The spleen is the main filter for blood-borne pathogens and antigens, as well as a key organ for iron metabolism and erythrocyte homeostasis. Also, immune and hematopoietic functions have been recently unveiled for the mouse spleen, suggesting additional roles for this secondary lymphoid organ. Here we discuss the integration of the spleen in the regulation of immune responses locally and in the whole body and present the relevance of findings for our understanding of inflammatory and degenerative diseases and their treatments. We consider whether equivalent activities in humans are known, as well as initial therapeutic attempts to target the spleen for modulating innate and adaptive immunity.

**Introduction**

The spleen is organized in regions called the red pulp and white pulp, which are separated by an interface called the marginal zone (MZ) (MacNeal, 1929). Blood circulation in the spleen is open: afferent arterial blood ends in sinusoids at the MZ that surrounds the white pulp. Blood flows through sinusoid spaces and red pulp into venous sinuses, which collect into efferent splenic veins. The splenic red pulp serves mostly to filter blood and recycle iron from aging red blood cells. The structural organization and multicellular composition of the organ also permits monitoring of most of the blood in the red pulp and MZ (Figure 1A). Diverse splenic populations not only trap and remove blood-borne antigens but also initiate innate and adaptive immune responses against pathogens. The white pulp is structurally similar to a lymph node, contains T cell and B cell zones (the latter are also called follicles), and allows generation of antigen-specific immune responses that protect the body against blood-borne bacterial, viral, and fungal infections. Additionally, the spleen is a site where immune responses that are deleterious to the host can be regulated.

Leukocytes in the spleen include various subsets of T and B cells, dendritic cells (DCs), and macrophages that exert discrete functions (Figure 1B). For example, red pulp macrophages are specialized to phagocytose aging red blood cells and regulate iron recycling and release, whereas MZ macrophages and metallophilic macrophages express a unique set of pattern-recognition receptors and remove at least certain types of blood-borne bacteria and viruses in the MZ. Beside specialized macrophages, the MZ also contains MZ B cells and DCs, which take up passing antigens and migrate to the white pulp to promote antigen presentation to lymphocytes. Access to the white pulp is largely restricted to B cells, CD4+ and CD8+ T cells, and DCs (Mebius and Kraal, 2005). Exit of leukocytes from the spleen occurs mostly through the splenic veins in the red pulp, although some cells in the white pulp can exit the organ locally via a network of efferent lymphatic vessels (Pellas and Weiss, 1990). Control of immune cell migration and functionality by several types of splenic stromal cells is reviewed elsewhere (Mueller and Germain, 2009).

In this review, we examine spleen functions and mechanisms of actions at the cellular and molecular levels, which are thought to regulate innate and adaptive immunity, control antigen tolerance, and either protect the host or contribute to diseases. To do so, we first address our current knowledge on the origins, behavioral activities, and dynamics of different splenic immune cell populations that: (1) exist in the spleen prior to immune activation, (2) are recruited in response to a diseased state, (3) are produced and/or further amplified locally, and (4) are mobilized from the spleen to other tissues. We then discuss splenic regulation of antigen tolerance, compare hematopoietic activities in mouse and human spleens, and report initial attempts to target the spleen for therapeutic purposes.

**Resident Lymphocytes**

Circulating T and B cells frequently gain access to secondary lymphoid organs in search for their cognate antigens. Trafficking and positioning of lymphocytes within defined splenic microenvironments enables scanning of antigen-presenting cells (APCs) and is guided by stromal cell networks (Mueller and Germain, 2009), integrins (Lu and Cyster, 2002), chemokines (Ngo et al., 1999), and other factors (Hannedouche et al., 2011). For instance, distinct chemokines attract and maintain B and T cells to their respective zones: Whereas chemokines such as CXCL13 attract B cells expressing the chemokine receptor CXCR5 to follicular B cell zones (Ansel et al., 2000), CCL19 and CCL21 attract CCR7+ T cells and antigen-presenting DCs in T cell zones (Gunn et al., 1999). Intravital lymph node imaging studies indicate that CCR7 ligand interactions not only guide T cell homing but also stimulate basal T cell motility inside the lymphoid organs (Worbs et al., 2007). Both processes facilitate T cell-DC interactions and thus antigen screening by T cells.

The spleen contains distinct B cell lineages, including follicular and MZ B cells. Whereas follicular B cells recirculate and participate mainly in T cell-dependent immune responses (Tarlinton and Good-Jacobson, 2013), MZ B cells reside between the MZ and red pulp, capture antigens carried in the blood via complement receptors, and promote both T cell-independent and dependent immune responses (Pilai and Cariappa, 2009).
Follicular and MZ B cells express the chemokine receptor CXCR5 and thus can be expected to respond to follicular-attracting activity mediated by its ligand CXCL13. However, MZ B cells overcome such activity by expressing high amounts of the sphingosine-1 phosphate-1 (S1PR1) and S1PR3 receptors (Cinamon et al., 2004). Binding of the lysophospholipid S1P to these receptors triggers a chemotactic response and promotes MZ B cell accumulation in the MZ and red pulp where S1P is found in higher concentrations. MZ B cells also express cannabinoid receptor 2 (CR2), which, together with S1PR1 and S1PR3, maintains these cells in the spleen (Muppidi et al., 2011).

