CELLULAR IMMUNOLOGY

Structure and function of the immune system in the spleen

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The spleen is the largest secondary lymphoid organ in the body and, as such, hosts a wide range of immunologic functions alongside its roles in hematopoiesis and red blood cell clearance. The physical organization of the spleen allows it to filter blood of pathogens and abnormal cells and facilitate low-probability interactions between antigen-presenting cells (APCs) and cognate lymphocytes. APCs specific to the spleen regulate the T and B cell response to these antigenic targets in the blood. This review will focus on cell types, cell organization, and immunologic functions specific to the spleen and how these affect initiation of adaptive immunity to systemic blood-borne antigens. Potential differences in structure and function between mouse and human spleen will also be discussed.

INTRODUCTION

The spleen is the primary site of blood filtration in the body. This review will first cover the cellular architecture relevant for the function of innate and adaptive immune cells, including how this differs between mice and humans. Second, we will highlight the cells specific to the spleen that are responsible for monitoring the blood and initiating adaptive immunity. Finally, we will cover recent developments in our understanding of three types of immune responses specific to the spleen in response to blood-borne bacteria, parasites, or foreign red blood cells.

SPLENIC ARCHITECTURE

The spleen is divided by function and structure into the red pulp (RP) and white pulp (WP); in between these two regions is the marginal zone (MZ) in rodents and the perifollicular zone in humans (Fig. 1) (*1*, *2*). The WP is the primary immunologic region of the spleen in both species; however, the WP makes up less than a quarter of splenic tissue. The RP makes up the majority of the tissue and has an immune function distinct from that of the WP. Unlike lymph nodes (LNs), the spleen lacks afferent lymphatic vessels, and therefore all cells and antigen enter the spleen via the blood.

Red pulp

The splenic RP extracts aged, dead, or opsonized cells from the circulation while simultaneously surveying for pathogens and tissue damage. Blood is delivered to the MZ by terminal arterioles, which release their contents into an open blood system without traditional endothelial linings. The RP filters out aged red blood cells (RBCs), which must traverse tortuous venous sinusoids to reenter the circulation. Aged, infected, or dysfunctional RBCs that cannot adequately deform have lost the "don't eat me" signal CD47, or that are opsonized by antibody or complement are removed from the circulation by RP macrophages (RPMs), and their iron is reclaimed for systemic use. After percolating through the RP cords, blood is recollected in sinuses to form the venous sinusoidal system and ultimately enters the efferent vein for return to the circulatory system.

Although adaptive immune responses to systemic antigens are initiated in the WP, immune effector function often takes place in the RP. Many leukocytes with innate functions reside in the RP, including neutrophils, monocytes, dendritic cells (DCs), gamma delta (γδ) T cells, and macrophages (*3*). These myeloid populations can change dynamically in both location and number during an inflammatory response to respond quickly to an insult and shape the adaptive immune response. Plasmablasts migrate from the WP to the RP following gradients of CXCL12 (which is higher in the RP) to produce antibodies that are carried throughout the circulatory system (*3*). Effector $CD8^+$ T cells emigrate to the RP to clear bacteria (*4*). Extramedullary hematopoiesis and storage of cellular reserves (such as monocytes, platelets, and RBCs) are other important functions of the splenic RP, but beyond the scope of this review [(*1*), review].

White pulp

The spleen can be considered a peripheral tissue of the circulatory system embedded with multiple LN-like structures (the WP) (*5*), with two important distinctions from LNs. The WP in both mouse and human does not contain a capsule to separate it from the parenchyma of the spleen (the RP). Instead, a cellular border primarily made up of innate immune cells demarcates the boundaries of the WP; this is well defined in mouse and only partially defined in humans (Fig. 1). Despite the lack of a capsule, antigen larger than 60 kDa does not freely flow into the WP and is instead carried in by cells from the MZ (*6*). Second, afferent lymphatics do not drain into the WP to deliver cells and antigens. Despite the widely held notion in mouse studies that the spleen is the draining secondary lymphoid organ (SLO) for the peritoneum, antigen injected intraperitoneally actually reaches the mediastinal LNs (*7*), although eventually some antigen reaches the spleen. Instead, the WP of the spleen functions as the SLO of the circulatory system, similar to how LNs drain and monitor antigens from tissues. In the WP, splenic naïve and central memory T cells are activated in response to cognate antigen and T cell–dependent B cell germinal center (GC) reactions, resulting in antibody production (*8*).

Inside the WP, compartmentalization separates the T and B cell areas into distinct zones, analogous to those found in LNs (Fig. 1). The T cell zone (TCZ) is also called the periarteriolar lymphoid sheath (PALS) because it forms around the central arteriole that runs through the WP on its way to the RP-WP border. From mouse studies, we know that CCR7 and its ligands CCL19 and CCL21 are

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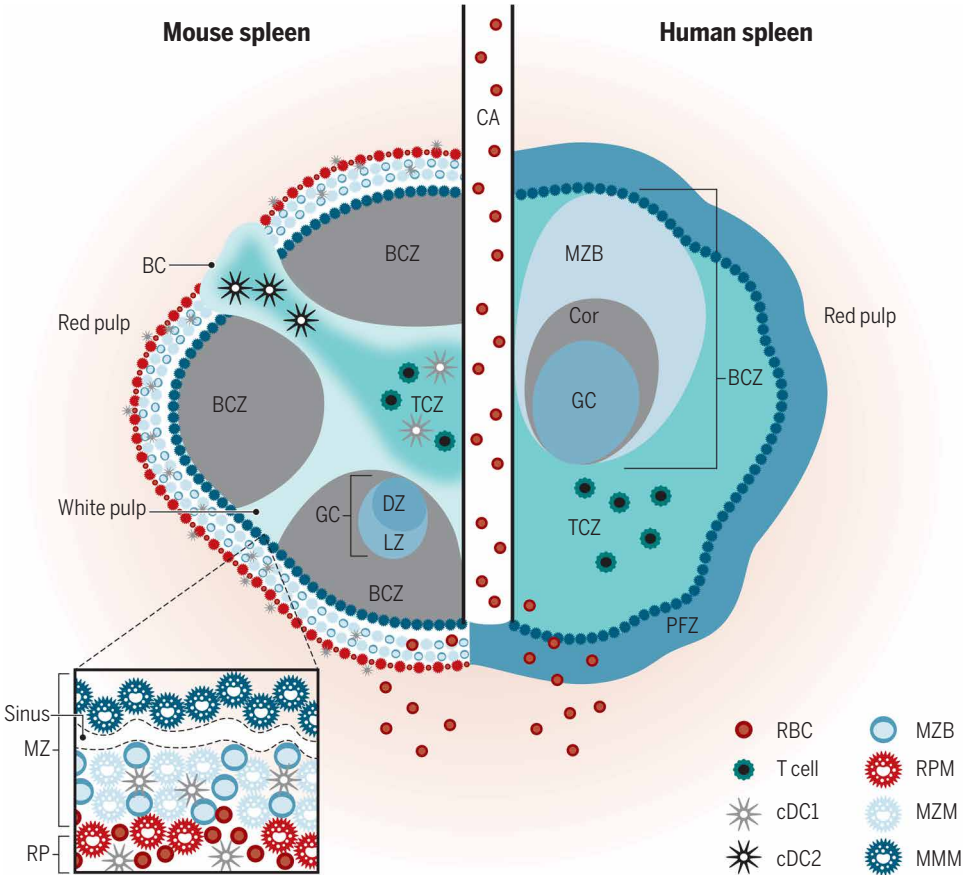

