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The Emerging Role of B Lymphocytes in Cardiovascular Disease

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Abstract

B cells are traditionally known for their ability to produce antibodies in the context of adaptive immune responses. However, over the last decade B cells have been increasingly recognized as modulators of both adaptive and innate immune responses, as well as players in an important role in the pathogenesis of a variety of human diseases. Here, after briefly summarizing our current understanding of B cell biology, we present a systematic review of the literature from both animal models and human studies that highlight the important role that B lymphocytes play in cardiac and vascular disease. While many aspects of B cell biology in the vasculature and, to an even greater extent, in the heart remain unclear, B cells are emerging as key regulators of cardiovascular adaptation to injury.

1. INTRODUCTION

It is increasingly recognized that chronic inflammation underlies the vast majority of acquired cardiovascular diseases in both industrialized and developing nations. Although the importance of B lymphocytes has long been recognized in the context of adaptive immune responses, there is a growing appreciation that B lymphocytes play an important role in health and disease that extends well beyond their ability to secrete antibodies. As will be discussed, recent studies have suggested a previously unrecognized role for B cells in the development of a broad variety of cardiovascular diseases. Here, we review the literature that has led to our current understanding of B cell regulation in the heart and vasculature. This review touches briefly on B cell ontogeny and the importance of different B cell subsets in order to provide a framework for understanding the role of B cells in cardiac and vascular disease. Given that the preponderance of data in the cardiovascular field is experimental, we first discuss the relevant experimental studies that describe the role of B cells in cardiac and vascular studies, and then discuss the relevant literature in human studies.

2. OVERVIEW OF B LYMPHOCYTE BIOLOGY

B lymphocytes have traditionally been defined as a "population of cells that express clonally diverse cell surface immunoglobulin (Ig) receptors recognizing specific antigenic epitopes" (1, p. 1570). Whereas invertebrates appear to lack bona fide humoral responses, lymphocytes and humoral immune responses have been observed in lower vertebrate (e.g., agnathans) species. However, B cell receptors (BCRs) are only present in higher vertebrates (gnathostomes). The subsequent evolutionary emergence of different B cell subsets was likely driven by the need for warm-blooded vertebrates to develop a more finely tuned immunological response to microbes, which were able to multiply and mutate much faster in warmer hosts (2). Although B cells are known classically for their ability to produce antibodies in the context of adaptive immune responses, recent studies have shown that B cells are also key modulators of the innate immune response and thus share attributes with primordial phagocytes, with which they likely share a common ancestry.

2.1. B Cells: One Name, Many Cell Types

B lymphocytes can be divided into two lineages, B1 and conventional B2 cells (3). In general, B1 cells (which include B1a and B1b subsets) (4) are produced mostly during prenatal life and are considered major contributors to production of natural antibodies, defined as "preimmune antibodies generated in the absence of exogenous antigenic stimulation, which are broadly reactive, low affinity, germline-like antibodies" (5, p. 4; 6–8). B2 cells are produced postnatally and, in the adult, represent the vast majority of B cells. B2 cells include a predominant population of follicular B cells and a smaller population of marginal zone (MGZ) B cells (9).

In the mouse, B1 cells are $CD19^+$, $CD11b^+$, IgM^+. B1a cells are also $CD5^+$, while B1b cells are $CD5^-$. B1 cells accumulate preferentially in the coelomic cavities such as the peritoneum but are found throughout the body. B2 cells are $CD19^+$ $CD11b^-$. In the mouse, two main subgroups of B2 cells can be differentiated based on expression of CD21 and CD23. MGZ B cells are $CD21^+$ $CD23^{low}$. Follicular B cells are $CD23^{high}$, $CD21^{low}$. MGZ B cells reside in lymphoid organs in between B cell follicles. Follicular cells are mostly found in the follicular region of lymphoid organs. Naive follicular B cells recirculate between lymphoid organs (further discussed below) (10, 11).

After encountering specific antigens recognized by the BCR, B1 cells, MGZ B cells, and follicular cells can differentiate into highly active antibody-producing cells referred to as short-lived plasma cells or plasmablasts. In the mouse, plasma cells downregulate CD20, the BCR, and MHC class II molecules and upregulate CD138 and CXCR4. Antigen-activated follicular B cells typically

proliferate rapidly within primary or secondary lymphoid organs to generate germinal centers, spherical structures in which the plasmablasts interact with helper T cells and usually undergo somatic hypermutation, class switching from IgM to IgG, and eventually produce long-lived plasma cells and memory B cells. B1 cells and MGZ B cells have also been shown to be able to produce long-term memory B cells through a process that is germinal center independent and less clearly mapped out. The memory cells derived from B1 and MGZ cells typically express IgM, do not undergo class switching, and are therefore referred to as unswitched memory B cells (12).

Additional B cell subtypes can be defined based on cytokine secretion pattern and function and are discussed in Section 2.3, B Cell Functions and Dynamics.

2.2. B Cell Ontogeny

B cell development begins with the differentiation of noncommitted hematopoietic stem cells (HSCs) to precursors with restricted lineage potential referred to as common lymphoid progenitors. These progenitors eventually mature into B cells through sequential stages. Immunoglobulin gene rearrangement is a defining feature of B cells and occurs throughout the various stages of B cell development via "an error-prone process involving the combinatorial rearrangement of the V, D, and J gene segments in the H chain locus and the V and J gene segments in the L chain loci" (1, p. 1571). The product of immunoglobulin gene rearrangement is the development of a multitude of unique B cells, each expressing a BCR with a unique specificity. During B cell maturation, autoreactive B cells are eliminated through a number of different mechanisms including deletion of autoreactive B cells, receptor editing, and anergy induction (13, 14).

During development, B cells are generated in waves that parallel the waves of development of HSCs. The first wave of primitive HSCs develops very early in development in extraembryonic tissues and gives rise to a first wave of B cell development that produces only B1a cells. Around the time at which the heart starts beating, a second wave of definitive HSCs is generated inside the embryo, within the aorto-gonads-mesonephros region (15). Definitive HSCs first colonize the fetal liver. Within the fetal liver definitive HSCs give rise to a second wave of B cell development of differentiation into B1a, B1b, and B2 cells. HSCs home to the bone marrow as hematopoiesis transitions from the hepatic phase to definitive bone marrow hematopoiesis. Within the bone marrow, HSCs produce a third and final wave of B cell development that gives rise to B1b cells and B2 cells. Since only the first and second waves of hematopoiesis produce B1a cells, B1a cells are not generated de novo after birth but are maintained throughout life through self-replication. In contrast, B1b cells and B2 cells are generated throughout life (9, 16).