MZ B cells were initially viewed as a sessile immune subset: they express high amounts of cell surface proteins, such as integrins VLA-4 and LFA-1 (Lu and Cyster, 2002), which confer binding to stromal cells and resistance to local shear forces of blood flow that would otherwise direct the cells to the circulation. Nevertheless, intravital microscopy studies indicate that MZ B cells shuttle continually between the MZ and follicles (Amon et al., 2013). The mechanisms that control such oscillatory...
migration involve transient S1PR1 desensitization (for migration from the marginal zone into the follicle) followed by S1PR1 resensitization (for migration back to the marginal zone). S1PR1 desensitization is regulated by guanine nucleotide–binding protein–coupled receptor kinase 2 (GRK2) (Amon et al., 2011). GRK2-mediated modulation of S1PR1 permits MZ B cell entry into white pulp follicles against the S1P gradient and provides a mechanism for the ability of these cells to deliver opsonized antigens from the open blood circulation to the follicles. Subsequent antigen presentation to follicular B cells, most notably by follicular DCs, establishes the humoral immune response.

Intravital microscopy studies have confirmed splenic follicular B cell recirculation, a process involving S1PR1-dependent cell transit from follicle to MZ and further removal from the spleen through the red pulp (Amon et al., 2013). Unlike MZ B cells, follicular B cells fail to express sufficient amounts of integrins and, for this reason, presumably cannot adhere to the MZ stroma. These findings suggest that distinct integrin-dependent adhesion capabilities of B cell subsets force follicular B cells to recirculate to distant follicles in search for antigens but enrich the MZ with a population of B cells that is equipped with specialized antigen-sensing functions.

The spleen also contains a sizable population of natural killer T (NKT) cells, which sense lipid antigens and are involved in a broad range of immune responses by secreting cytokines and inducing downstream activation of adaptive immune cell types. Lipid antigen presentation is facilitated by CD1d, which is expressed at elevated amounts by MZ B cells. Intravital microscopy studies indicate that splenic NKT cells locate mostly in the MZ and red pulp. These cells respond to lipid antigens specifically in these regions but only in presence of MZ B cells (Barral et al., 2012). Presumably, MZ B cells undergo physical interactions with NKT cells to facilitate sensing of blood-borne antigens and NKT cell stimulation. Activation of both T and NKT cells likely depends on the positioning of B cell lineages in distinct splenic compartments. Manipulating B cell compartmentalization should also considerably alter antigen presentation and the outcome of adaptive immune responses.

**Resident Phagocytes**

The spleen hosts all major types of mononuclear phagocytes, including macrophages, DCs, and monocytes. These cells are key protectors of the organism because they identify pathogens and cellular stress, remove dying cells and foreign material, regulate tissue homeostasis and inflammatory responses, and shape adaptive immunity. Additionally, they can contribute to many diseases, as discussed below. Mononuclear phagocytes remain actively studied >130 years after their discovery (Metschnikoff, 1884) and recent reports continue to reveal unexpected findings on their origins and functions in various tissues including the spleen.

Until recently, tissue-resident macrophages were mostly viewed as descendants of circulating monocytes; however, genetic and cell-fate mapping studies suggest that in the steady-state, most macrophages and monocytes represent distinct phagocyte lineages (Schulz et al., 2012; Hashimoto et al., 2013; Yona et al., 2013). Whereas circulating monocytes derive from hematopoietic stem cells (HSCs) and discrete intermediary progenitors, which occupy specialized niches of the bone marrow (Mercier et al., 2012), many tissue-resident macrophage populations, including lung, liver, brain, peritoneal, bone marrow, and red pulp splenic macrophages, are established prior to birth either from elements present in the yolk-sac (Schulz et al., 2012) or from embryonic fetal liver precursors (Hoeffel et al., 2012; Guilliams et al., 2013). Consequently, under steady-state, most splenic and other macrophages can be generated independently of adult HSCs and, by extension, of HSC-derived circulating monocytes. Such macrophages demonstrate the capacity to self-maintain throughout adult life (Hashimoto et al., 2013). Macrophage maintenance is not restricted to the steady-state but can be preserved after enhanced cell turnover triggered by exogenous challenges (Jenkins et al., 2011; Robbins et al., 2013).

As notable exceptions, discrete splenic macrophage populations were recently proposed to derive from CX3CR1<sup>hi</sup>Ly6C<sup>lo</sup> monocytes, possibly after their transition to CX3CR1<sup>hi</sup>Ly6C<sup>lo</sup> monocytes. These include MZ-resident SIGN-R1<sup>-</sup> MARCO<sup>+</sup> and metallophilic CD169<sup>+</sup> macrophages (A-Gonzalez et al., 2013). The development and maintenance of these cells, but not of other splenic macrophage populations, depends in part on liver X receptor α (LXRα) signaling because the spleen of LXRα-deficient mice selectively lacks MZ macrophages. Conversely, adoptive transfer of LXRα-sufficient monocytes into LXRα-deficient mice partially restores the MZ macrophage population (A-Gonzalez et al., 2013). LXRα-dependent accumulation of MZ macrophages also promotes MZ B cell retention and pathogen clearance, which suggests that LXRα signaling regulates systemic antimicrobial immunity by selectively controlling the splenic MZ niche. LXRα and LXRβ are members of the nuclear receptor superfamily of transcription factors responsive to oxysterols, which are oxidized derivatives of cholesterol. Characterizing the spectrum of effects mediated by oxysterols and other LXR ligands in the spleen promises to be interesting because initial studies already indicate that these agents might (1) control the maintenance of MZ macrophages (A-Gonzalez et al., 2013) and DCs (Gatto et al., 2013), (2) chemoattract activated B cells to the outer follicle to promote plasma cell responses (Hannedouche et al., 2011; Liu et al., 2011), and (3) suppress splenic myelopoiesis (Yvan-Charvet et al., 2010; Murphy et al., 2011).

