetal and early postnatal life, but the nature of the innate signals that regulate this process remains unclear[59,75]. During adulthood, innate immune signals enhance the survival and diversification of MZ B cells, but the origin of these signals is also poorly understood. Self antigens and/or microbial TLR ligands from the commensal microbiota may have a role[18,35,60,145]. The latter possibility echoes earlier studies suggesting that microbial TLR signals are central to the establishment of long-term serological memory and natural antibody production[40,131,143].

Of note, some natural antibodies target key microbial epitopes, blood-group antigens and self antigens, and a better understanding of the innate signals underpinning their production by MZ B cells may have a beneficial impact in multiple clinical settings, including immunodeficiencies, autoimmunity, transplantation and transfusion medicine. More generally, a deeper knowledge of the innate signals involved in the ontogeny, reactivity, diversification and activation of MZ B cells will be important for fully elucidating the protective role of MZ B cells against infections. This information could be used to develop novel MZ B cell-harnessing strategies aimed at improving the protective activity of vaccines.

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Glossary

Toll-like receptors (TLRs)	A large family of germline-encoded pattern-recognition receptors that initiate innate and adaptive immune responses after recognizing conserved molecular signatures associated with microorganisms
B cell receptors (BCRs)	A term that refers to transmembrane immunoglobulin molecules irrespective of their heavy-chain constant region
B-1 cells	$IgM^{hi}IgD^{low}MAC1^{+}B220^{low}CD23^{-}$ cells that are dominant in the peritoneal and pleural cavities. Their precursors develop in the fetal liver and omentum, and in adult mice the size of the B-1 cell population is kept constant owing to the self-renewing capacity of these cells. B-1 cells recognize self components, as well as common bacterial antigens, and they secrete antibodies that tend to have a low affinity and broad specificity

Metallophilic macrophages	A subset of macrophages that is located at the border of the white pulp and the marginal zone of the spleen. These cells are stained by silver impregnation, which explains the name
C-type lectin receptors (CLRs)	A large family of germline-encoded pattern-recognition receptors that initiate innate and adaptive immune responses after recognizing specific conserved molecular signatures associated with microorganisms
Peyer's patches	Groups of lymphoid nodules present in the small intestine that are massed together on the intestinal wall, opposite the line of attachment of the mesentery. Peyer's patches consist of a subepithelial dome area, B cell follicles and interfollicular T cell zones
Class-switch recombination (CSR)	A switch in the DNA that encodes the constant region of the immunoglobulin heavy chain, from Cμ (which encodes the constant region of IgM) to Cγ, Cα or Cε, which encode the constant regions of IgG, IgA and IgE, respectively. This is accomplished through an intrachromosomal deletional rearrangement
Somatic hypermutation (SHM)	A unique mutation mechanism that is targeted to the variable regions of rearranged immunoglobulin gene segments. Combined with selection for B cells that produce high-affinity antibodies, SHM leads to the affinity maturation of B cells in germinal centres
Congenital asplenia	A genetic deficiency of unknown origin in humans that results in the absence of splenic development and is frequently associated with cardiovascular malformations
Natural antibodies	Antibodies spontaneously released by B-1 cells and marginal zone B cells in the absence of immunization or infection. These antibodies have a low affinity and broad specificity for conserved self and microbial antigens and mostly belong to the IgM class, but also include IgG and IgA, particularly in humans
Transitional B cells	Refers to transitional stage 1 (T1) and transitional stage 2 (T2) B cells, which are immature, newly formed B cells that are present in the bone marrow, blood and spleen and give rise to marginal zone and follicular B cells
Follicular dendritic cells (FDCs)	Cells with a dendritic morphology that are present in lymph nodes. These cells display on their surface intact antigens that are held in immune complexes, and B cells in the lymph node can interact with these antigens. FDCs are of non-haematopoietic origin and are not related to dendritic cells
T cell-independent pathway (TI pathway)	A pathway followed by B cells to produce antibodies specific for TI antigens such as microbial LPS and capsular polysaccharides. The pathway usually involves extrafollicular marginal zone B cells and B-1 cells and does not require help from T cells
T cell-dependent pathway (TD pathway)	A pathway followed by B cells to produce antibodies specific for TD antigens such as microbial proteins. The pathway usually involves follicular B cells and requires help from T cells expressing CD40