In the bone marrow, in order to differentiate into B cells, the common lymphoid progenitor matures through at least three stages. Pre-pro-B cells are the earliest stage and precede the rearrangement of the immunoglobulin genes. As they rearrange their immunoglobulin genes, pre-pro-B cells mature into pro-B cells, pre-B cells, and eventually small resting naive B cells that are classically described as expressing IgM but not IgD. In mice, B cells express B220 from the pre-pro-B cell stage onward and CD19 from the pro-B cell stage onward (17). B1 and B2 cells both originate from the common lymphoid progenitor. However, B1 cell differentiation diverges early on, likely before the pro-B cell stage. As they mature, B1 cells rearrange immunoglobulin genes less than B2 cells and ultimately have a BCR repertoire that is comparatively restricted in terms of heavy chain V segment use and modification (9).

Once immature B cells exit the bone marrow they are referred to as transitional B cells (T1, T2, and T3). B1 and B2 cells appear to have different transitional cell stages (9). In the B2 lineage, T1 cells are IgM^{high} IgD^{-} $CD21^{-}$ $CD23^{-}$. T2 B cells retain high levels of surface IgM but are also IgD^{+} $CD21^{+}$ and $CD23^{+}$. Allman et al. (18) described a third nonproliferating population,

termed T3, that resembles T2 with the exception of a lower level of surface IgM. Transitional B2 cells home to the spleen and other lymphoid organs to complete maturation. T1 cells are not found in lymphoid follicles, whereas T2 and T3 cells are found in follicles along with mature B cells. The maturation of transitional B cells in extramedullary sites is still the subject of much investigation (14); however, it is clear that only a portion of cells progress from each step to the next until becoming mature B cells. As they mature, B cells acquire a greater ability to produce antigen-specific immune responses (19).

2.3. B Cell Functions and Dynamics

While the prototypical and arguably most studied function of B cells is antibody production, B cells can perform other critical functions in the context of the immune response. Namely, they can present antigens to T cells and they produce cytokines and chemokines that ultimately modulate the function of other leukocytes (**Figure 1**).

2.3.1. Antibody production. Antibodies are secreted by relatively rare populations of B cells. B1 cells can produce natural antibodies in the absence of specific stimulation through a poorly characterized mechanism (20). In addition, MGZ B cells, B1 cells, and follicular cells can differentiate into plasmablasts when their BCR is engaged by antigen. This is typically an extrafollicular

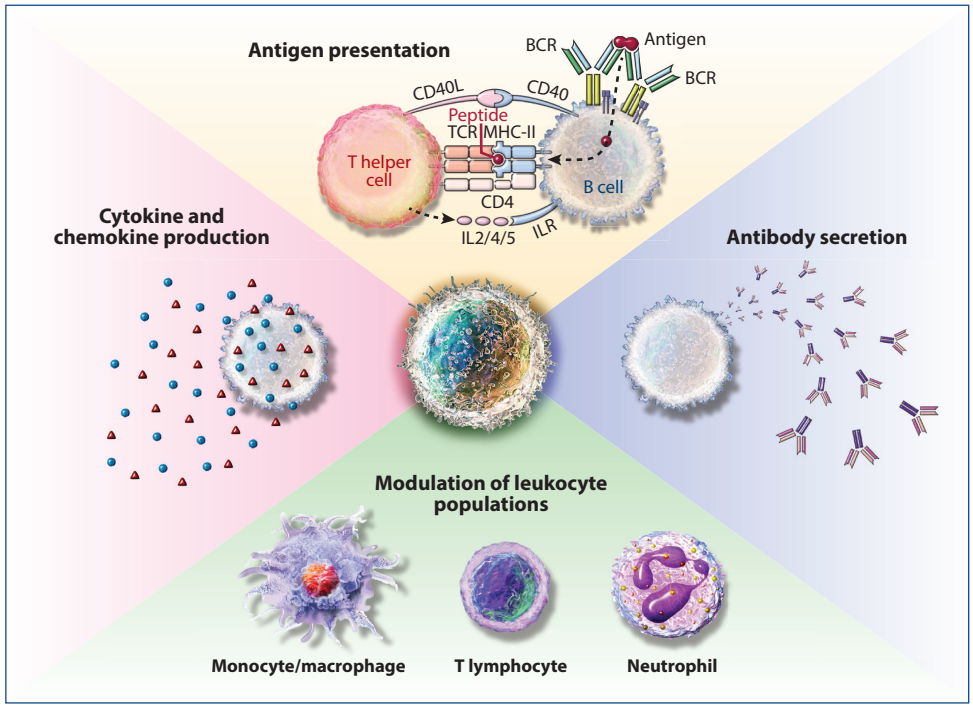

Figure 1

Biology of B lymphocytes. B cells play an important role in both innate and adaptive immune responses by presenting antigens to T cells, producing antibodies, and producing cytokines and chemokines, as well as by modulating the function of macrophages, T lymphocytes, and dendritic cells. Abbreviations: BCR, B cell receptor; ILR, interleukin receptor; TCR, T cell receptor.

event. Follicular cell–derived plasmablasts can enter lymphoid follicles and, under the influence of specialized T helper cells, undergo vigorous proliferation and BCR hypermutation, which results in maturation of the affinity of the BCR for its target antigen, which is associated with class switching of the immunoglobulin genes. The germinal center ultimately produces long-lasting antibody-producing plasma cells that produce high levels of antibodies and long-lived memory B cells (1, 21, 22). Importantly, while most antigen-specific responses take place within lymphoid organs, in the context of nonresolving inflammation, lymphocytes can organize in tertiary lymphoid organs within tissues. Tertiary lymphoid organs are accumulations of B cells and T cells that mimic the structure of lymphoid organs. It is clear that tertiary lymphoid organs can support germinal center reactions, but many aspects of their biology and function remain poorly elucidated (23). Classically, only follicular cells have been considered able to produce long-lived plasma cells through the germinal cell reaction (in a response commonly referred to as response to T cell–dependent antigens). However, recent evidence suggests that also MGZ cells and possibly B1 cells might produce long-lived unswitched plasma cells through a T cell–independent process (long-lived plasma cells produced in response to T-independent antigens and expressing IgM) (12, 24).