Fig. 1. Mouse and human splenic immune cellular architecture at steady state. There are structural differences between the murine (left) and human (right) splenic immune system, most notably, the organization of T cell zone (TCZ, turquoise; also known as PALS) and B cell zone (BCZ) follicles (gray and shades of blue, shown with light zone, LZ, and dark zone, DZ, organization in mouse spleen) within the WP and the border between the WP and RP, the MZ (marginal zone) in mouse or perifollicular zone (PFZ) in human (dark blue outer ring). Because applications of advanced imaging techniques to the human spleen have been limited, the extent to which the mouse MZ and human PFZ are analogous remains unknown. For example, the precise layering and composition of macrophage subsets in the MZ is known for mice (see bottom left box)—$CD169^+$ MMMs (dark blue) form a concentric ring around the WP with MZMs (light blue) and MZB cells (darker blue)—but not for humans. In humans, MZB cells surround activated B cells, containing a GC (light blue in the human spleen on the right) and Corona (gray, "Cor"). The homeostatic location of dendritic cell (DC) subsets in mice is shown (with cDC2s in the bridging channel, BC, and cDC1s in the TCZ, MZ and RP, red pulp). Release of blood into the MZ of the WP from a central arteriole (CA) is shown.

crucial for this concentration of T cells; loss of CCR7 results in a scattering of T cells throughout the spleen (*5*, *9*). The B cell zones (BCZs) are the follicles (and GCs during an active immune response), which contain a mixture of cells important for the activation and survival of B cells. Nonhematopoietic follicular DCs (FDCs) present antigen to follicular B cells while also producing CXCL13 to help organize the B cell follicle (*10*).

This basic structure, which is slightly different between mouse and human (Fig. 1), is enforced by regions of chemoattractant produced primarily by nonhematopoietic cells (*10*). Integrins (αLβ2 and α4β1) are required for T and B cells to enter the splenic WP (*5*); then, fibroblast channels that possibly originate in the MZ help direct T cell movement into the WP through the bridging channel (BC) (*11*), which is consistent with older work demonstrating a conduit for chemokine-directed cellular trafficking through the splenic WP (*6*). Fibroblast reticular cells are lined by the CCR7 ligand CCL21, which acts to direct T cells through the TCZ and interfollicular zone, where this network terminates. Similarly, B cells migrate along the same network but switch to an FDC network in follicles lined by the CXCR5 ligand CXCL13.

Marginal zone

Part of the blood released by terminal arterioles drains into the MZ (mouse) or perifollicular zone (humans). A distinct type of innate-like B cell, MZ B cells, resides here at steady state in mice anchored by integrins LFA-1 and α4β7 binding to intercellular adhesion molecule–1 (ICAM-1) and vascular cell adhesion molecule–1 (VCAM-1) along with chemoattract signals from sphingosine 1-phosphate (S1P) (*12*, *13*). At this RP-WP interface, specialized leukocytes including DCs and MZB cells capture and transport blood-borne antigens to the WP for surveillance by T and B cells (*9*, *14*, *15*). Two populations of macrophages populate the mouse MZ, retained by CCL21 chemokine signals (*5*): marginal metallophilic macrophages (MMMs) and MZ macrophages (MZMs). Together, these populations form the MZ borders (Fig. 1) and help filter the blood as it is released into the MZ (*16*). The MZ is also where lymphocytes are released from the circulation to enter the WP.

Bridging channel

A conduit exists between the RP and WP: the BC (Fig. 1). This direct extension of the TCZ into the RP contains many T cells as well as antibody-producing cells. The BC, as will be discussed in the section on DC subsets, is also home to a particular population of splenic DCs with defined functions in $CD4^+$ T cell activation. It is widely believed that naïve and activated lymphocytes enter and exit the WP via the BC (*11*), migrate through the MZ to the RP, and ultimately rejoin the circulation (*4*, *5*). Follicular B cells might have additional escape routes through the MZ (*14*). However, some work has proposed—but remains to be validated by using newer imaging techniques—that efferent lymphatics exist in the spleen, originating in the WP and carrying a small fraction of lymphocytes out of the spleen by means of lymph (*1*, *17*).

Pulp fiction: Perceived differences and similarities between mouse and human splenic structure

Although the function of specific immune cell types and regions of the spleen is largely similar between mouse and human, some fundamental differences in splenic structure and cell types are nevertheless thought to exist between mouse and human spleen (*18*). The lack of detailed cellular characterizations of the human spleen further complicates a proper comparison (*19*). Most comparisons between the

two species use nonspecific cellular architecture based on hematoxylin and eosin (H&E) staining or single antigenic characterization with immunohistochemistry of human spleen sections compared with multicolor immunofluorescence and multiparameter flow cytometry in mice. Some differences do arise. From early human studies, we know that the TCZ and BCZ within the human WP are organized like grapes on a vine rather than layers of follicles surrounding a central TCZ, as in mouse. However, the most striking difference between mouse and human spleen is not the cells contained in or the organization of either the RP or WP but rather the border between the two. What is called the MZ in rodents is composed of multiple cellular layers. A border of MMMs surrounds the WP, and a marginal sinus is formed by nonhematopoietic sinus-lining cells (*5*). Then, a ring of MZM and DCs concentrically forms around the WP (*16*). An innate-like B cell population exists in this area of the mouse spleen, called MZB cells, with specific functions such as circulatory migration patterns into and out of follicles for antigen delivery to follicular B cells and production of rapid, T cell–independent immunoglobulin M (IgM) during immunization (*20*, *21*). By contrast, the region referred to as the MZ in humans is defined by H&E staining of secondary follicles but is not the RP-WP border; it contains a potentially heterogeneous memory IgM^+ B cell population called MZB cells that is embedded within the BCZ and is not clearly flanked by the macrophage populations observed in the mouse MZ (*22*). Unlike their mouse counterparts, human MZB cells show evidence of somatic hypermutation and systemic recirculation (*23*). Therefore, mouse and human MZB cells resemble each other in name only and might represent very different B cell populations. Future work will be needed to determine whether mouse-equivalent innate-like MZB (iMZB) cells exist in humans and whether human-equivalent memory, affinity-matured MZB cells adjacent to GCs exist in mice.