The reason why MZ macrophages might have a distinct monocytic origin remains unclear. Possibly, monocyte precursors confer unique attributes to these cells. For instance, monocye-derived macrophages appear better equipped than their yolk-sac-derived counterparts to trigger and shape immune responses (Schulz et al., 2012), a feature that might be required for efficient MZ macrophage activity. Additionally, MZ macrophages—like gut macrophages (Hashimoto et al., 2013; Yona et al., 2013)—might never face a truly resting environment but instead be repeatedly exposed to exogenous antigens, which trigger monocyte accumulation and local differentiation into “activated” macrophages. Endogenous circulating ligands, such as apoptotic cells, might also promote the tonic maintenance of tolerogenic macrophages, as discussed below.

Another longstanding paradigm states that monocytes are cells that either circulate freely in blood (CX3CR1<sup>hi</sup>Ly6C<sup>lo</sup> cells) or patrol blood vessels (CX3CR1<sup>hi</sup>Ly6C<sup>lo</sup> cells) (Geissmann et al., 2010) but irreversibly differentiate into DCs or macrophages...
upon recruitment to either lymphoid (Serbina et al., 2003; Cheong et al., 2010) or nonlymphoid tissues (Nahrendorf et al., 2007; Grainger et al., 2013). Departing from this paradigm, bona fide undifferentiated monocytes, including CX3CR1intLy6Chi and CX3CR1intLy6Clo subsets, accumulate in the spleen under steady-state and outnumber circulating monocytes (Swirski et al., 2009). Intravital imaging of the spleen (Pittet and Weissleder, 2011) indicates that monocytes assemble in clusters of ~20–50 cells in the subcapsular red pulp, near venous sinuses and collecting veins, along the perimeter of the organ; the cells closely resemble their blood monocyte counterparts and are morphologically, phenotypically, and functionally distinct from splenic macrophages and DCs. How splenic monocytes are maintained and whether they contribute to the replenishment of MZ macrophages is unknown; however, inflammatory signals can mobilize these cells en masse to distant tissues, as discussed below.

Splenic DCs originate from bone marrow HSCs, specialize in antigen processing and presentation, and are key initiators and controllers of adaptive immunity (Merad et al., 2013). Besides interferon (IFN)-producing plasmacytoid cells, splenic DCs contain at least two classical subsets: CD8α–CD11b+ cells, present in T cell zones and responsible for the uptake of dying cells and cross-presentation of antigens to CD8+ T cells, and CD8α+CD11b+ cells, preferentially found in the red pulp and MZ, expressing major histocompatibility complex class II glycoprotein (MHCII)-peptide complexes and presenting antigens to CD4+ T cells (Dudziak et al., 2007; Sancho et al., 2009). The mechanisms that define splenic DC phenotypic and functional heterogeneity implicate several molecular pathways, including at least lymphoxygenase-1 (LT1), Notch2, retinoic acid (RA), and chemotactic receptor EBI2 signaling. LT1 receptor signaling acts by generating and maintaining CD8α+CD11b+ DCs (Kabashima et al., 2005) and by promoting M2 B cell homeostasis (Hozumi et al., 2004). Notch2 receptor signaling more specifically controls differentiation of a subset of splenic CD8α–CD11b+ DCs that express the endothelial cell-selective adhesion molecule (Esam). Esam+CD8α–CD11b+ DCs are specialized in CD4+ T cell priming, whereas their Esam− counterparts secrete cytokines and are phenotypically closer to, but distinct from, monocytes and macrophages (Lewis et al., 2011). The vitamin A derivative RA is also critical for maintenance of the Esam+CD8α–CD11b+ DC subset (Klebanoff et al., 2013). Because mammals lack the capacity to synthesize vitamin A, malnutrition or other events that alter RA amounts (e.g., total body irradiation, which impairs the small intestine where RA is obtained) might reduce the numbers of splenic CD8α–CD11b+ DCs and limit CD4+ T cell activity, but these impairments might be restored with RA infusion. Finally, the chemokine receptor EBI2, which binds 7α,25-dihydroxycholesterol and other closely related oxysterols, regulates positioning of a subset of splenic MZ DCs expressing the CD8α–CD4+ phenotype. It is not clear how many of these cells express Esam but, like Esam+ DCs, they appear required for full-fledged CD4+ T cell activation and antibody response induction (Gatto et al., 2013; Yi and Cyster, 2013).

Cells Recruited to the Spleen

The spleen has no afferent lymph vessels and collects its leukocytes directly from blood. Besides circulating immune cells that continuously migrate into and out of the resting spleen, diseases can recruit additional cells to the organ. For instance, the effects of *Listeria monocytogenes*, a Gram+ bacterium that infects the spleen and liver, involve a sequence of actions that includes CCR2-dependent mobilization of CX3CR1intLy6Chi monocytes from the bone marrow to the blood stream, followed by CX3CR1-dependent accumulation in the spleen MZ and T cell zones and differentiation into DC-like cells that produce tumor necrosis factor-α (TNF-α) and inducible nitric oxide synthase (iNOS or NOS2) (Tip-DCs) (Serbina et al., 2003; Serbina and Pamer, 2006; Auffray et al., 2009). These events are important for early control of the infection. *Listeria* also induces NK cell recruitment to the spleen, at least in part in a CCR5-dependent manner; NK cells locally produce IFN-γ, which is necessary for differentiating monocytes into cytokine-producing DCs (Kang et al., 2008). CX3CR1intLy6Clo monocytes accumulate in the spleen in response to other infections, but the fate of these cells might diverge depending on the pathogen’s identity. For instance, different strains of lymphocytic choriomeningitis virus (LCMV), which trigger either acute or chronic infections, induce splenic accumulation of monocytes and neutrophils; however, only the chronic strain sustains a prolonged accumulation of these cells, which eventually acquire suppressor functions and chronically inhibit virus-specific T cell immunity (Norris et al., 2013). This process also involves inflammatory monocyte-derived DCs activated by LCMV to phagocytose apoptotic erythrocytes and produce interleukin-10 (IL-10) (Ohyagi et al., 2013).