2.3.2. Antigen presentation to T cells. B cells express high levels of MHC-II and are efficient antigen-presenting cells. B cells engage antigens through the BCR and then internalize antigens in order to efficiently concentrate and respond to antigens that are present at low concentrations. It is well established that B cells efficiently present antigens to $CD4^+$ T cells in the context of the formation of germinal centers. In this context, B cells receive help from T cells commonly referred to as follicular helper T (Tfh) cells (25). However, there is also evidence that B cells present antigen to $CD4^+$ T cells and regulate their function outside of germinal centers. Yet, the evidence in this area is conflicting. B cells seem to be able to both activate naive T cells and suppress them through MHC-II-restricted interactions, likely depending on the antigen presented (26). Recent studies suggest that B cells might be more efficient than dendritic cells in activating T cells in response to low-concentration, particulate antigens (27).

2.3.3. Production of cytokines/chemokines and effect on other leukocytes. B cells secrete cytokines in response to several signals including activation of the Toll-like receptors (TLRs) expressed on their surface, BCR engagement, etc. During lymphoid development, B cells secrete $LT\alpha1\beta2$ and thus modulate maturation and growth of lymphoid structures both during homeostasis and in the context of an immune response. B cell–mediated $LT\alpha1\beta2$ secretion can also promote the formation of tertiary lymphoid structures, which are ectopic lymphoid organs that develop in nonlymphoid tissues at sites of chronic inflammation (28). B cell–mediated cytokine secretion plays a key role in the polarization of $CD4^+$ T cells toward various phenotypes, through both MHC-II-dependent and MHC-II-independent mechanisms. B cells can also activate innate immune cells through secretion of cytokines such as IFN-γ, IL-6, and IL-17 (28), and they can modulate the mobilization of monocytes through secretion of CCL-7 (29). A subset of B cells have been reported to express GM-CSF, IL-3, and natural IgMs in response to bacterial infections and have been named innate response activator (IRA) B cells (30).

B cells can also act as negative regulators of the immune response, mostly through the production of IL-10, IL-35, and TFG-β. B cells with anti-inflammatory properties develop or expand in response to immune activation (28). These B cells, often referred to as Bregs or B10 cells, are immunosuppressive cells that support immunologic tolerance and resolution of the acute inflammatory response. Bregs suppress inflammation by promoting T regulatory cells (Tregs). However, it has also been proposed that they maintain the homeostasis of certain types of natural killer cells and suppress proinflammatory activity of other cell types including monocytes, dendritic cells,

and $CD8^+$ T cells (31). The exact phenotype of Bregs is still a matter of debate. In mice, multiple types of IL-10-producing B cells have been identified including MGZ cells, plasmablasts, plasma cells, and $CD5^+$ B1-like B cells; some of these cell types have also been identified in humans (31).

B cells have also been shown to regulate T cell recruitment to inflamed tissue through secretion of a specific peptide named PEPITEM (32). The complexity of the interaction between B cells and other immune cells reminds us that it is impossible to deplete B cells without affecting significantly the function of other immune cells and highlights the challenges of interpreting the results of B cell–depleting experiments.

2.3.4. B cell trafficking. The current model of B cell trafficking posits that, after maturing in the bone marrow, B cells home to primary and secondary lymphoid organs (spleen, lymph nodes, and mucosa-associated lymphoid tissue). A significant portion of B cells are thought to recirculate between lymphoid organs to constitute a pool of recirculating B cells (33). According to this model, B cells exit the circulation after binding to specialized endothelial cells within the postcapillary venules of the lymph nodes. They reenter the circulation through lymphatic ducts that drain into the thoracic duct. After encountering an antigen, activated B cells migrate to inflamed tissues or back to the lymph nodes and spleen, where they differentiate into plasmablasts, plasma cells, or memory cells. Plasma cells eventually migrate to the bone marrow, albeit a low number of B cells can also be found in other lymphoid and nonlymphoid organs (34, 35). Recruitment to the inflamed tissue is likely the result of specific modifications in the endothelium (36). It bears emphasis that the majority of the studies on tissue lymphocytes have focused on T cells, and therefore the homing, dynamics, phenotype, and function of tissue B cells remain for the most part unclear. In addition, the model of B cell trafficking described above does not explain the observation that B cells are present in relatively high numbers in the naive heart and in the naive aorta (see below).

3. B LYMPHOCYTES AND CARDIAC DISEASE

The role that B lymphocytes play in the heart in health and disease has been investigated in a relatively small number of experimental studies and clinical studies. Insofar as the preponderance of data have been derived from murine models, we first review the experimental evidence that suggests that B cells play a critical role in the context of the myocardial adaptation to injury.

3.1. B Cells in the Naive Myocardium

B cells have been reported to be one of the most prevalent subpopulations of $CD45^+$ myocardial leukocytes within the naive murine heart (10, 37). Ramos et al. (38) identified two populations of murine $B220^+$ (CD45R) lymphocytes: a larger population of IgM^{high} IgD^{low} cells and a smaller population of IgM^{low} IgD^{high} cells. The authors showed that most myocardial $B220^+$ cells are also $CD19^+$. Adamo et al. (10) observed these same two populations but further characterized myocardial B cells using a larger panel of common B cell cluster differentiation markers. They reported that a portion of myocardial B cells (~10%) are $CD11b^+$. Of these, the majority are IgM^+ $CD5^-$, which is consistent with B1b cells, while a very small portion are IgM^+ $CD5^+$, which is consistent with B1a cells. Within the larger $CD11b^-$ population of myocardial B cells, the authors identified a population of $CD19^+$, $CD11b^-$, $CD21^-$, $CD23^-$ cells. The identity of this population remains unclear. Horckmans et al. (39) reported that the pericardial adipose tissue harbors a subpopulation of GM-CSF-producing B cells that is compatible with that of IRA B cells (40). However, at the time of this writing, it is unclear what percentage of the B cells in the naive

heart are intravascular, or associated with the microvasculature, and what percentage are within the parenchyma.