In humans, but not mice, the perifollicular zone is the border between the WP and RP (*2*); however, the immune cellular constituents of this region have not been well characterized. It is reported that MMMs do not exist in the perifollicular zone, although $CD169^+$ macrophages have been identified in an isolated study by using immunohistochemistry (*24*). In this region, humans have sheathed capillaries rather than a marginal sinus, as assessed by light microscopy. However, a human marginal sinus was identified using electron microscopy (*25*). Therefore, newer imaging and cellular characterization techniques need to be applied to the human spleen to reconcile these ambiguities; in particular, the function of different cells and regions of the spleen in textbooks of human anatomy relies almost completely on work done with the mouse or other rodent spleens. Whether these perceived or real differences in cellular organization alter the initiation or execution of an immune response in the mouse versus human spleen is not known. Clarifying MZ similarities and differences between mouse and human will aid in discussions of the applicability of mouse immunology research to human biology and disease.

THE CELLULAR INITIATORS AND EFFECTORS OF THE ADAPTIVE SPLENIC IMMUNE RESPONSE

In this section, cellular functions and organization specific to the spleen will be discussed. Most of the work identifying cell subsets, location, and function has been performed in mice, and how these compare to humans remains to be clarified.

Innate immune cell function and organization

Recognition of infection or host damage in the spleen activates a plethora of pattern recognition receptors (PRRs) on myeloid cells, which, in turn, induce requisite T cell activation signals on antigen-presenting cells (APCs), cytokine secretion, and pathogen clearance in phagocytes. PRRs include Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I–like RNA helicases, and C-type lectin receptors (CLRs). TLRs recognize extracellular or phagocytosed pathogen-associated molecular patterns. A number of cytosolic NLRs sense both microbial products that gain access to the cell interior (such as intracellular flagellin) and host molecules released during states of cell stress or damage (e.g., extracellular adenosine 5′-triphosphate) called damage-associated molecular patterns (DAMPs). A number of CLRs are used as molecular markers for specific DC subsets in the spleen, such as Langerin and dendritic cell-specific ICAM-3-grabbing non-integrin 1 (DC-SIGN), and help these particular subsets recognize carbohydrate moieties and internalize pathogens such as fungi and the exoskeleton of insects. PRRs are selectively expressed on different cell types in particular regions of the spleen, helping tailor the nature of both the early innate and subsequent adaptive immune response (*5*, *16*, *20*, *26*).

Splenic DC subsets

In part because of dynamic and overlapping marker expression by different DC subsets, it has historically been challenging to segregate DCs into clear lineages. Further complicating categorization is the expression of classic T cell markers by DC subsets, which has led to one of the most widely used classification systems of splenic murine DCs as $CD4^+$, $CD8^+$, or double-negative ($CD4^-CD8^-$) DCs (*27*). Multiple types of myeloid cells also express CD11c without acting as classical or conventional DCs (cDCs). Discovery of particular transcription factors that define the ontogeny of mouse DC and monocytic lineages has recently allowed for a clearer separation of both monocytes from DCs and of DC subsets from each other (*28*, *29*). All DCs are derived from the bone marrow (BM) and, as such, must arrive at the various tissues via the bloodstream; further, they have a high turnover rate (hours to a few weeks). cDCs, which make up about 3% of all $CD45^+$ cells in the spleen, are derived from a common DC progenitor (CDP), express the transcription factor ZBTB46, and act almost exclusively as "professional" APCs (*30*).

cDCs can be divided into two major subsets: cDC1s and cDC2s (Table 1). All splenic cDC1s express XCR1, and most express CD8αα. Within this subset, some reside at steady state in the WP and express the lectin receptor DEC205 (*31*). The other cDC1s reside in the MZ and RP at steady state and express CD103, and some express Langerin (*15*, *27*). After immunization, all cDC1s colocalize with and preferentially activate $CD8^+$ T cells in the WP. In part, this is due to the selective ability of murine cDC1s to cross-present antigen. By contrast, all cDC2s reside in the BC at steady state and express signal regulatory protein alpha (SIRPα), and most express CD11b. The chemotactic receptor EBI2, which recognizes oxysterol ligands, and S1P are important cues that localize and help maintain cDC2s at the BC (*12*, *32*–*34*). Two major subpopulations of cDC2s exist in the spleen. One requires neurogenic locus Notch homolog protein 2 (NOTCH2) and recombination signal binding protein for immunoglobulin kappa J region (RBPJ) signaling for development and expresses the adhesion molecule ESAM (endothelial cell-selective adhesion molecule) (*35*–*37*). The $ESAM^{hi}$ subset also expresses CD11b, CD4, and the CLR DC-inhibitor receptor 2 (DCIR2)

Table 1. Innate immune cells and markers specific to the spleen. Selected innate immune cells adapted to the mouse spleen are shown with key markers, developmental origin and transcription factors, and location in the spleen at steady state. Markers in italics are expressed in subsets of the population. CSF1R (colony-stimulating factor 1 receptor)/CD115/M-CSFR (macrophage colony-stimulating factor receptor); CCR2 (chemokine, cc motif receptor 2)/MCP-1R (monocyte chemoattractant protein 1 receptor); XCR1 (X-C motif chemokine receptor 1); CLEC9A (C-type lectin domain containing 9A)/DNGR1 (DC NK lectin group receptor 1); DEC205/CD205; SIRPα (signal regulatory protein alpha)/CD172α; DCIR2 (DC immunoreceptor 2)/33D1 staining/CLEC4A4 (C-type lectin domain family 4, member 4a); CX3CR1(C-X3-C motif chemokine receptor 1); CD317/BST2 (BM stromal cell antigen 2)/PDCA1 (plasmacytoid DC Ag-1); SIGLECH (sialic acid binding Ig-like lectin H); B220/PTPRC (protein tyrosine phosphatase receptor type c)/LY5.

	Markers	Origin	Splenic location	Key transcription factors	Function
RPM (*16*, *26*)	CD11b$^+$ F4/80$^+$ CD68$^+$ CD206$^+$ Dectin-2$^+$ VCAM$^+$	BM monocyte and fetal monocytes from yolk sac	RP	SPIC	Clearance of aged or foreign RBCs, iron recycling, heme breakdown
MZM (*16*, *26*)	CD209b/SIGN-R1$^+$ MARCO$^+$ SR-A$^+$ ER-TR9$^+$ CD68$^+$ Dectin-2$^+$ Tim4$^+$	BM monocyte	MZ	LXRα	Recognition of pathogens, activation of MZB cells
MMM (*16*, *26*)	CD169/Siglec-1$^+$/ MOMA1 staining CD68$^+$	BM monocyte	At the interface of the WP and MZ	LXRα	Recognition of pathogens, speculated transfer of antigen from blood to WP
Tingible body macrophages (*16*)	MERTK$^+$ TIM4$^+$ F4/80$^-$CD11b$^-$ CD68$^+$MFG-E8$^+$	?	GC DZ in B cell follicle	?	Removal of apoptotic B cells
MZBs (mouse) (*20*, *21*)	IgM$^+$ IgDlo CD21$^+$CD23$^-$ CD1d$^+$ B220lo	Transitional B cells	MZ	AIOLOS	Rapid production of IgM antibodies and antigen trafficking to follicle
Inflammatory monocytes/TIP-DC (*59*)	CCR2$^+$ CD115/CSF1R$^+$ Ly6C$^+$ Ly6G$^-$ F4/80$^+$ CD11b$^+$ MAC3$^+$ *CD11c*$^+$	BM	Recruited to spleen from BM via CCR2	Mafb	TNF and NO production, bacterial clearance
cDC1 (*28*, *30*)	XCR1$^+$ CD11c$^+$ CD8αα$^+$ CLEC9A/DNGR1$^+$ *DEC205*$^+$ *CD207*$^+$ CD24$^+$ *CD103*$^+$	BM CDP (common DC precursor)	RP/MZ and PALS of WP	BATF3, IRF8	Cross-presentation, CD8$^+$ T cell activation
cDC2 (*28*, *30*)	CD11c$^+$ CD11b$^+$ DCIR2/33D1 staining SIRPα/CD172$^+$ *ESAM*$^+$ *CD4*$^+$ *CX3CR1*$^+$	BM CDP	BC	NOTCH2, IRF4, KLF4	MHC II antigen processing, CD4$^+$ T cell activation