	ligand. However, extrafollicular marginal zone B cells can also respond to TD antigens
AID (Activation-induced cytidine deaminase)	A DNA cytidine deaminase that mediates somatic hypermutation and class-switch recombination
T follicular helper cells	(T_{FH} cells). $CD4^+$ T helper cells that are essential for the induction of class switching in the germinal centres of secondary follicles during antibody responses to T cell-dependent antigens
Hyper-IgM syndrome (HIGM syndrome)	A congenital immunodeficiency characterized by defective immunoglobulin heavy-chain class switching and increased IgM production. The underlying molecular defect involves CD40 ligand (in HIGM1), activation-induced cytidine deaminase (in HIGM2), CD40 (in HIGM3), uracil DNA glycosylase (in HIGM4) or other unknown B cell proteins (in HIGM5)
Germinal centre	A lymphoid structure that arises in follicles after exposure to T cell-dependent protein antigens. This structure fosters the induction of B cell clonal expansion, class-switch recombination and somatic hypermutation, followed by the development of memory B cells and long-lived plasma cells that express high-affinity antibodies
Invariant natural killer T cells (iNKT cells)	A subset of innate-like lymphocytes expressing an invariant $V\alpha14^+$ T cell receptor that recognizes soluble glycolipids loaded on the non-polymorphic MHC class I-like molecule CD1d. In humans, CD1c might serve the same function that CD1d has in mice
Innate response activator B cells	B-1 cell-derived plasmablasts that populate perifollicular areas of both mouse and human spleens and secrete the cytokine GM-CSF
Neutrophil extracellular trap (NET)	A set of extracellular fibres produced by an activated neutrophil to ensnare invading microorganisms. NETs enhance neutrophil-mediated killing of extracellular pathogens while minimizing damage to host cells

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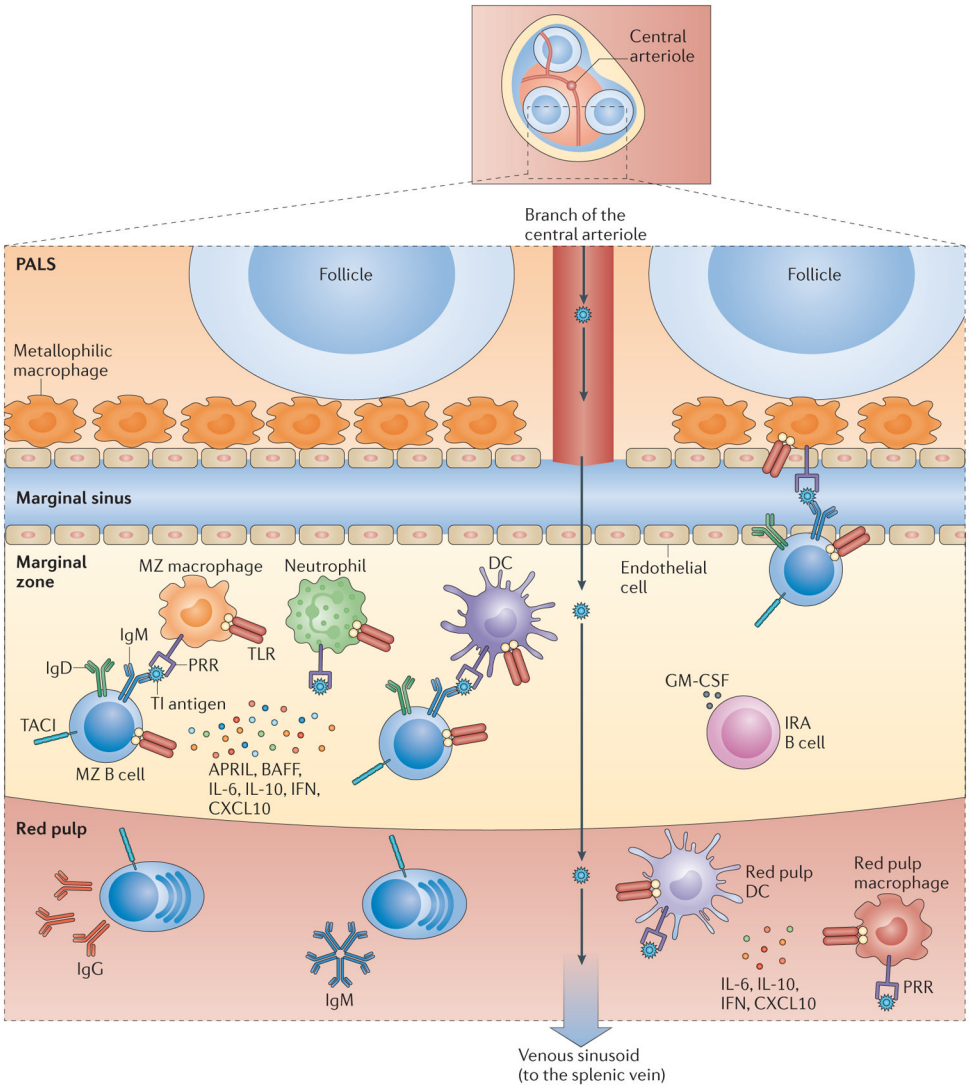