Additionally, bacterial infection can induce the recruitment of a unique population of B cells to the spleen (Rauch et al., 2012). These cells, called innate response activator (IRA) B cells, likely derive from peritoneal B1a B cells, accumulate mainly in the red pulp, are retained locally in a VLA-4- and LFA-1-dependent manner, and are the main producer of granulocyte-macrophage colony-stimulating factor (GM-CSF). Mice in which GM-CSF production is only completely deficient in B cells show a severe IL-1β, IL-6, and TNF-α cytokine storm, impaired bacterial clearance, and decreased mouse survival in response to experimental sepsis, suggesting that IRA-B cells represent important regulators of innate immune activity. How GM-CSF produced by IRA-B cells precisely controls splenic innate immune cells remains unexplored.

**Cells Amplified or Produced in the Spleen**

It is well appreciated that hematopoietic stem and progenitor cells (HSPCs) exist in bone marrow niches where they produce their lineage descendants (Weissman et al., 2001). It has also been established for more than 5 decades that at least a fraction of bone marrow HSPCs can enter circulation in the steady-state (Goodman and Hodgson, 1962). Circulating HSPCs, which resist cell death by upregulating the don’t-eat-me signal CD47 (Jaiswal et al., 2009), traffic through peripheral tissues and lymph (Massberg et al., 2007) and can re-engraft bone marrow niches (Wright et al., 2001). If conditions permit, circulating HSPCs produce lineage-descendant cells outside the bone marrow (Massberg et al., 2007). This process, called extramedullary hematopoiesis, occurs predominantly in the liver in the developing embryo (in this case, before the inception of medullary hematopoiesis) but also in adult tissues including the spleen (Figure 2). Indeed, under specific disease conditions, splenic HSPCs profoundly expand and...
produce progeny locally. Splenic hematopoiesis is enhanced at least by GM-CSF, IL-1β, and IL-3 (Marigo et al., 2010; Leuschner et al., 2012; Robbins et al., 2012; Bayne et al., 2012; Pylayeva-Gupta et al., 2012; Griseri et al., 2012), depends on the C/EBPβ transcription factor (Marigo et al., 2010), and might be inhibited by prostaglandin E2 (PGE2) (Young et al., 1988).

Splenic hematopoiesis has been reported in several animal models of disease including cancer (Bronte et al., 2000; Marigo et al., 2010; Cortez-Retamozo et al., 2012), atherosclerosis (Murphy et al., 2011; Robbins et al., 2012; Dutta et al., 2012), myocardial infarction (Leuschner et al., 2012), and colitis (Griseri et al., 2012). These studies suggest that HSPCs originally described in bone marrow accumulate in high numbers in the splenic red pulp of diseased animals and are more skewed toward myelopoiesis at the expense of erythropoiesis and lymphopoiesis. The myeloid progenitors proliferate similarly to their bone marrow counterparts, form normal colonies in vitro, and produce their progeny in vivo.

Whether cells produced in the spleen differ from those derived from the bone marrow will require further study. Monocyte- and granulocyte-like cells newly produced in the spleen might derive from unique circulating progenitors or receive microenvironmental education signals that are specific to the spleen. In this first scenario, the splenocytes might become distinct from their bone marrow counterparts. A second scenario postulates that the splenic niches reproduces all essential features of bone marrow niches and produce monocytes and neutrophils that are otherwise comparable to their bone marrow counterparts. Remarkably, many recent studies have reported the accumulation of immature myeloid-derived suppressor cells (MDSCs) in tumor-bearing animals, most notably in the spleen and also in the tumor stroma (Gabrilovich et al., 2012). Both MDSCs and granulocyte-macrophage progenitor-derived splenic monocytes and neutrophils are CD11b+Gr-1+ in mice and likely overlap with each other. MDSCs express immunosuppressive enzymes...
such as arginase 1 (ARG1) and inducible nitric oxide synthase (NOS2), produce reactive oxygen species (ROS), inhibit T cell proliferation and IFN-γ production in coculture experiments, and suppress antitumor T cell activity in vivo. The full spectrum of MDSCs’ suppressive functions might not be acquired in the spleen but upon recruitment to the diseased sites (Haverkamp et al., 2011). Interestingly, CD11b+Gr-1+ cells accumulating in the bone marrow of tumor-bearing mice are not immunosuppressive (Ugel et al., 2012). It is thus likely that distinct factors might be delivered to MDSCs at different locations and times during their ontogenic development.

**Cells Mobilized from the Spleen**

The spleen, by mobilizing its motile constituents, can establish connections with other body locations. A prototypical example would be CD4+ and CD8+ T cell redistribution to nonlymphoid tissues following recognition of cognate antigens in the splenic white pulp. In addition to lymphocytes, the spleen contributes mononuclear phagocytes in various disease settings (Figure 2). For instance, ischemic myocardial injury increases the motility of reservoir monocytes and induces a massive exit of this population to the circulation (Swirski et al., 2009). A fraction of the deployed splenic monocytes accumulates in the ischemic myocardium where it contributes to wound healing. Mechanisms of monocyte release are distinct in bone marrow and spleen: emigration of bone marrow monocytes depends on CCR2 signaling, whereas emigration of splenic monocytes is controlled by angiotensin II. This hormone, whose concentration increases in serum after myocardial infarction, directly contributes to macrophage emigration via signaling through the angiotensin type-1 receptor expressed on the surface of reservoir monocytes (Swirski et al., 2009). Other studies suggest that splenic monocytes can be mobilized to the ovaries to enhance the ovulatory process (Oakley et al., 2010); identification of a causal link between splenocytes, ovulation, and fertility, and factors that induce splenocyte mobilization during ovulation, will require further examination.