stained by 33D1 but are negative for CX3CR1 (*32*). Lymphotoxin B receptor, activated by B cell–derived lymphotoxin, retinoic acid, a vitamin A derivative, and the transcriptional regulators interferon regulatory factor–4 (IRF4) and Kruppel like factor 4 (KLF4) play crucial roles in the development and homeostasis of this cDC2 subset (*36*–*39*). The CD4$^+$ ESAM^{hi} cDC2 subset excels in priming naïve CD4$^+$ T cells. By contrast, ESAM^{lo} cDC2s express CD11b, CX3CR1, and DCIR2 but less CD4, and probably constitute most of the double-negative DCs, as defined in the older classification system (*30*, *40*, *41*). Functionally, this NOTCH2- and IRF4-independent cDC2 subset seems to be more proficient at producing inflammatory cytokines such as tumor necrosis factor–α (TNFα) and interleukin-12 (IL-12) (*36*).

A major nonconventional DC subset in the spleen is plasmacytoid DCs (pDCs), which are derived from both CDPs and CLPs (common lymphoid progenitors), particularly IL-7R$^+$ CLPs (*42*). Mouse pDCs are identified through expression of B220 and PDCA-1 (plasmacytoid DC Ag-1) along with lower levels of CD11c. pDCs express TLR7 and TLR9, which enables them to recognize viruses. After activation, pDCs rapidly secrete large amounts of type I interferons, IL-12 and IL-18, leading to increased natural killer T (NKT) cell activity, apoptosis of virally infected cells, and enhanced cross-priming [(*43*), review].

Although fewer analyses of human splenic DCs exist, grossly equivalent DC subsets exist to the mouse, albeit expressing some markers specific to humans (*44*). cDC1s express BDCA3 (blood DC antigen 3), also known as CD141 or thrombomodulin, in addition to the overlapping XCR1 and CLEC9A/DNGR-1 markers. cDC2s express BDCA1, also known as CD1c, in addition to the common SIRPα expression. pDCs express BDCA2, also known as CD303, along with CD304 and CD123. Differences between mouse and human DC specialization do exist; however, similar transcription factors have been identified in the mouse-equivalent DC populations, and the human DC subsets have overlapping functions to their mouse counterparts, including cytokine production predilection (*45*).

Migratory and resident splenic DC subsets

The functional specialization of cDC1s in $CD8^+$ T cell priming and cDC2s in $CD4^+$ T cell priming can only be partially explained by differences in innate immune receptors, phagocytosis pathways, and major histocompatibility complex (MHC) class I versus class II machinery [(*46*), review]. In vitro or even ex vivo, all DC subsets can activate $CD4^+$ or $CD8^+$ T cells; however, loss in vivo of cDC1s through basic leucine zipper transcriptional factor ATF-like 3 (BATF3) or IRF8 deficiency impairs primarily $CD8^+$ but not $CD4^+$ T cell responses, and conversely, loss of cDC2s typically impairs $CD4^+$ but not $CD8^+$ T cell responses (*9*, *28*, *32*, *33*, *35*, *47*). Accumulating data from a variety of studies suggest that DC subset specificity for activating particular T cell lineages is driven by the spatiotemporal cellular organization of T cells and DC subsets [(*30*), review].

DCs collect antigen in peripheral tissues and, once they receive a licensing signal, primarily from PRR activation, modulate their chemokine receptor expression and migrate through the lymphatics to LNs, where they activate naïve T cells. For systemic blood-borne antigens such as transfused RBCs or bacteremia, this entire process must occur in the spleen, which, unlike all other secondary lymphoid structures, does not contain afferent lymphatics (*1*). Because splenic cDC1s reside in the TCZ at steady state and the architecture of the spleen juxtaposes antigen-exposed tissue (MZ and RP) directly with the lymphoid compartment (WP), it was assumed that movement of DCs within the spleen was not essential for T cell priming. Yet, splenic DC migration does occur, and we are only just beginning to understand how this process is regulated and how it affects immunity. As in the periphery, DC migration within the spleen is initiated through PRR activation (*9*, *48*–*50*). Using models to disrupt the ability of splenic DCs to reposition within the spleen, we and others recently demonstrated that DC migration from the BC or MZ/RP into the TCZ of the WP is required for $CD4^+$ T cell priming (*9*, *12*). Again, similar to migratory DCs in the periphery, this relocalization to the WP is CCR7 dependent (*9*, *32*). Therefore, splenic DCs mirror DCs in peripheral tissues: Resident (LN/WP) and migratory (tissue/RP) DCs exist at steady state in different locations and then, upon activation, undergo chemokine-directed relocalization, which brings both resident and migratory DCs into segregated regions of the TCZ—in this case, the splenic PALS.

Macrophages

Macrophages are tissue-resident phagocytes that maintain homeostasis by clearing cell debris and regulating the function of neighboring cells but, during infection or tissue damage, can respond with inflammatory cytokine responses that activate other leukocytes. Multiple subsets of macrophages exist in the spleen, each scanning distinct anatomical regions and expressing particular sets of PRRs and scavenger receptors. The four most studied subsets include the MZM, MMM, RPMs, and tingible body macrophages [(*26*), review] (Table 1). Two macrophage populations reside in the MZ at steady state, MZM and MMM, both of which clear debris (including apoptotic cells through specialized sets of phagocytic receptors), share BM monocyte ontogeny, and are important for the induction of tolerance to self-antigens (*16*). MZMs and MMMs are macrophage colony-stimulating factor (M-CSF)– and liver X receptor α (LXRα)–dependent cells (*16*); they can rapidly turn over during inflammatory states, replenished by BM-derived monocytes. MZMs express macrophage receptor with collagenous structure (MARCO) and SIGN-R1 and can interact with MZB cells. By contrast, MMMs express SIGLEC1/CD169 and MHC II, have processes that can penetrate into the WP from the MZ, and can share antigen with DCs in the MZ (*26*, *51*). RPMs reside throughout the RP and filter the blood of bacteria, apoptotic or fragmented cells, and other debris as well as "groom" RBCs of inappropriate inclusions, such as denatured hemoglobin. RPMs are long-lived, self-renewing cells derived from a yolk sac progenitor and are M-CSF independent; however, during states of inflammation, some can also be replenished by BM-derived monocytes (*52*). Tingible body macrophages are B cell follicle–resident macrophages responsible for removing apoptotic B cell debris that accumulates during B cell selection (affinity maturation and class switching) in the light zone of the GC (*26*). The ontogeny of tingible body macrophages requires further investigation.