Figure 1. T cell-independent responses by mouse MZ B cells

T cell-independent (TI) antigens are captured by metallophilic macrophages and marginal zone (MZ) macrophages after entering the splenic MZ via the marginal sinus. Alternatively, TI antigens are captured by dendritic cells (DCs) and neutrophils in the circulation. Innate response activator (IRA) B cells may enhance the survival and activation of these antigen-capturing cells by releasing granulocyte–macrophage colony-stimulating factor (GM-CSF). Antigen-sampling cells stimulate MZB cells via the B cell receptor (BCR), Toll-like receptors (TLRs) and transmembrane activator and CAML interactor (TACI). TACI delivers signals that induce class-switch recombination and antibody production after ligating B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL), which are released by antigen-capturing cells in response to microbial TLR ligands. Antigen-capturing cells, including red pulp DCs and macrophages, also secrete interleukin-6 (IL-6), IL-10, type I interferons (IFNs) and CXC-chemokine ligand 10 (CXCL10), which cooperate with BAFF and APRIL to promote the differentiation and survival of plasmablasts secreting IgM or class-switched IgG. Arrows indicate the path followed by antigens through the spleen. PALS, periarteriolar lymphoid sheath; PRR, pattern-recognition receptor.

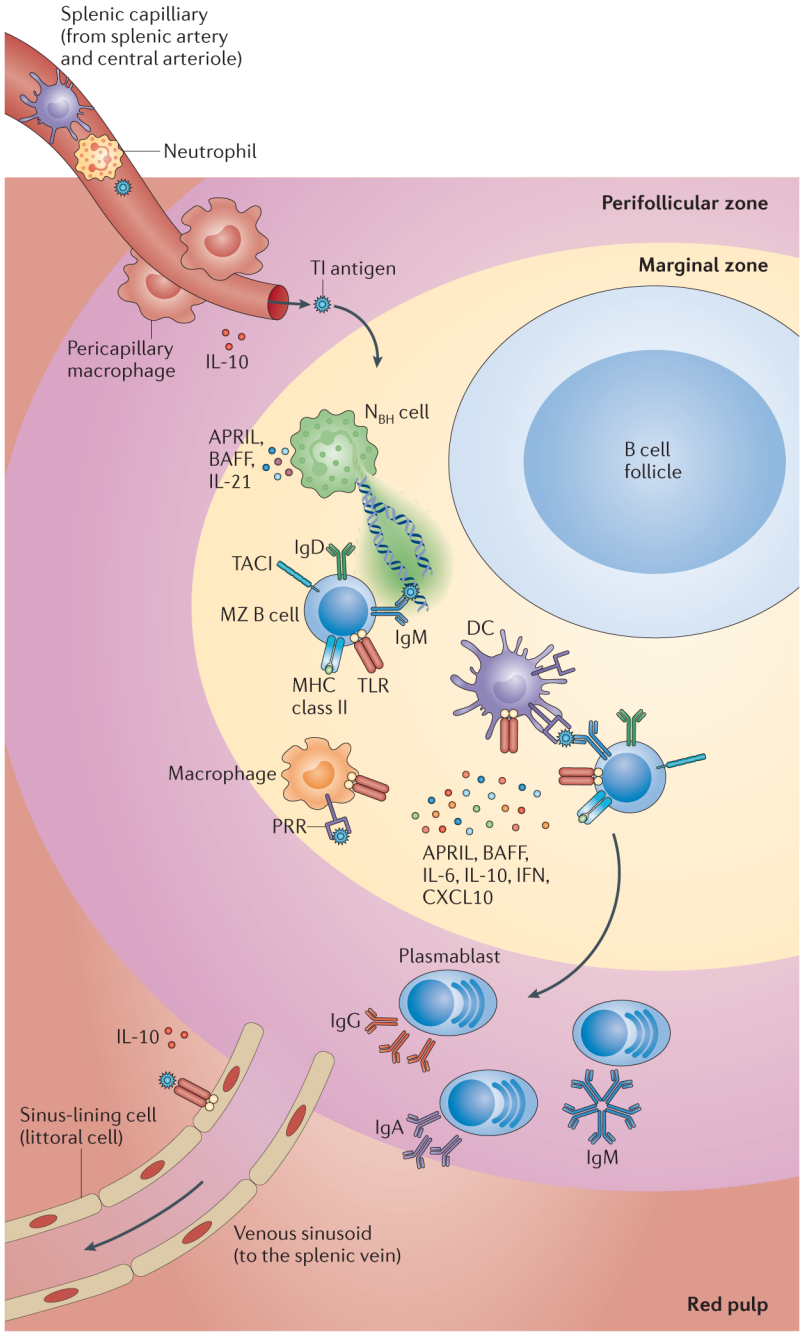