In addition to bona-fide reservoir monocytes, CX3CR1int Ly6Cint monocytic cells newly produced in the spleen (discussed above) also enter the circulation and relocate to distant tissues. In the context of cancer, these cells can migrate to the tumor stroma and contribute new tumor-associated macrophages (TAMs) throughout cancer progression (Cortez-Retamozo et al., 2012; Cortez-Retamozo et al., 2013). This activity might be deleterious to the host because TAMs can stimulate tumor growth, and their density predicts patient survival in several cancer types (Qian and Pollard, 2010; Steidl et al., 2010; DeNardo et al., 2011). Newly made CX3CR1intLy6Cint splenic monocytes contribute macrophages in other diseases, including atherosclerosis (Dutta et al., 2012; Robbins et al., 2012) and myocardial infarction (Leuschner et al., 2012). These findings suggest that certain diseases elicit macrophage responses by inducing the recruitment of monocytes both from bone marrow and spleen. This notion is divergent from reports from the past 50 years, which indicated that circulating monocytes are bone marrow-derived cells (van Furth and Cohn, 1968; Geissmann et al., 2010). To reconcile these findings, we propose that the bone marrow autonomously maintains monocytes in circulation in steady-state; under inflammatory conditions, the spleen and perhaps other extramedullary tissues are also amenable to produce monocyte-like cells and continuously mobilize these cells in the circulation (Figure 2).

**Spleen and Antigen Tolerance**

Peripheral tolerance is characterized by systemic immune unresponsiveness to repeatedly applied, or large amounts of, antigen (high antigen zone tolerance), or to mucosal contact with the antigen (oral tolerance). Splenectomy can selectively abrogate high antigen zone tolerance (Buettner et al., 2013), indicating that splenocytes are involved in this process. Anterior chamber-associated immune deviation (ACAID) is a form of tolerance that ensues when antigens are placed in the anterior chamber, vitreous cavity, and in the subretinal space of the eyes (Streilein and Niederkorn, 1981). Antigen inoculation in these immune privileged sites elicits a systemic immune response characterized by the induction of antigen-specific antibodies, failure to mount cell-mediated immunity, and allograft acceptance (Streilein, 2003). Spleenic macrophages are likely an important component of this response because both splenectomized and F4/80-deficient mice do not develop tolerance to antigens injected in the eye (Lin et al., 2005). Data suggest that intraocular APCs, mostly F4/80+ macrophages, capture antigens in the anterior chamber, cross the ocular trabecular meshwork, reach the bloodstream, and home to the MZ of the spleen, where they release chemokines, such as CXCL2, and attract NKT cells and other cell types (Faunce and Stein-Streilein, 2002; Masli et al., 2002). Macrophages and NKT cells, as well as local MZ B cells and macrophages, orchestrate a promiscuous environment that is enriched in immunosuppressive soluble factors such as thrombospondin, TGF-β, and IL-10 (Faunce and Stein-Streilein, 2002; Masli et al., 2002; Sonoda et al., 1999). The process eventually involves conversion of eye antigen-specific CD4+ and CD8+ T cells into regulatory (Treg) cells, which are dispensable for early immune unresponsiveness but essential for maintenance of long-term tolerance (Getts et al., 2011).

Macrophages, particularly those in the MZ, do not only trap particulate materials and circulating apoptotic cells but also regulate tolerance to antigens (Figure 3). For example, deletion of CD169+ macrophages causes delayed clearance of injected dying cells in the MZ and abrogates tolerance to an encephalitogenic peptide delivered by apoptotic cells (Miyake et al., 2007). Similarly, MZ macrophage depletion by chlodronate liposomes accelerates systemic tolerance breakdown in mouse models of systemic lupus erythematosus and promotes immunity toward apoptotic cell antigens (McGaha et al., 2011). The molecular events triggering the tolerogenic process in MZ macrophages remain largely unknown but might involve at least LXR and indoleamine 2,3 deoxyxygenase (IDO) signaling. Apoptotic cell engulfment activates LXRx and LXrβ and induces the expression of the receptor tyrosine kinase Mer, which is required for phagocytosis (A-Gonzalez et al., 2009). Full Lxr null macrophages show a selective defect in phagocytosis of apoptotic cells that results in aberrant proinflammatory immune activity (A-Gonzalez et al., 2013). Mice lacking LXRs develop autoantibodies and autoimmune glomerulonephritis, and LXR agonist treatment ameliorates disease progression in mouse models of lupus-like autoimmunity (A-Gonzalez et al., 2009). Considering that LXRs control MZ macrophage homeostasis (A-Gonzalez et al., 2013).

Immunity 39, November 14, 2013 ©2013 Elsevier Inc. 811
et al., 2013), we can speculate that LXR signaling that occurs during apoptotic cell delivery promotes MZ macrophage self-renewal and maintenance. Additionally, the immunoregulatory enzyme IDO1, produced by SignR1+ MARCO+ MZ macrophages within 24 hr from the administration of apoptotic cells, promotes CD4+ T cell-mediated tolerance to apoptotic cell antigens (Ravishankar et al., 2012).