NK cells

NK cells localize in the RP but can contribute to T cell polarization by migrating into the WP after infection and by producing interferon-γ (IFNγ), which can also affect the early innate response by promoting TNF and iNOS producing DC (Tip-DC) differentiation (*53*, *54*). Most studies into NK cell function have focused on their role in defending against viral infection (using PRRs), tumors, or damaged self-cells (using NKG2D). However, their role in regulating splenic adaptive immunity is still an area of active research [(*55*), review].

Monocytes

Monocytes are present in the BM, blood, and spleen and can differentiate into multiple specialized myeloid subsets (*56*). Two major monocyte populations in the blood have been identified, $Ly6C^{lo}$ and $Ly6C^{hi}$, which perform different functions: One circulates and mobilizes to tissues when needed, and the other is the patrolling monocyte whose job is to monitor the vasculature. These blood-derived monocytes, after encountering bacteria in the blood, can exit into the MZ and induce T cell–independent MZB responses (*48*). Monocytes in the spleen can also help maintain self-tolerance through the clearance of apoptotic bodies and production of immunosuppressive factors such as indoleamine 2,3-dioxygenase (IDO), transforming growth factor–β (TGF-β), and IL-10 (*1*). During an inflammatory response, CCR2 triggered by MCP-1 and MCP-3 (presumably provided by macrophages in the MZ) (*57*) also recruits $Ly6C^{hi}$ monocytes from the BM to the spleen. There, they can differentiate into multiple myeloid cell types, including certain types of nonconventional DCs and macrophages (*58*). A particular type of monocyte develops in the RP from these precursors, analogous to inflammatory monocytes in other tissues, $CD11c^+$ "TIP (TNF-iNOS)–DCs". TIP-DCs are highly inflammatory, producing TNFα and nitric oxide (NO), but are not proficient APCs for naive T cells (*59*); however, these inflammatory monocytes are capable of reactivating effector or memory T and NK cells (*60*). The spleen also acts as a reservoir for undifferentiated monocytes that can be mobilized to other organs under inflammatory conditions likely to augment the monocytes released from the BM. These reservoir monocytes are marked by CX3CR1 along with variable amounts of Ly6C and are concentrated in the subcapsular RP (*1*).

Adaptive immune cell function and organization

T and B cells, the key effectors of the adaptive immune system, are present throughout the spleen. Their localization changes with activation state and is organized by expression of cell surface receptors and chemotactic gradients. B-2, follicular B cells are the canonical

T cell–dependent antibody-producing B cells in the spleen (*61*). Naïve follicular B cells reside in WP follicles, but once activated, B cells rapidly cycle between the light and dark zone of the GC. CXCR5 drives B cells to the light zone, where they receive T cell help, whereas CXCR4 drives them into the dark zone, where they undergo rapid proliferation, class switching, and somatic hypermutation (*8*). Epstein-Barr virus–induced gene 2 (EBI2; GPR183) is another important receptor that organizes B cells within the BCZ based on oxysterol chemoattractants (*8*). After this GC reaction, some B cells depart into the BC and RP to become antibody-secreting cells. Some also become long-lived plasma cells in the RP.

Recently, it was shown that $CD4^+$ and $CD8^+$ T cells segregate within the PALS of the mouse WP at steady state and after systemic infection (*9*, *12*, *62*); whether a similar pattern exists in the human spleen is unknown. $CD4^+$ T cells are concentrated in the outer border of the PALS, adjacent to B cell follicles. $CD4^+$ T cells, particularly T follicular helper (T_{fh}) cells, are found in the splenic TCZ/PALS and provide help to B cells in the follicle for the production of high-affinity antibodies through cytokine production (such as IL-21) and direct costimulation (such as ICOS-ICOS ligand) (*63*). During an active immune response, T_{fh} cells up-regulate CXCR5 to reach the T-B border, whereas B cells up-regulate CCR7 to move from the BCZ to the T-B border [(*30*), review]. Oxysterols (EBI2 ligands) are also expressed at the T-B border, which recruits T_{fh} cells, cDCs, and B cells.

In contrast, naïve $CD8^+$ T cells reside in the central PALS of the WP, awaiting antigen presentation by APCs (*64*). After priming, $CD8^+$ T cell responses evolve during the course of splenic infection, and different fates are compartmentalized (*4*). Once primed, activated cytotoxic T lymphocytes (CTLs) leave the WP through the BC to the MZ and RP, ultimately helping to clear the infection in the "periphery" of the spleen (RP) (*4*). Later, some memory $CD8^+$ T cells return to the PALS of the WP (*4*, *65*), whereas CD62L-$CXCR3^+$ memory $CD8^+$ T cells remain in the RP (*64*, *66*).

HYBRID CELLS: INNATE-LIKE LYMPHOCYTES

NKT cells and γδ T cells

A specialized type of innate lymphocyte called the NKT cell expresses an invariant T cell receptor (TCR) and recognizes glycolipid antigens on the MHC I–like molecule, CD1d. CD1d is highly expressed by MZB cells, which can activate NKT cells. Activated NKT cells produce inflammatory cytokines and can license splenic DCs for cross-priming of $CD8^+$ T cells (*67*, *68*). γδ T cells also exhibit innate-like function because of their lack of MHC restriction, rapid cytokine secretion, expression of PRRs, and ability to directly lyse infected cells (*68*, *69*). γδ T cells are relatively rare in the spleen but are important for the early response to infection. NKT and γδ T cells in the spleen promote T helper 1 (T_H1) polarization and $CD8^+$ T cell activation by producing cytokines such as IFNγ, TNFα, and IL-12, which also help to activate cDC1s (*70*).

Innate lymphoid cells

Innate lymphoid cells (ILCs) are defined by their CLP ontogeny and lack of recombination-activating genes or TCR expression; they are further subdivided by their cytokine secretion profile (*71*). A vital function of ILCs is to rapidly produce cytokines in response to infection or tissue injury. T_H2-like cytokines—IL-5, IL-9, and IL-13—are produced by ILC2s found in the spleen (*72*). ILC3s can activate MZB cells and neutrophils by producing TNFα, lymphotoxin, and granulocyte-macrophage colony-stimulating factor and can provide costimulation to splenic $CD4^+$ T cells through production of IL-2, IL-6, and macrophage inflammatory protein 1α. The role of ILCs in direct antigen presentation remains controversial (*73*).