3.2. B Cell Animal Models of Myocardial Injury

Different murine models have been used to study changes in the myocardial B cell compartment following tissue injury. Thus far, three groups have studied changes in myocardial B lymphocytes following acute coronary ligation [myocardial infarction (MI)]. Yan et al. (41) reported a careful time course of myocardial B cell numbers after ischemic injury. Using flow cytometry, they found that myocardial $CD19^+$ cells increase in number after MI, peaking between day 5 and day 7 after injury and returning to baseline soon after. Using the permanent coronary ligation model, Zouggari et al. (29) showed that $B220^+$ B lymphocytes influx into the myocardium and localize around the infarct zone by day 3. Horckmans et al. (39) also studied a murine model of permanent coronary ligation in young mice, before the age at which epicardial fat is typically considered to develop. They found that after MI the number of myocardial B cells increases in the pericardial fat, especially within the GM-CSF^+ compartment and not in the myocardium (39). The reason for the discrepancy between these two studies is unclear, but it may be model dependent. Whereas Horckmans et al. studied a murine model of permanent coronary ligation, Yan et al. (41) and Adamo et al. (10) studied a model of ischemia reperfusion (I/R) injury. Yan et al. found that the time course of B cell changes in the myocardium after I/R injury is different than that observed after permanent coronary ligation, with B cells peaking numerically approximately 2 days earlier and returning to baseline faster (41). Adamo et al. studied a model of closed-chest I/R injury and found that on day 4 after I/R there is a significant increase in myocardial $CD19^+$ $CD11b^-$ cells. The authors observed a trend toward increases in myocardial B1a and B1b cells that did not reach statistical significance (10). It should be noted that Adamo et al. also studied a genetic model of cardiac injury in which administration of diphtheria toxin (DT) triggers both necrotic and apoptotic myocyte death. The authors demonstrated that DT-induced cell death results in striking changes in the patterns of gene expression in cardiac $CD11b^+$ and $CD11b^-$ cells. Interestingly, acute injury induces activation of the BCR signaling pathway and of antigen presentation in $CD11b^-$ cells, whereas myocardial injury is associated with activation of the TLR signaling pathway and of chemokine-chemokine signaling pathways in the $CD11b^+$ cell compartment. These findings suggest that specific subpopulations of myocardial B cells might have different functions within the injured heart (10). Transverse aortic constriction has been reported to induce an increase in myocardial $CD19^+$ cells at 4 weeks after surgery (42).

3.2.1. B cells as modulators of the myocardial response to injury. Several groups, using different models of myocardial injury, have reported that B cell depletion improves cardiac function. Zouggari et al. (29) were the first to suggest a link between B lymphocytes and adverse left ventricular (LV) remodeling. In a seminal paper, the authors showed that B cell–depleted mice (depleted both genetically and via treatment with monoclonal antibodies) have reduced cardiac adverse LV remodeling following permanent coronary artery ligation of the left anterior descending artery (LAD). The authors suggested that B cells contribute to adverse LV remodeling by recruiting $Ly6C^+$ monocytes from the bone marrow through a CCL7-dependent mechanism (29). The role of B cells in the permanent coronary ligation model was also studied by two other groups. Horckmans et al. (39) reported that B cells orchestrate the amplification of several immune cell types in the pericardial adipose tissue and in the heart after MI. They complemented these studies by showing that $CB2^{-/-}$ mice (a genetically modified strain with increased number of B cells) have worse adverse LV remodeling after MI. Using a rat model of myocardial infarction following LAD

ligation, Goodchild et al. (43) showed that intramyocardial injection of bone marrow–derived $B220^+$, $CD5^-$ cells into early postischemic myocardium preserves cardiac function by preventing cardiac myocyte apoptosis. Intriguingly, intramyocardial injection of other bone marrow–derived cells was either ineffective or blunted the cardioprotective effects conferred by an enriched population of B cells. The findings with intramyocardial injection are in sharp contrast to the aforementioned studies wherein B cell depletion was shown to be cardioprotective. The reason for the discrepancy between these studies is unclear, but it may relate to the specific properties of B lymphocytes isolated from the bone marrow–derived cells, which are likely immature B cells (43). Based on these encouraging preclinical data, small-scale clinical trials investigating the effect of B cell depletion in the context of heart failure with a reduced ejection fraction are being planned (ClinicalTrials.gov Identifier: NCT03332888) (44), and clinical trials in patients with ST-segment myocardial infarction are ongoing (ClinicalTrials.gov Identifier: NCT03072199).

Using an experimental model where angiotensin II is infused to create a pressure overload on the heart, Cordero-Reyes et al. (45) showed that antibody-dependent (anti-CD22) depletion of B cells leads to decreased myocyte hypertrophy and myocardial fibrosis, as well as reduced levels of several inflammatory cytokines, including IL-1β and tumor necrosis factor alpha (TNF-α). The authors further showed that angiotensin II–treated mice with severe combined immune deficiency (SCID) are protected from myocyte hypertrophy and myocardial fibrosis and LV dysfunction, whereas B cell reconstitution in the SCID mice results in increased cardiac myocyte hypertrophy and myocardial fibrosis. Adamo et al. (10) reported that the small molecule pirfenidone reduces adverse LV remodeling following myocardial injury through a B cell–dependent mechanism. These authors presented evidence suggesting that, in the context of myocardial injury, B cells might be activated by a TLR-dependent mechanism triggered by the release of damage-associated molecular patterns (DAMPs) from the injured myocardium (10). Studying a transaortic constriction (TAC) pressure overload model of myocardial hypertrophy, Kallikourdis et al. (46) showed that IL-10-producing B cells reduce pathologic myocardial hypertrophy inhibiting T cell–mediated responses.

3.2.2. Antibodies and cardiac dysfunction. Although none of the above experimental studies that linked B cells and cardiac remodeling identified antibody production as an essential part of B cell–mediated effects, there is a substantial body of literature that suggests that antibodies play an important role in the context of myocardial injury and heart failure. Zhang et al. (47) showed that natural IgMs play a role in myocardial I/R injury and identified a specific clone of B1 cells that produce a natural antibody that promotes complement-dependent amplification of myocardial damage in the context of I/R injury (48). Autoantibodies have also been shown to be sufficient to produce cardiomyopathy in several animal models. For example, immunization with specific cardiac proteins was shown to be sufficient to induce cardiomyopathy (49), and antibodies against troponin I were found to produce a severe dilated cardiomyopathy phenotype in PD-1-deficient mice (50). Moreover, infection with cardiotropic viruses provokes high levels of myocardium-specific antibodies (51).