Figure 2. T cell-independent responses by human MZ B cells

T cell-independent (TI) antigens are thought to enter the marginal zone (MZ) through the perifollicular zone. Once in the MZ, they may be captured by neutrophil extracellular trap (NET)-like structures emanating from B cell-helper neutrophils (N_{BH} cells). These cells may differentiate from circulating neutrophils as a result of the production of interleukin-10 (IL-10) by perifollicular sinus-lining cells and macrophages in response to microbial Toll-like receptor (TLR) ligands. Antigen capture may also involve reticular cells, macrophages, sinus-lining cells and dendritic cells (DCs). In addition to making TI antigens available to the B cell receptor (BCR) and TLRs on MZ B cells, antigen-capturing cells release B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL), which engage

transmembrane activator and CAML interactor (TACI) on MZ B cells. N_{BH} cells also release IL-21, thereby inducing class-switch recombination, somatic hypermutation and antibody production in MZ B cells. The generation of plasmablasts secreting IgM or class-switched IgG and IgA involves the production of IL-6, IL-10, IL-21 and CXC-chemokine ligand 10 (CXCL10) by antigen-capturing cells. Arrows indicate the putative path followed by antigens through the spleen. IFN, interferon; PALS, periarteriolar lymphoid sheath; PRR, pattern-recognition receptor.