MZB cells

Mouse MZB cells are an innate-like B cell subset specific to the spleen, characterized by both location and function. They exhibit higher expression of PRRs than other B cells, have polyreactive B cell receptors, and rapidly produce low-affinity T cell–independent antibodies, primarily IgM (*20*). MZB cells originate from B cells in the BM and differentiate in the spleen after receiving survival signals through B cell–activating factor (BAFF) (*22*). They are NOTCH2, RBPJ, AIOLOS, dedicator of cytokinesis 8 (DOCK8), phosphatidylinositol 3-kinase, and CD19-dependent (*21*). In the absence of any of these molecules, MZB cells are substantially reduced in the spleen, whereas follicular B cell development remains intact (*22*). MZB cells capture antigen via complement receptors and migrate into the WP, both into the follicles to deliver opsonized antigen to B cells (*14*) and into the TCZ, where they have been suggested to activate $CD4^+$ T cells (*20*). This shuttling of antigen into the WP is followed by the return of MZB cells to the MZ and depends on cyclical expression of S1P receptors and CXCR5 (*74*). Therefore, MZB cells are important in both T cell–dependent and T cell–independent antibody production. The extent to which human MZB cells map onto the above description of mouse MZB cells remains unclear.

B-1 cells

B-1 cells are another innate-like B cell; this population is most abundant in the peritoneum, but a small population also exists in the spleen. Like MZB cells, B-1 cells produce IgM antibodies and additionally contribute to circulating "natural" antibodies (those produced without antecedent exposure to the antigen) (*75*). B-1 cells can be further divided into B-1a cells ($CD5^+$, produce natural antibodies) and B-1b cells ($CD5^-$, less common, produce T cell–independent antibodies, and participate in memory responses). The difference in function of their produced antibodies inspired the "division of labor hypothesis" to describe the observed phenotypic differences between B-1a and B-1b cells (*76*). Some antibodies produced by B-1a cells are self-reactive—for example, IgM antibodies that recognize oxidized lipids and aid in removal of apoptotic cells (*75*). Whether B-1 cell counterparts exist in the human spleen is still under debate.

INNATE CONTROL OF ADAPTIVE IMMUNITY TO INSULTS IN THE SPLEEN

The functional importance of the spleen in controlling systemic infections is evidenced by splenectomized patients, who show decreased clearance of malaria-parasitized RBCs and are at greater risk of meningitis and sepsis after infection with *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenza* type B (*1*). This section will review how foreign pathogens or cells in the blood lead to coordinated splenic innate and adaptive immune responses and how pathogens target immune cells to prevent linkage of the two immune arms.

The spleen in bacterial infection

Listeria monocytogenes is a Gram-positive, food-borne bacterium that, when injected intravenously in mice, provides a model pathogen

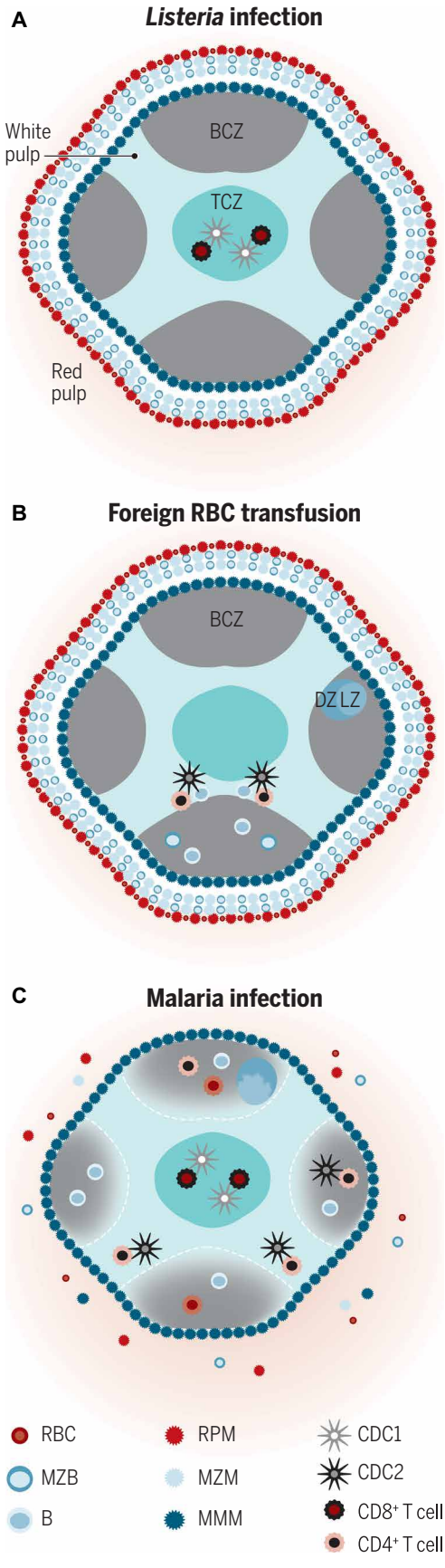

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Fig. 2. The cellular organization of the murine WP is dynamic. The cells of the TCZ (turquoise) are organized into $CD8^+$ T cells (maroon) in the center and $CD4^+$ T cells (orange) in the outer TCZ. This organization enables rapid formation of necessary cellular contacts, after migration within the WP, for the response to an immunologic insult. Shown are selected, dominant immune reactions in the spleen relevant to each insult. (**A**) *Listeria* induces rapid migration of cDC1s (silver) into the central TCZ where they present antigen and prime $CD8^+$ T cells. (**B**) Transfusion of xenogeneic or allogeneic RBCs induces migration of cDC2s (black) to the outer TCZ, for $CD4^+$ T cell priming and effective B cell antibody production (with the LZ, light zone, and DZ, dark zone, of a germinal center, GC, shown). (**C**) Malaria-infected RBCs induce a poorly coordinated and slow humoral immune response that includes disintegration of WP-RP border, MZ disruption and GC disorganization. After repeated infections, protective antibodies are ultimately produced.

for understanding splenic filtering of bacteria from blood. During physiologic infection, the pathogen is ingested and travels from the gastrointestinal tract to the liver and spleen. Because *Listeria* is a facultative intracellular pathogen, antibody-mediated immunity is not particularly effective for clearance. Instead, the innate branch of the immune system is crucial for controlling early infection, particularly monocyte-derived TIP-DCs, neutrophils, and multiple macrophage populations (*59*, *77*, *78*). Although depletion of macrophages impairs the early response to infection, it does not impair activation of T cells, which is accomplished by DCs (*79*). However, macrophages can facilitate the process of live *Listeria* delivery into DCs through transinfection of $CD8^+$ T cells by MMMs (*51*). As will be discussed, this can potentiate $CD8^+$ T cell priming but paradoxically helps propagate the bacterial infection.