3.2.3. B cells and cardiac transplantation. A handful of studies have investigated the role that B cells play in the context of cardiac transplantation. The generation of donor-specific antibodies in organ transplant recipients is a well-characterized issue in the context of transplant medicine. However, animal studies have clarified that B cells play a key role in organ rejection beyond their antibody-secreting properties (52). This has been characterized in the context of renal transplants but, to some extent, seems to apply to cardiac transplants as well. In particular, in murine models of allogeneic cardiac transplantation, B cell–mediated MHC-II-restricted antigen presentation was

found to play a key role in both acute and chronic allograft rejection (53, 54). Interestingly, while these data suggest that B cell depletion might be an effective strategy to improve graft survival, data from models of renal transplantation suggest that IL-10-expressing Bregs might play an important role in the maintenance of organ tolerance and that therefore depletion of all B cells might have negative effects (52).

3.3. B Cells and the Human Heart

Surprisingly, studies on the role of B cells in the human heart are very limited. Data from histological analysis of autopsies of patients who did not experience cardiac death suggest that in normal human hearts B cells are present at a frequency approximately similar to that of $CD4^+$ and $CD8^+$ T cells (55). These authors also studied biopsies from patients with dilated cardiomyopathy and found that, similar to the case of murine models, the frequency of intramyocardial B cells is independent of cardiac dysfunction. Moreover, histological analyses of patients with dilated cardiomyopathy revealed an increase in T lymphocytes, whereas the number of B cells did not increase above baseline. However, the authors analyzed a small sample, and therefore their findings should be interpreted with caution (55). The fact that B cells are present in the human heart is also supported by reports in the literature of rare cases of primary cardiac B cell lymphoma (56–59). In human specimens, B cells have also been described within the pericardial fat, with a relative increase in the size of pericardial-fat-associated lymphoid clusters in patients with coronary artery disease (39).

3.3.1. Heart failure. Unfortunately, data on B cells in human ischemic cardiomyopathy are scant. Patients with ST-segment myocardial infarction have a slight drop in circulating $B220^+$ cells 90 minutes after myocardial reperfusion that is followed by an increase in circulating $B220^+$ cells above pre-reperfusion levels at 24 h after reperfusion (60). There are no data on B cells in chronic ischemic heart failure. Further, very little is known with respect to B cells in the context of human nonischemic cardiomyopathy. Analyzing the peripheral blood of a cohort of 56 patients with dilated nonischemic cardiomyopathy via flow cytometry, Yu et al. (61) found that heart failure patients have a higher frequency of $CD19^+$ cells and actively replicating $CD19^+$ cells, when compared to healthy volunteers. In addition, the authors reported that patients with dilated cardiomyopathy have an increase in the percentage of TNF-producing B cells, but no change in the percentage of IL-10-producing B cells as compared to healthy controls. The frequency of TNF-producing B cells correlated directly with indices of cardiac dysfunction and myocardial fibrosis, in a subset of patients in whom cardiac MRI data were available (61). In another study with a different, smaller cohort of patients with heart failure, Guo et al. (62) found that patients with nonischemic cardiomyopathy but not patients with ischemic cardiomyopathy have an increase in the percentage of circulating Bregs ($CD19^+$ $CD5^+$ $CD1d^+$ $IL\text{-}10^+$ cells). Jiao et al. (63) studied IL-10 secretion in mononuclear cells isolated from patients with dilated cardiomyopathy and cultured in vitro for 48 h under stimulating conditions. They observed that, under these experimental conditions, patients with dilated cardiomyopathy have lower prevalence of IL-10-producing B cells and that the presence of cardiac dysfunction is associated with reduced efficiency in IL-10 production, suggesting that Bregs are not only reduced in number but are also dysfunctional (63). In a limited case series, Tschope et al. (64) showed that in five out of six patients with treatment-refractory dilated cardiomyopathy with endomyocardial biopsy-proven persistence of $CD20^+$ B lymphocytes, treatment with rituximab resulted in improved left ventricle function.

3.3.2. Autoantibodies. Several studies have reported that patients with advanced heart failure of any etiology and patients with myocarditis and dilated cardiomyopathy are much more likely to

have autoantibodies against myocardial proteins than healthy controls (65–68). In small studies, removal of autoantibodies via immunoadsorption was associated with transient hemodynamic improvements (69). These findings in patients are consistent overall with findings from the murine models of autoimmune dilated cardiomyopathy discussed above. Interestingly, heart failure patients were found to have a reduction in B cell function as measured by their ability to produce antibodies in response to pneumococcal vaccination (70).

3.3.3. Natural antibodies. The role of natural-antibody-mediated complement activation has also been investigated in humans. Based on the animal data suggesting that complement activation induced an amplification of myocardial damage in the context of I/R injury, complement inhibitors were tested in clinical trials as adjuvant therapy in patients undergoing ST-segment myocardial infarction. The use of pexelizumab, a humanized monoclonal antibody that binds the C5 component of complement, does not lead to an improvement in the primary end point of all-cause mortality through day 30 [hazard ratio (HR), 1.04; 95% confidence interval (CI), 0.80–1.35; log-rank $P = 0.78$] when used as an adjunctive therapy to percutaneous coronary intervention in the phase III APEX AMI trial. Moreover, the composite end points of death, shock, or heart failure are not different between the patients receiving pexelizumab or placebo at 30 days (HR, 0.98; 95% CI, 0.83–1.16; $P = 0.81$) and at 90 days (HR, 1.01; 95% CI, 0.86–1.19; $P = 0.91$) (71). However, in a substudy of the APEX AMI, the investigators showed that intravenous pexelizumab prevents the increase in C5a following ST-segment myocardial infarction but does not prevent an increase in serum membrane attack complex (SC5b-9), suggesting that late administration of pexelizumab precludes adequate complement inhibition, resulting in a negative clinical trial (72).

3.3.4. Cardiac transplantation. In contrast, B cells have received a fair amount of attention in the context of human cardiac transplantation. Transplanted hearts are monitored for rejection with routine biopsies, especially in the first several weeks after transplantation. In 10–20% of biopsies the pathologists describe a subendocardial accumulation of B and T cells, which is referred to as the Quilty effect (73). However, the significance of these B cell clusters remains unclear. Epidemiological evidence suggests that these clusters are not related to acute transplant rejection; however, they may be related to chronic graft rejection (73). For obvious reasons, B cells have been universally recognized to play a role in antibody-mediated graft rejection (AMR). Despite the fact that plasma cells downregulate CD20 and can be CD20 negative, the anti-CD20-depleting antibody rituximab has entered clinical practice for the treatment of AMR (74). However, this practice may change based on the recent results of the CTOT-11 [Prevention of Cardiac Allograft Vasculopathy Using Rituximab Therapy in Cardiac Transplantation (Clinical Trials in Organ Transplantation 11)] trial. This randomized, placebo-controlled, multicenter, double-blind clinical trial in non-sensitized primary heart transplant recipients showed that treatment with rituximab resulted in an unexpected significant increase in coronary atheroma during the first year following cardiac transplantation (75).