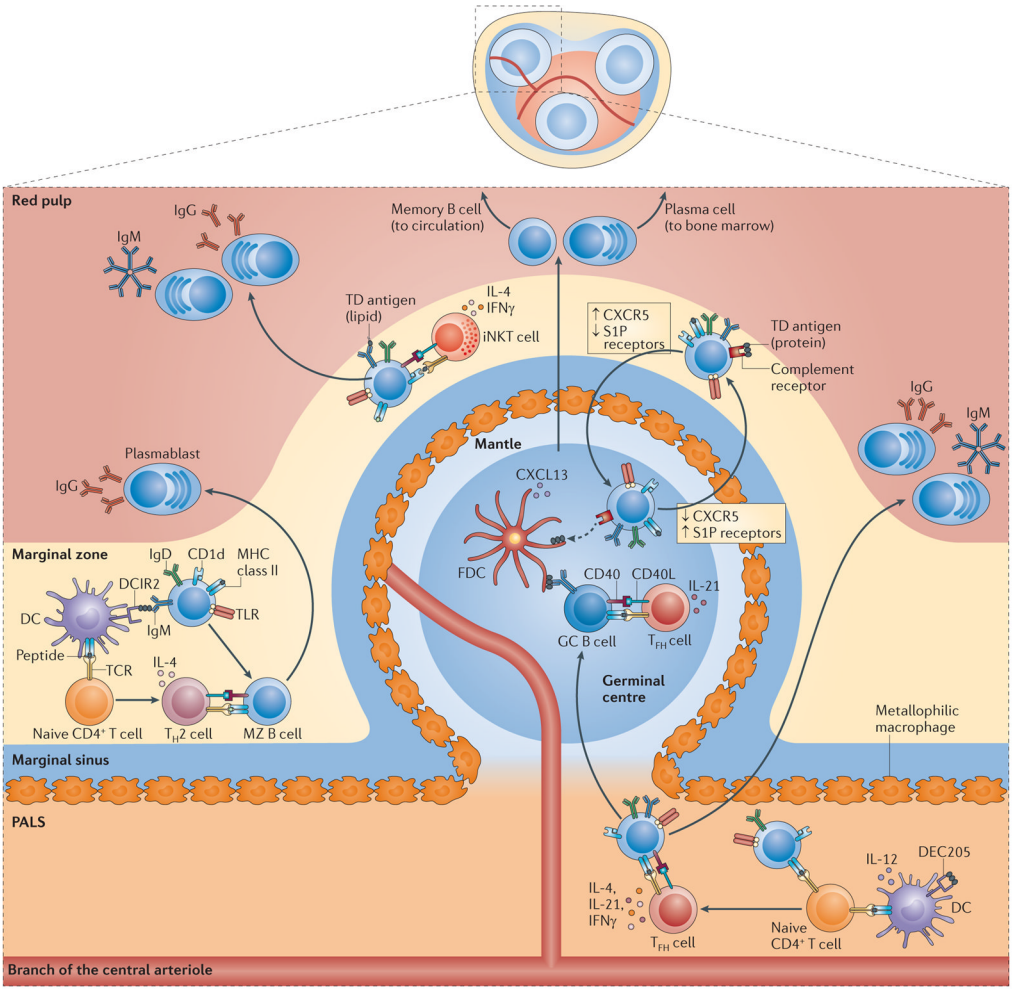

Figure 3. T cell-dependent responses by mouse MZ B cells

In the extrafollicular T cell-dependent (TD) pathway, marginal zone (MZ) dendritic cells (DCs) expressing DC inhibitory receptor 2 (DCIR2) capture protein antigens and present them to naive $CD4^+$ T cells, which subsequently differentiate into T helper 2 (TH2) cells expressing CD40 ligand (CD40L) and interleukin-4 (IL-4). $DCIR2^+$ DCs also expose antigens to MZ B cells, and signals from the B cell receptor (BCR) convert MZB cells into antigen-presenting cells. By establishing cognate interactions with T_H2 cells, activated MZ B cells rapidly produce low-affinity IgGl but not IgM. ln an alternative pathway, lipid-reactive MZ B cells activate invariant natural killer T (iNKT) cells by presenting CDld-loaded glycolipids. The expression of CD40L, IL-4 and interferon-γ(IFNγ) by activated iNKT cells induces MZ B cells to differentiate into plasma blasts that secrete low-affinity IgM or IgG. In the follicular TD pathway, MZB cells downregulate receptors for sphin-gosine-1-phosphate (SIP) after capturing complement-decorated protein antigens via CD21 and CD3B. The interruption of MZ-retaining signals from SIP receptors stimulates MZ B cells to enter the follicle in response to CXC-chemokine receptor 5 (CXCRB) signals. In the follicle, MZ B cells deposit antigens on follicular dendritic cells (FDCs) and thereafter upregulate SI P receptor expression to exit the follicle and return to the MZ. Protein antigens displayed by FDCs enhance TD antibody responses by promoting the selection of follicular germinal centre (GC) B cells expressing a high-affinity BCR. This selection also involves cognate interactions between GC B cells and T follicular helper (T_{FH}) cells expressing CD40L and IL-21. Ultimately, selected GC B cells differentiate into long-lived memory B