Apoptotic cell clearance by MZ macrophages might also prevent apoptotic material to reach the white pulp and induce proinflammatory immune activity (McGaha et al., 2011). For instance, IDO blockade with the pharmacologic agent D-1-methyl-trytophan reduces TGF-β production but increases TNF-α and IL-12 transcripts in DCs and macrophages, respectively, resulting in loss of CD4+ T cell-mediated tolerance and induction of a lupus-like disease (Ravishankar et al., 2012). MZ macrophages might also regulate the functional activation status of other APCs that come in contact with the apoptotic material. In particular, CD8α+ DCs are required for tolerance initiation following phagocytosis of apoptotic debris through a wide array of receptors, including PSR, βιA integrin, CD36, scavenger receptor BI, CD14, and CD68 (Iyoda et al., 2002). The population of CD8α+ DCs responsible for apoptotic cell tolerance is constituted by a limited subset of langerin+ DCs that are physically close to MZ macrophages; these DCs cross-present antigen to CD8+ T cells (Qiu et al., 2009) and can induce suppressive FoxP3+ Treg cells from the naive T cell population (Yamazaki et al., 2008). Splenic pDCs are conditioned by soluble factors (like TGF-β) released from macrophages processing apoptotic material and contribute to antigen tolerance, e.g., in the context of allogeneic bone marrow transplantation (Bonnefoy et al., 2011).

Besides sensing apoptotic death, the spleen might regulate peripheral tolerance by expressing the autoimmune regulator gene (AIRE). This transcription factor regulates negative selection of autoreactive T cells in the medulla of the thymus by inducing ectopic expression of tissue-specific antigens (Mathis and Benoist, 2009). Splenocytes expressing AIRE and tissue-specific antigens have been suggested to promote self-tolerance (Gardner et al., 2008). Reported AIRE+ splenic cells include a CD11c+ DC subset (Lindmark et al., 2013) and bone marrow-derived epithelial cell adhesion molecule (EpCAM)+ CD45+ APCs (Gardner et al., 2013). The latter cells might mediate tolerance by inactivating autoreactive T cells and without involving Treg cells. This mechanism of tolerance induction might be important for controlling autoreactive T cells that escape thymic selection.

Antigen tolerance might also be triggered in the spleen by tumor-derived signals. In line with this notion, splenectomy before or early after tumor implantation can retard tumor growth in colon carcinoma, mammary carcinoma, and melanoma-bearing mice (Fotiadi et al., 1999; Schwarz and Hiserodt, 1990). The paradoxical, beneficial effect of splenectomy may be viewed in light of the recent findings indicating that the spleen produces myeloid cells that are recruited to the tumor stroma (Cortez-Retamozo et al., 2012; Cortez-Retamozo et al., 2013), but also because the spleen locally orchestrates tolerance induction to tumor-antigens (Figure 3). As discussed above, the spleen of tumor-bearing mice often contains increased numbers of Ly6C+ monocytic cells (Ugel et al., 2012). These cells share markers with granulocyte-macrophage progenitors (GMPs), are enriched in the MZ, and might produce not only mononuclear phagocytes but also granulocytes through a tumor-induced pathway involving transcriptional silencing of the retinoblastoma gene and epigenetic modification operated by histone deacetylation 2 (Ugel et al., 2012; Youn et al., 2013). These cells capture tumor-derived material and cross-present tumor-associated antigens to memory CD8+ T cells transiting the spleen MZ; this process causes T cell unresponsiveness to tumor antigens (Ugel et al., 2012). Splenic accumulation of Ly6C+ monocytes depends on the CCR2-CCL2 axis because MZ anatomical alteration, splenomegaly, and tumor-antigen tolerance are absent in either Ccr2 or Ccl2 genetically ablated mice; moreover, CCL2 serum concentrations in cancer patients correlate with accumulation of myeloid progenitors and predict overall survival of patients who respond to cancer vaccines (Ugel et al., 2012). Tumor-derived apoptotic bodies and microvesicles might contribute to this process and involve several cells in the MZ, including MZ macrophages considering their derivation from Ly6C+ monocytes (A-Gonzalez et al., 2013).

The mouse spleen also accumulates immunosuppressive myeloid cells after trauma and sepsis (Makarenkova et al., 2006; Delano et al., 2007); in particular, ARG1+ myeloid cells can be detected as early as 6 hr after traumatic stress in T cell zones around germinal centers (Makarenkova et al., 2006). Thus, the spleen apparently maintains peripheral tolerance induced by tissue remodeling in various contexts including ACAID, tissue injury, and cancer. In particular, the MZ likely continuously samples blood for the presence of signals delivered as particulate fractions in the blood, including apoptotic bodies and microvesicles. Resident elements including specialized macrophages and DCs assure a steady-state control of autoimmune reactivity, further supported by local myelopoiesis during cancer or intense traumatic or inflammatory stress (Figure 3).

The Spleen in Mice and Humans

Mouse and human spleens are mostly similar anatomically, although the human MZ is more organized, with clearly identifiable outer and inner layers surrounded by a large peri-follicular zone (Mebius and Kraal, 2005). Although a vast array of diseases such as infections, vascular alterations, autoimmune disorders, hematologic malignancies, and metabolic syndromes can cause splenomegaly in mice, this main sign of hypersplenism is not commonly associated with the development of human solid tumors. Interestingly, spleen enlargement is commonly seen in tumor cell transplantable models (Gabrilovich et al., 2012) but to a lesser extent or not at all in genetically engineered models of cancers in which, nonetheless, splenic HSPC and myeloid activity significantly increase (Cortez-Retamozo et al., 2012; Cortez-Retamozo et al., 2013). So splenomegaly and extramedullary hematopoiesis are not necessarily connected.