4. B LYMPHOCYTES AND VASCULAR DISEASE

The majority of our knowledge about the role of B cells in vascular disease comes from animal models. Accordingly, in this section we also first discuss data from animal models and then review human data.

4.1. B Cells in Normal Vasculature

B cells home to the adventitia of normal arteries through a physiological process that is dependent, at least in part, on L-selectin. Flow cytometry analysis of aortic cell suspensions revealed that

there is a significant population of $CD19^+$ B cells within the adventitia of naive noninflamed aortas (76). This finding was confirmed by immunohistochemistry of the aorta in which $CD20^+$ cells were detected. Subsequent studies investigated B cell trafficking to the naive aorta through adoptive transfer experiments, which revealed that adoptively transferred $CD19^+$ B cells appear in the aorta of recipient mice (B cell– and T cell–deficient Rag-$1^{-/-}$ mice) within 24 h after adoptive transfer, reaching a peak 3 days after the transfer. Interestingly, B lymphocyte migration to the aortic wall is significantly reduced in B cells from L-selectin knockout mice.

4.2. B Cells in Animal Models of Vascular Disease

B cells are recruited to the inflamed vasculature and have been implicated in the pathogenesis of several vascular diseases including vasculitis (77), aneurysm (78), arterial injury (79), hypertension (80), and atherosclerosis (81). In a murine model of coronary vasculitis, Martinez et al. (82) showed that vasculitis is significantly reduced in mice lacking B and T lymphocytes (Rag-$1^{-/-}$ mice), suggesting a potential deleterious effect of B cells in the vasculature. Schaheen et al. (83) investigated the effects of B cells in the formation of abdominal aortic aneurysms (AAAs) in ApoE (apolipoprotein E)-deficient mice. $ApoE^{-/-}$ mice depleted of B cells through injection of anti-CD20 antibody are protected from AAA formation in comparison to nondepleted $ApoE^{-/-}$ mice. Interestingly, the authors reported that anti-CD20 administration is associated with an increase in infiltration of plasmacytoid dendritic cells and Tregs in the inflamed aorta. These findings were confirmed by Furusho et al. (84), who demonstrated that B cell–deficient μMT mice are also protected from AAA. Using a model of periadventitial cuff injury in the right carotid of wild-type and Rag-$1^{-/-}$ mice, Dimayuga et al. (79) investigated the modulatory role of B cells in neointimal formation. They showed that neointimal formation induced by injury is aggravated in Rag-$1^{-/-}$ mice in comparison to wild-type mice. Notably, Rag-$1^{-/-}$ mice reconstituted with B cells showed a reduction in the neointima area in comparison to Rag-$1^{-/-}$ mice that were not reconstituted with B cells, suggesting that B cells have a protective role following vascular injury.

Chan et al. (80) reported that B cells play a permissive role in the development of hypertension using B cell–deficient BAFF-R (B cell–activating factor receptor) mice and anti-CD20-mediated B cell depletion. These authors observed that the B cell–deficient animals used in the study were resistant to angiotensin II (Ang-II)-induced hypertension. Importantly, Ang-II-induced hypertension was restored in BAFF-$R^{-/-}$ mice after adoptive transfer of B cells. However, in other studies, B cell–depleted mice did not show a resistance to Ang-II-induced hypertension (45). The explanation for the discrepancy between these studies is unknown, but it may relate to differences in the methods used for Ang-II treatment and/or methods for achieving B cell depletion.

4.3. B Cells in Animal Models of Atherosclerosis

The function of B cells has been most extensively characterized in atherosclerosis. B cells represent a minor component of the tunica intima in atherosclerotic vessels; however, B lymphocytes and plasma cells accumulate in the tunica adventitia adjacent to arterial plaques of atherosclerosis-prone mice and human patients. Moreover, their numbers parallel the severity of vascular lesions. The abundant body of knowledge accumulated in this area has been reviewed recently by several groups (81, 85, 86). Here, we focus on the role that distinct B cell subpopulations and different B cell effector functions play in atherosclerosis, in order to highlight the complex relationship between B cells and vascular disease.

Atherosclerosis is an inflammatory disease characterized by the accumulation of low-density lipoprotein (LDL) in the intimal layer of major arteries (87). Oxidative modification of LDL (ox-LDL) leads to the formation of oxidation-specific epitopes (OSEs) that are recognized by the

immune system as DAMPs, thus triggering a complex inflammatory response (88–90). The first observation that B cells might play a role in atherosclerosis came from the work of Yla-Herttuala et al. (91), who observed that rabbit and human atherosclerotic lesions contain immunoglobulins specific for OSEs. Interestingly, B cells are not commonly found in atherosclerotic plaques. However, they are present in significant numbers in the adventitial layer of the arterial wall, where they also participate in the formation of artery tertiary lymphoid organs (ATLOs) (85, 92), which are atherosclerosis-associated lymphoid aggregates that range in complexity from small T and B cell clusters to well-structured lymph node–like structures. ATLOs are believed to be involved in the local humoral immune response (93).