At later stages of infection, primed $CD8^+$ cytotoxic T cells promote phagocyte clearance of bacteria, directly lyse infected cells, and prevent reinfection (*80*). These cytotoxic T cells are primed by cDC1s; however, cDC1s also act as a "Trojan Horse" to propagate *Listeria* infection in the spleen (Fig. 2) (*80*, *81*). cDC1s harboring *Listeria* migrate to the TCZ, where few phagocytes or neutrophils reside at steady state, enabling the pathogen to temporarily avoid immune clearance (*82*, *83*), although ultimately, NK cells and TIP-DCs cluster in the WP to help contain the infection (*54*). Thus, by co-opting cDC1s' natural ability to hold onto intact antigens in nonacidified phagosomes and migrate to the WP, *Listeria* effectively uses the immune system itself to briefly escape the innate immune response. The importance of this mechanism in infection is highlighted by cDC1 depletion in BATF3-deficient mice, which actually confers resistance to *Listeria* (*81*).

Several innate immune mechanisms have evolved for *Listeria* recognition in different cellular locations. Before entering the cytosol, *Listeria* is sensed by TLR5 (for flagellin) and TLR2 (for cell wall lipoproteins) in a myeloid differentiation primary response 88 (MyD88)–dependent pathway, leading to nuclear factor κB–driven production of pro–IL-1β and TNFα (*84*). The presence of *Listeria* peptidoglycan in the cytosol activates NOD1 and NOD2 to amplify the activation signals from TLRs via RIP2, resulting in IL-6 and TNFα production (*84*). Other cytosolic receptors, such as cGAS-STING (cyclic GMP-AMP synthase and stimulator of interferon genes protein) and RIG-I, are required for sensing *Listeria* after escape from endosomes (*85*, *86*). Multiple aspects of *Listeria* or its effect on host cells are detected by inflammasomes, which induce caspase-1 activity and IL-1β and IL-18 production (*84*). Loss of any one of the above innate immune pathways typically has little impact on initiation of adaptive immunity, likely because of this redundancy; however, these pathways are particularly important for innate immune

cell activity, including recruitment and effector function of TIP-DCs (*59*). The study of *Listeria* in the spleen highlights both the myriad of innate and adaptive effector cells in the spleen that cooperate to control a bacterial pathogen and how the pathogen circumvents many of these attempts to replicate in an SLO.

The splenic response to foreign RBCs

Another immune response regulated by the spleen is the transfusion of foreign RBCs. Blood transfusion is a lifesaving therapy, but because of the myriad of RBC non-ABO antigen polymorphisms between individuals, some patients generate a deleterious antibody response to alloantigens on nonself RBCs. The fundamental rules that govern when someone will generate an antibody response to transfused RBCs remain unclear. Identifying such rules from human studies has been challenging, and mice have a poorly understood system of blood group antigens (Ea1 to Ea10); further, mice do not express the same minor antigens as human RBCs. Therefore, transgenic mice expressing well-defined foreign antigens on RBCs such as the human KEL glycoprotein, glycophorin A, or model protein antigens have recently enabled the study of alloimmunization in mouse models (*87*). The humoral alloantigen response to transfused leukoreduced RBCs requires an intact spleen and, in some models, requires T cell help (*47*, *87*, *88*); however, certain antigens have been recently shown to induce a T cell–independent MZB antibody response (*88*). Although RBCs are consumed in both the spleen and the liver, T cell activation does not occur in the liver (or LNs) in these models (*89*). The spleen is the main SLO regulating this humoral response to RBCs, and accordingly, splenectomized mice generally fail to generate alloantibodies after transfusion (*87*). Both splenic DCs and macrophages phagocytose allogeneic mouse RBCs (*90*, *91*), and because splenic macrophages clear endogenous aged RBCs, it had been assumed that macrophages also regulate the adaptive immune response (*92*). However, more recent studies have concluded that DCs are the primary APC for initiating the allogeneic response to RBCs (*47*, *93*).

Historically, mouse models have used transfusion of xenogeneic sheep RBCs rather than allogeneic RBCs to study GC responses. In contrast to the allogeneic RBC immune response in which transfused RBCs circulate for weeks, sheep RBCs are cleared within hours (*32*, *47*). Both forms of RBCs induce DC activation and migration to the WP (*32*, *47*, *50*, *94*). Antibody generation to both types of RBCs requires the same subset of cDCs. Impairment of 33D1$^+$ BC cDC2s in EBI2-, IRF4-, CD47-, or DOCK8-deficient mice results in impaired T cell–dependent antibodies to sheep RBCs or mouse allogeneic RBCs (*32*, *33*, *47*, *50*). More specifically, a subset of cDC2s regulated by NOTCH2 signaling likely regulates this T_{fh} cell–dependent antibody response (*35*). Because either cDC1 or cDC2 subsets can activate CD4$^+$ or CD8$^+$ T cells in vitro (*9*), we searched for a mechanism that could explain this restricted in vivo DC subset requirement. We found that CD4$^+$ T cells segregated within the WP with cDC2s and that CD8$^+$ T cells colocalized with cDC1s (Fig. 2); disrupting this pairing selectively blocked naïve CD4$^+$ or CD8$^+$ T cell activation to multiple antigenic targets, including sheep and mouse RBCs (*9*, *12*). The GC reaction to sheep RBCs requires EBI2 expression on both T_{fh} cells and cDC2s, recognizing 7α,25-dihydroxycholesterol for proper positioning, further supporting a model in which chemoattractant-driven colocalization is necessary for adaptive immune induction (*12*). The response to transfused RBCs demonstrates the importance of spatiotemporal cellular organization of splenic T cell and DC subsets in regulating the humoral immune response to systemic antigens.

cDC2s recognize sheep RBCs as foreign through species differences in the "don't eat me" signal CD47, an integrin-associated protein that binds SIRPα and inhibits phagocytosis (*50*). DC priming of T_{fh} cells after recognition of CD47-deficient sheep RBCs is dependent on the integrin-signaling adaptor protein Talin-1 (*95*). However, the mechanism by which cDC2s sense allogeneic RBCs as foreign remains unknown. Processing and storage of RBCs for transfusion result in biochemical and morphological modifications known as the "storage lesion." Whether this confers immunogenicity to stored RBCs (such as DAMP production) is controversial and requires further study (*96*). Nonetheless, transfusion of stored but not fresh mouse RBCs induces innate cytokine production, including IL-6, which is relevant for the subsequent alloantibody response and DC activation (*94*). Mouse RBCs lack inhibitory ligands for the B cell lectin CD22, and therefore, if they additionally contain an antigen recognized by the B cell receptor, they can directly activate B cell responses (*97*). Hemoglobin-derived hemin has been proposed to stimulate human monocytes to promote a regulatory T cell response to damaged RBCs (*98*). By contrast, heme released from lysed RBCs has been shown to induce NLR family pyrin domain containing 3 (NLRP3) activation in macrophages and proinflammatory cytokine production (*99*). However, NLRP3, caspase-1, or caspase-11 inflammasomes are not required for alloantibody production to one tested strain of transgenic RBCs (*94*). Although CD47 declines with aging of RBCs in vivo, it is not clear that the same is true of processed and stored RBCs (*91*); therefore, how transfused allogeneic RBCs trigger requisite innate immunity is a mystery that remains to be solved.