On the other hand, several examples of increased presence and activity of splenic HSPCs in human pathology suggest that the embryonic role of the spleen in blood formation can be conserved in adult life. Splenic hematopoiesis is observed in osteopetrosis (Freedman and Saunders, 1981) and in metastatic carcinomas of different origins, including lung, breast, prostate, and kidney cancers (O’Keane et al., 1989). These findings were recently confirmed and extended. Splenic GMP-like cells are found in elevated numbers in patients with invasive lung
carcinoma and can generate monocytes and granulocytes both in vitro and following in vivo engraftment in immunodeficient mice (Cortez-Retamozo et al., 2012). When scale differences between the species are taken into account, the precursors’ quantity in humans and mice bearing cancer is similar. Moreover, HSPCs’ presence in patients shortly after heart attack supports the notion that the spleen acts as a reservoir and source of monocyte progenitors (Dutta et al., 2012; Leuschner et al.,

Figure 3. The Spleen Is a Site of Immune Tolerance Induction
The spleen’s tolerogenic role depends on MZ cellular interactions between different macrophage subsets, CD8+ DCs, pDCs, inflammatory Ly6C+ monocytes, NKT cells, TNF-related apoptosis inducing ligand (TRAIL)+ CD8+ T cells and CD4+Foxp3+ Tregs. Orange boxes show molecules involved with tolerance regulation. These include cytokines and soluble molecules (thrombospondin, TGFβ, IL-10), membrane-bound molecules (TRAIL and programmed death ligand 1 [PD-L1]), enzymes (IDO, ARG, NOS, and 12/15 lipoxigenase [12/15 LO]) and reactive oxygen species (ROS). These circuits operate: (1) in steady-state by sensing apoptotic remnants released by privileged tissues such as the eye (ACAID) or during normal cell turn-over in conventional tissues, and (2) under inflammatory conditions such as trauma, acute, and chronic inflammation including cancer, which result in increased loads of apoptotic materials and microvesicles, as well as stimulation of splenic myelopoiesis (see Figure 2).
that of normal HSPCs (Wang et al., 2012). Additionally, transformed human myeloid cells can select the spleen for their survival. The spleen of myelofibrosis patients contains more malignant CD34+ HSPCs than bone marrow and blood; these cells support a prolonged myeloid and lymphoid reconstitution and retain a differentiation program similar to that of normal HSPCs (Wang et al., 2012). Activated myeloid cells are also found in higher proportions in the spleen’s MZ of patients after trauma or severe sepsis (Cuena et al., 2011).

Splenectomy predisposes patients to death from ischemic heart disease and increases the risk to develop sepsis and meningitis in response to Streptococcus pneumoniae, Neisseria meningitidis, and Hemophilus influenza type B infections (Amlot and Hayes, 1985; Ram et al., 2010; Robinette and Fraumeni, 1977). Humans with acute Plasmodium falciparum malaria who had previously undergone splenectomy show decreased clearance of parasitized red blood cells from the circulation (Chotvaniich et al., 2002). Splenectomy might also protect against blunt trauma complications by dampening systemic inflammation (Crandall et al., 2009). However, the question of whether asplenia predisposes to either decreased or increased risk of cancer growth or recurrence has not received clear answers from clinical studies, with most of the data indicating a modest if any effect on overall risk after splenectomy (Cadili and de Gara, 2008; Robinette and Fraumeni, 1977). Only few studies indicate an increased incidence of cancer in patients splenectomized for nontraumatic causes, but treatments for underlying disease and lifestyle habits, such as cigarette smoking, could not be ruled out in explaining these increased risks (Mellemkjør et al., 1995). On the other hand, studies on concomitant rather than preceding splenectomy in patients with gastric, colon, and pancreatic cancers have unveiled a decreased disease-free and overall survival but suffer from the confounding biases of more locally advanced tumors, increased operative time, and blood loss that are usually associated with the clinical indication of splenectomy (Cadili and de Gara, 2008). In summary, analysis of clinical outcomes of splenectomy in humans confirms its relevance in controlling immune responses to bacteria and parasites, but the impact on cancer progression has not yet been clarified.

**Therapeutic Targeting**

Targeting the spleen for therapeutic purposes offers several advantages. First, the large blood flow through the organ should facilitate delivery of systemically administered drugs and cells. Second, splenic reservoir precursors could be intercepted before they are mobilized and enter pharmacodynamical unfavorable compartments, such as the tumor stroma; these precursors could be used as “Trojan horses” to deliver drugs or to shape immune repertoires in the tumor stroma. Third, the spleen’s anatomical structure is naturally fit to regulate responses to blood-borne antigens. The main goals pursued in targeting the spleen are inducing tolerance to “self” peripheral antigens, restraining monocyte-dependent inflammatory responses, or controlling tumor-induced myelopoiesis and immune suppression. Below we discuss how targeting the spleen could serve to manipulate tolerance induction, tumor-induced myelopoiesis, and immune suppression in therapy.

From the first demonstration, dating >30 years ago, that allogeneic splenocytes treated with common crosslinking chemicals induce tolerance to protein antigens administered intravenously (Miller and Hanson, 1979), several studies in experimental autoimmune diseases driven by T helper 1 (Th1) and/or Th17 T lymphocytes (such as rheumatoid arthritis, type 1 diabetes, and multiple sclerosis) have proven that a single intravenous injection of either syngeneic splenocytes or erythrocytes chemically coupled with “self” peptides or proteins can induce potent antigen-specific tolerance in vivo. This effect relies on apoptotic cell and erythrocyte clearance from recipient splenic phagocytes, IL-10 production, and expansion of antigen-induced Treg cells (reviewed in Ravishankar and McGaha, 2013). Even though the spleen’s contribution remains to be formally assessed, treatment’s efficacy seems promising for patients with multiple sclerosis: in a first-in-human study, high doses of autologous peripheral blood mononuclear cells chemically coupled with seven myelin antigens showed decreased T cell reactivity against these antigens (Lutterotti et al., 2013). Widespread clinical use of apoptotic cells might be difficult because it requires large cell numbers and standardized protocols, and because of the complexity and unknown nature of the antigenic mixture. For these reasons, recent attempts have focused on manufacturing artificial mimics of apoptotic cells, endowed with equivalent ability to trigger in vivo tolerance. Clearance of apoptotic debris can be substituted by artificially engineering microparticles of either polystyrene or biodegradable poly(lactide-co-glycolide) beads with encephalitogenic peptides. This approach induces T cell tolerance in mice with relapsing experimental autoimmune encephalomyelitis. Microparticles are uptaken by MARCO+SIGN-R1+ MZ macrophages, which trigger a tolerance circuit involving Treg cell activation (Getts et al., 2011).