A functional role of B cells in atherosclerosis was first shown in an elegant study that demonstrated that splenectomy aggravates atherosclerosis in hypercholesterolemic ApoE knockout mice (94). Transfer of spleen cells from either wild-type or nonatherosclerotic young ApoE knockout mice (that had not developed disease) reduces the size of fibrofatty atherosclerotic plaques to that observed in sham-operated ApoE knockout mice, rescuing the recipients from the proatherogenic effect of splenectomy. Adoptive transfer of splenic T and B cells from the ApoE knockout mice into splenectomized young nonatherosclerotic ApoE knockout mice revealed that $CD3^+$ T cells reduce the disease-aggravating effect of splenectomy, whereas B cells protect mice from developing disease. Of note, circulating antibodies to oxidized LDL are significantly reduced by splenectomy and normalized by reconstitution with spleen cells from atherosclerotic ApoE knockout mice. Studies in LDL receptor–deficient ($LDLR^{-/-}$) mice that underwent bone marrow irradiation followed by reconstitution with μMT or wild-type bone marrow revealed that there was an increase in atherosclerotic lesions in the $LDLR^{-/-}$ mice that were reconstituted with μMT bone marrow, when compared to those reconstituted with wild-type bone marrow (95). In a different study, Doran et al. (96) showed that the helix-loop-helix transcription factor inhibitor of differentiation 3 (Id3) is a critical regulator of B cell aortic trafficking and B cell–mediated atheroprotection and identified *Ccr6* as an Id3 target gene mediating these effects. A subsequent study reported that B cell depletion with an anti-CD20 antibody reduces the size of atherosclerotic lesions in both $ApoE^{-/-}$ and $LDLR^{-/-}$ mice, suggesting a proatherogenic role of B cells (97). Notably, anti-CD20-mediated B cell depletion resulted in preservation of natural anti-ox-LDL IgM antibodies, whereas IgG anti-ox-LDL antibodies were decreased, suggesting that anti-CD20 administration predominantly depleted conventional B2 lymphocytes and preserved B1 cells. In agreement with these findings, Kyaw et al. (98) showed that adoptive transfer of B2 cells but not B1 cells augments atherosclerosis in lymphocyte-deficient $ApoE^{-/-}$ $Rag\text{-}2^{-/-}$ mice, suggesting that different B cell subsets play distinct roles in atherosclerosis. Together, these observations have resulted in a detailed investigation of the role that different B cell subsets and effector functions play in the context of atherosclerosis, which is discussed below.

4.3.1. Role of B1 cells. The role of innate-like B1 cells in atherosclerosis was initially studied through adoptive transfer of B1a or B1b cells in B cell–deficient mice. Kyaw et al. (99) demonstrated that the transfer of B1a but not B2 lymphocytes reduces atherosclerosis in splenectomized mice. The authors found that adoptive transfer of B1a cells from secretory IgM (sIgM)-deficient mice does not reduce atherosclerosis in splenectomized mice, suggesting that the mechanism for B1a-induced atheroprotection is dependent on the production of IgM. A different group observed that B1b cells attenuate atherosclerosis when transferred to $Rag\text{-}1^{-/-}ApoE^{-/-}$ mice through secretion of IgM anti-OSEs (100). Using a murine model of B1a cell expansion (Siglec-G deficient), Gruber et al. (101) demonstrated that the number of B1a cells and natural IgM are increased in $LDLR^{-/-}$ $Siglec\text{-}G^{-/-}$ mice. Of note, the increase in B1a cells and increased IgM levels were associated with a reduction in atherosclerosis and systemic inflammation. These findings were

further supported by the observation that administration of an anti-TIM-1 monoclonal antibody (RMT1–10) that increases the numbers of IgM-producing B1a cells reduces the development and progression of atherosclerosis (102). RMT1–10 administration also reduces the number of apoptotic and necrotic cells in the plaque. The literature further suggests that the B1 cells that secrete atheroprotective natural IgM anti-OSEs home to atherosclerotic lesions (81, 92). Little is known about the signaling pathways that activate IgM production in the context of atherosclerosis; however, it has recently been reported that suppression of atherosclerosis and IgM secretion by B1a cells is dependent on the expression of TLR4 and MyD88 (103).

4.3.2. Role of B2 cells. To determine whether B2 cells are proatherogenic, two groups studied models of B2 cell depletion. B cell–activating factor (BAFF) is a critical factor for B2 cell maturation and survival. BAFF-R$^{-/-}$ mice manifest impaired B cell maturation with marked depletion of B2 cells, with relatively preserved numbers of B1 cells. When LDLR$^{-/-}$ mice are lethally irradiated and reconstituted with either wild-type or BAFF-R$^{-/-}$ bone marrow, reconstitution with BAFF-R-deficient bone marrow is associated with protection from atherosclerosis (104). Kyaw et al. (105) confirmed the important role of BAFF signaling in atherosclerosis by demonstrating that antibody-mediated depletion of BAFF-R^{+} cells results in smaller lesions and fewer immune cells in the atherosclerotic plaques. The observation that strategies that predominantly deplete B2 cells (anti-CD20 antibody, anti-BAFF-R antibody, and BAFF-R deletion) reduce atherosclerosis is in agreement with the general concept that B2 cells are proatherogenic, whereas B1 cells are atheroprotective. However, it should be kept in mind that the B2 pool is heterogeneous and that not all B2 cells may play the same role in atherosclerosis. This was recently demonstrated by Nus et al. (106) who showed that MGZ B cells control the response of helper T cells in hypercholesteremic mice and have an atheroprotective role.

4.3.3. Role of Bregs. Using a model of neointima formation in which a perivascular collar is placed around the murine carotid artery, Strom et al. (107) showed that lymph node–derived Bregs confer protection from the development of vascular lesions through a mechanism that is sensitive to IL-10 inhibition. These authors demonstrated that the CD21^{high} CD23^{high} CD24^{high} Breg cells are increased in the draining lymph nodes of ApoE$^{-/-}$ mice, and that adoptive transfer of lymph node–derived B2 cells or purified CD21^{high} CD23^{high} CD24^{high} B cells reduces lesion size and inflammation without changing serum cholesterol levels. Blockade of IL-10 or transfer of B cells from IL10-deficient mice prevents lymph node–derived B cell–mediated protection. Sage et al. (108) did not find any difference in the size and cellular composition of atherosclerotic plaques in LDLR$^{-/-}$ mice that underwent radiation and bone marrow reconstitution with bone marrow either from μMT/wild-type animals or μMT/IL-10$^{-/-}$ animals. These authors concluded that Bregs are dispensable for atherosclerosis development. Further studies are needed to understand the origin of this discrepancy and determine the role of Bregs in vascular disease.

4.3.4. Role of innate response activator B cells. Hilgendorf et al. (109) showed that the number of GM-CSF-producing IRA B cells expands considerably in experimental models of atherosclerosis and that this impacts the populations of T helper 1 effector and classic dendritic cells. Through adoptive transfer and depletion studies, the authors revealed that IRA B cells aggravate atherosclerosis (109). This is the first study to demonstrate a proatherogenic role of a subtype of B1 cells.