cells or plasma cells that produce high-affinity IgG. Alternatively, MZ B cells capture protein antigens with or without the help of metallophilic macrophages and migrate to the border between the periarteriolar lymphoid sheath (PALS) and the follicle to initiate a GC reaction after establishing a cognate interaction with T_{FH} cells expressing CD40L, IL-4 or interferon-γ(IFNγ). These T_{FH} cells probably emerge from the priming of naive $CD4^{+}$T cells by either MZ B cells or DCs expressing DEC205. A subset of these T_{FH} cells can also promote the generation of low-affinity extrafollicular IgM and IgG plasmablasts. CXCL13, CXC-chemokine ligand 13; TCR. T cell receptor; TLR, Toll-like receptor.

Table 1

Species-specific features of MZ B cells

Parameter	Mouse	Human	Refs
Phenotype	$IgM^{hi}IgD^{low}CD21^{hi}CD23^{-}CD1d^{hi}$	$IgM^{hi}IgD^{low}CD1c^{+}CD21^{hi}CD23^{-}CD27^{+}$	5,22,36
Mutational status and reactivity	Non-mutated, polyreactive	Mutated, monoreactive, probably also polyreactive	3,5,22
Localization	Splenic MZ	Splenic MZ, subcapsular sinus in lymph nodes, tonsillar epithelium, subepithelial dome of Peyer's patches	5,9,22
Site of development	Postnatal spleen	Fetal liver, fetal mesenteric lymph nodes, postnatal spleen	22,36
Precursors	T2B cells, some T1B cells	Fetal B cells, transitional B cells, follicular B cells	22,36
Recirculation	Limited to follicular shuttling	Extensive	5,22
Homing receptors	$S1P_1$, $S1P_3$, CXCR5, αLβ2 integrin, α4β1 integrin	Unclear	61–65
Antibodies produced in T cell-dependent responses	IgG1	IgM, IgG1, IgG2, IgA1	5,22, 138,139
Antibodies produced in T cell-independent responses	IgM, IgG2b, IgG3, IgA	IgM, IgG1, IgG2, IgA2	5,22, 138,139
Helper cells	DCs, MZ macrophages, marginal metallophilic macrophages, iNKT cells, T_{FH} cells	N_{BH} cells, Tcells	5,9,148
Activating signals	Capsular polysaccharides, TLR ligands, BAFF, APRIL, CD40L, complement proteins, IFNγ	Capsular polysaccharides, TLR ligands, BAFF, APRIL, CD40L, IL-21	5,9,148

APRIL, a proliferation-inducing ligand; BAFF, B cell-activating factor; CD40L, CD40 ligand; CXCR5, CXC-chemokine receptor 5; DC, dendritic cell; IFNγ, interferon-γ; iNKT, invariant natural killer T; MZ, marginal zone; N_{BH} cell, B cell-helper neutrophil; $S1P_1$ sphinqosine-1-phosphate receptor 1; T1, transitional stage 1; T_{FH}, T follicular helper; TLR, Toll-like receptor.