It is often viewed as a potential limitation for drug delivery that many nanoparticle formulations are retained in the spleen. However, this limitation might represent an opportunity to therapeutically target splenocytes. For example, nanoparticles encapsulating short interfering RNA (siRNA) against CCR2 reach inflammatory Ly6Cmonocyte progenitors in the spleen (and to a lesser extent the bone marrow) upon intravenous injection and efficiently silence expression of the chemokine receptor. The treatment decreases the number of inflammatory monocytes in atherosclerotic plaques, reduces the infarct size after coronary artery occlusion, prolongs survival of allogeneic pancreatic islet transplantation in diabetic mice, and controls tumor growth by lowering the numbers of tumor-associated macrophages (Leuschner et al., 2011).

Another strategy consists in creating a barrier between the precursors within the splenic niche and their target site. The peptide hormone Angiotensin II (AngII) is crucial to maintain self-renewing HSCs and macrophage progenitors in the spleen of tumor-bearing hosts. By binding its receptor, AGTR1A, AngII interferes with the signaling through S1PR necessary for cells to sense the S1P hematic gradient and migrate to blood from tissues. Interfering with the AngII pathway, by either administering AGTR1A antagonist losartan or blocking its production through the angiotensin converting enzyme inhibitor enalapril, allowed to interrupt the continuous flux of tumor myeloid cells at its source, preventing accumulation of Ly-6Cmonocyte-derived, tumor-promoting macrophages, reducing the number of detectable lung tumor nodules, and increasing survival of...
mice with a conditional genetic lung adenocarcinoma (Cortez-Retamozo et al., 2013). Myocardial infarction and atherosclerosis in humans are also associated with increased Ang II activity (McAlpine and Cobbe, 1988; Schieffer et al., 2000); thus blocking Ang II signaling could restrain the amplification of deleterious macrophages in these diseases as well.

Conventional chemotherapy can also be optimized to alter the tolerogenic splenic niche. Several chemotherapeutic agents (gemcitabine, fluorarabine, suten, sorafenib, bortezomib, cyclophosphamide, and 5-flourouracil), when administered at low dose in tumor-bearing hosts, assure a prolonged depletion of splenic Ly6Chi monocytes and restore impaired cytotoxic T lymphocyte (CTL) functions. In fact, chemotherapy allows the occupation of the splenic niche by CD8+ T cells that actively restrain replenishment by Ly6Cmonocytes (Ugel et al., 2012). These findings provide a rationale to explain the often empirical and paradoxical observations that chemotherapeutics can be useful adjuvants for adoptive cell therapy of cancer with antigen-specific CD8+ T cells. Indeed, a single inoculation of 5-flourouracil before adoptive transfer of toleromerase-specific CD8+ T lymphocytes was found to prolong overall survival in both immune competent and deficient tumor-bearing mice; moreover, reconstitution of 5-fluorouracil-treated mice with Ly6Chi but not with Ly6Cmonocytes fully abrogates the immune adjuvant activity of chemotherapy (Ugel et al., 2012). The drug trabectedin, a DNA binder of marine origin approved for treating pancreatic cancer, also shows selective activity on mononuclear phagocytes as a main component of its antitumor activity. Trabectedin activates caspase-8-dependent apoptosis, and its selectivity for monocytes is due to differential expression of signaling and decoy TRAIL receptors (Germano et al., 2013). It is not clear whether other chemotherapeutic drugs share a similar mechanism of action, but these results open the possibility to use chemoinmunomodulation to halt the pathological consequences of altered myelopoiesis in cancer. Chemotherapeutics that affect immunosuppressive circuits and induce immunogenic cell death (Kroemer et al., 2013) could act synergistically in combination and might further increase antitumor immune responses.

**Concluding Remarks**

Emerging studies are forcing investigators to reconsider the temporal and spatial distribution of hematopoiesis and immune responses. Tissue- and lymphoid-related circuits promote innate and adaptive immune cell positioning and reciprocal interplay with unexpected specificity and complexity. It appears that distinct sites have evolved to serve specific purposes but our perception is still limited. For example, the advantage for the host to establish distinct sites of monocyte production is not fully understood. Identifying unique splenic signals that instruct myeloid cells and their precursors locally, and/or functional differences between bone marrow and spleen-derived circulating myelomonocytic cells, might help to further define whether inflammatory conditions support locational production of circulating cells with unique functions. Analogously, we need to understand which environmental factors control positioning and differentiation of DC populations and other immune cell types in the spleen. Moreover, targeting receptors and/or their corresponding ligands could modulate immune cell positioning and, by extension, the unfolding of undesired adaptive immune responses, as initial studies seem to suggest. Finally, it is clear that more studies are needed on human spleen before dismissing its importance on the basis of the few epidemiology analyses evaluating the long-term consequences of splenectomy in patients, often based on the consultation of national registries and biases related to the criteria used for the statistics.

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