The spleen in parasitic infections

Plasmodium falciparum is a parasite that infects RBCs, causing malaria, a disease of substantial morbidity and mortality (*100*). Both protective and harmful inflammatory responses to malaria are coordinated in the spleen that contribute not only to cellular and humoral parasite clearance but also to inflammation-induced host damage [(*19*), review]. The malaria parasite enters the human host through a mosquito vector and reproduces in the liver for 7 to 10 days (*101*). After this stage, the parasite infects and replicates inside RBCs and triggers innate and adaptive immune responses in the spleen. Although CD8$^+$ T cell–mediated immunity can restrict infection during the non-RBC phase, infected RBCs can evade CD8$^+$ T cell–mediated killing because they mostly lack MHC expression. CTL control of infected reticulocytes (immature RBCs) might partially help control parasite burden (*102*), but antibody-mediated immunity is most efficacious at clearing parasitized RBCs (*103*). Mice with T cell–specific B cell lymphoma 6 protein (BCL6) deficiency or ICOS blockade, and therefore loss of T_{fh} cell differentiation, have impaired T cell–dependent antibody production and are unable to clear malaria infection, showing that T_{fh} cells are required for the protective humoral response (*104*, *105*). Reduced antibody production in IL-12–deficient mice suggests that these CD4$^+$ T cells are IFNγ-producing type 1 T_{fh} cells (*106*). Type 1 immunity characterizes the first erythrocytic stage of infection with elevated IL-6, IL-12, IFNγ, NO, and TNFα production by both NK cells and T_H1 cells [(*107*), review]. However, induction of T_H1 cells in fact might worsen symptoms during infection rather than enable parasite clearance (*108*). Originally, it was thought that the nature of the cellular response evolves over the course of infection to a type 2–skewed

T_H2-dominated response. New evidence suggests that mixed $CD4^+$ T effector cell fates along with regulatory T cell subsets better characterize this phase of immunity (*104*).

The coordinated immune response to malaria involves multiple splenic myeloid populations. Work using CD11c-DTR (diphtheria toxin receptor) mice demonstrated that $CD11c^+$ cells selectively phagocytose infected RBCs and are required for presenting parasite antigens to $CD4^+$ T cells (*109*). Using methods to target subsets of DCs, it was found that the relevant APC shifts during the two stages of infection. During the early blood stage, cDC1s are required for T_H1 cell priming (*110*). During the late blood stage, cDC2s support antigen-specific $CD4^+$ T cell responses and T cell–dependent antibody production; cDC1s appear to be more sensitive to apoptosis during this later stage (*111*). Early in the immune response, monocytes are also recruited from the BM to the spleen in both mice and humans, where they play an important role in controlling parasite burden but less of a role in T cell priming (*112*).

Multiple aspects of the malaria parasite are sensed by the splenic innate immune system. Once inside host RBCs, the pathogen uses proteases to digest hemoglobin, converting it into an inert crystal, hemozoin (*100*, *113*). Mouse and human monocytes respond to this DAMP through the NLRP3 inflammasome, resulting in production of the fever-promoting cytokine IL-1β (*107*). *P. falciparum* DNA engages multiple PRRs, including TLR9, which drives TNFα and IL-12 production by DCs and IFNγ production by NK cells through MyD88 (*107*), as well as cGAS and STING (*114*, *115*). TLR2, TLR4, and TLR7 also sense different aspects of the parasite or its effect on RBCs, including glycosylphosphatidylinositols in the parasite membrane, heme from parasitized RBCs, and RNA (*116*–*118*). pDC activation by these PRRs induces type 1 IFN production, which facilitates cDC function (*119*). Possibly because numerous innate immune pathways are triggered by this parasite, deletion of individual pathways does not substantially impair the T cell response (*114*, *120*). However, many of the cytokines important for promoting adaptive immunity or the symptoms of malaria are induced by particular PRRs or their downstream pathways and have been directly linked to APC activation (*119*). Different groups use different *Plasmodium* strains, and this might favor different mechanisms of innate sensing or responses from particular subpopulations of cells (*121*).

Unlike many infections, malaria requires repeated or prolonged infection before protective humoral immunity is established (*122*). This may be due to the severe disruption of the splenic architecture induced by *Plasmodium* infection, including loss of the MZ, blurring of the RP-WP distinction, and disrupted GCs (Fig. 2) (*114*, *123*). Parasitized RBCs also impair DC activation and antigen presentation and survival, possibly through the production of hemozoin, resulting in reduced T cell priming (*19*). However, other studies have argued that in vivo DC activation and antigen presentation are intact (*124*). The nature of DCs used and the methods of study, along with different *Plasmodium* species, may account for some of these differences. Regardless, T_{fh} cell induction and GC formation appear to be inhibited by *Plasmodium* in part through modulation of DC and other innate cell function. One proposed mechanism for blunted T_{fh} cell priming is type 1 IFN stimulation of DCs (*105*), but likely multiple pathways result in the delayed humoral response, including direct B cell effects (*120*). Further, CXCL10 production by inflammatory monocytes and parasite-induced IFNγ and TNFα have been associated with impaired T_{fh} cell differentiation, GC formation, antibody production, and an increase in parasite burden (*125*). Together, these effects diminish the potentially protective humoral adaptive immune response.

The response to malaria highlights the delicate balance between redundant immune sensing and activation mechanisms in controlling systemic infection and the detrimental effect of excessive cytokine production in causing tissue damage and inefficient adaptive responses.

CONCLUSIONS AND FUTURE PERSPECTIVES

The intricate positioning of immune cells within the spleen and the ways in which their migration is orchestrated allow specific tailoring of the immune response to match the insult. However, we are only just beginning to identify the mechanisms that control the location of splenic immune cells, both at steady state and upon immune insult. Intravital imaging has revolutionized our understanding of how immune cells interact; however, a challenge in applying this technology to the spleen is the inability to penetrate deeply into the WP by means of two-photon microscopy. Some limited glimpses of the outer border of the WP—including pDC–T cell and DC–apoptotic cell interactions in the MZ, T cell entry from the blood, as well as follicular B cell–T_{fh} cell interactions—have been visualized (*11*, *14*). Early neutrophil and myeloid cell responses to bacterial infection in the RP have also been visualized (*77*). Use of fluorescent protein-expressing *Plasmodium* parasites has provided insights into the three-dimensional structural changes in the spleen that result from infection (*19*). Two-photon imaging ex vivo has been used to show infection of DCs by *Listeria* and trafficking into the $CD8^+$ T cell dense PALS (*83*). However, we have not yet achieved a complete picture of how lymphocytes throughout the WP in vivo interact with each other and with other cells of the innate immune system. Despite these limitations, application of this technology holds promise for understanding single-cell dynamics that govern innate cellular regulation of adaptive immunity in the spleen. Lastly, a concerted effort must be made to understand how different immune cell subsets are similarly or distinctly organized in the human spleen as compared with mouse.

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