4.4. Antibody-Mediated Functions of B Cells in Atherosclerosis

As discussed above, the literature suggests that B cells modulate atherosclerosis predominantly through the production of antibodies that target OSEs (**Figure 2**). Palinski et al. (111) described

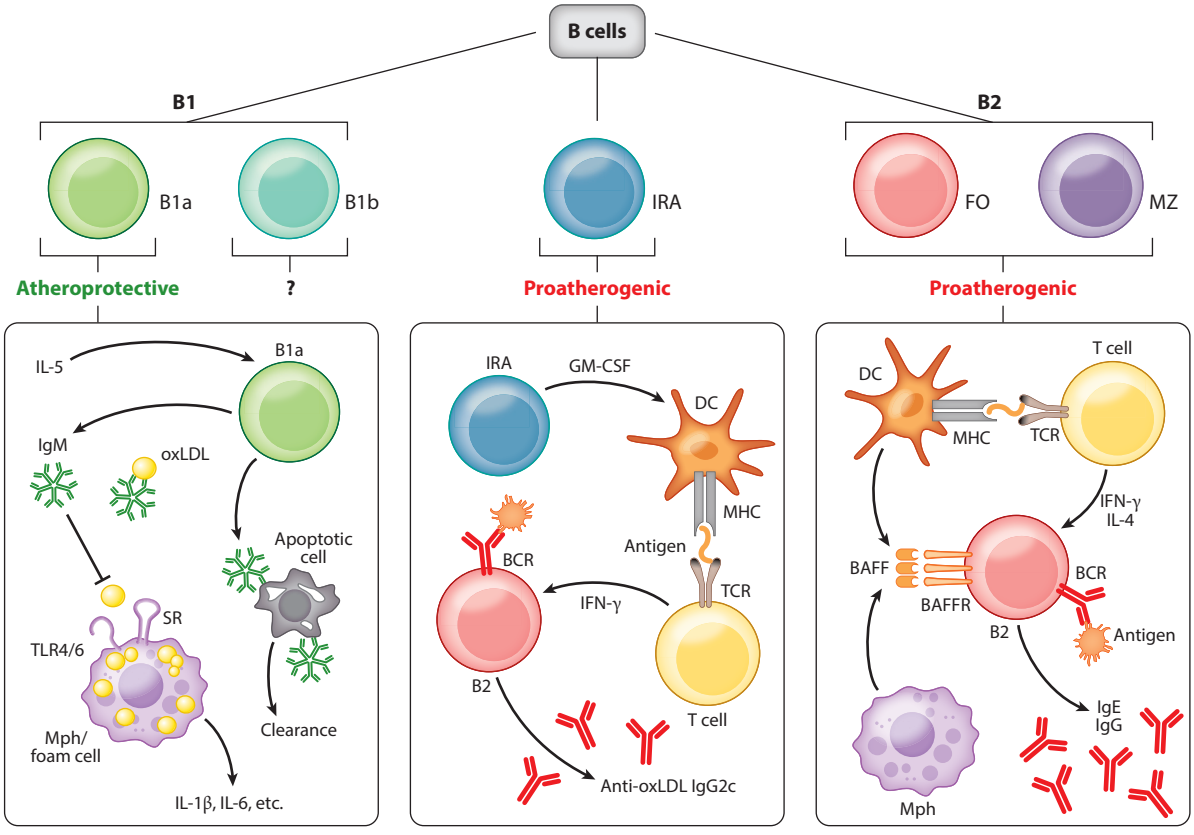

Figure 2

Roles of different B cell subsets in murine atherosclerosis. B cells consist of two major cell subsets, named B1 and B2 cells, which are characterized by different localization properties and activation requirements. B1 cells reside predominately in the peritoneum and are subdivided into B1a and B1b cells. B1a cells protect from atherosclerosis. Their atheroprotective properties depend mainly on the capacity to produce oxLDL-specific natural IgM antibodies, which can be enhanced by IL-5 stimulation and which block oxLDL uptake and foam cell formation. In addition, natural IgM has also been shown to promote apoptotic cell clearance. The role of B1b in atherosclerosis remains elusive. B2 cells are mainly found in the spleen and consist of MZ and FO B cells. B2 cells seem to be proatherogenic. Although the underlying mechanism is yet to be identified, this may include production of proatherogenic IgG and IgE antibodies. IRA B cells are a subset of B1a-derived B cells that are characterized by GM-CSF secretion, promote the expansion of IgG2c anti-oxLDL antibodies and aggravate atherosclerosis. Abbreviations: BAFFR, B cell–activating factor receptor; BCR, B cell receptor; DC, dendritic cell; FO, follicular; GM-CSF, granulocyte-macrophage colony-stimulating factor; IRA, innate response activator; Mph, macrophage; MZ, marginal zone; oxLDL, oxidized low-density lipoprotein; SR, scavenger receptor; TCR, T cell receptor; and TLR, Toll-like receptor. Adapted from Reference 110.

the presence of OSEs and an increase in anti-OSE antibodies in atherosclerotic lesions in the ApoE$^{-/-}$ mouse model. Subsequent studies disclosed that OSEs are the target of innate natural IgM antibodies in mice and humans (112). Anti-OSE antibodies are believed to prevent ox-LDL uptake by foam cells (86), and IgM secretion is essential for atheroprotection in LDLR$^{-/-}$ mice (113). These observations have prompted several authors to explore vaccination as a potential strategy to treat atherosclerosis. Nicoletti et al. (114) showed that immunoglobulin treatment decreased atherosclerosis in ApoE$^{-/-}$ mice and that immunization with anti-OSE antibodies was atheroprotective (115, 116). It is important to recognize that binding of OSEs might not be the only explanation for the atheroprotective role of IgMs. Tsiantoulas et al. (117) found that there is

an increase in IgE levels in sIgM$^{-/-}$ mice, which was shown to aggravate atherosclerosis. Treatment with an IgE-neutralizing antibody was sufficient to reverse the development of atherosclerosis in LDLR$^{-/-}$ sIgM$^{-/-}$ mice.

4.5. Non-Antibody-Mediated Functions of B Cells in Atherosclerosis

Although most of the data discussed above point to antibody production as the key effector function of B cells in atherosclerosis (for a recent review on the topic see Reference 86), the non-antibody-mediated functions of B cells have also been shown to be important. In fact, depletion of TNF in B cells attenuates atherosclerosis development in LDLR$^{-/-}$ mice, revealing a cytokine-dependent atherogenic pathway (118). In addition, B cells have been implicated in the regulation of the balance between regulatory and effector memory CD4^{+} T cells (119) and in modulating the response of follicular helper T cells (106). These findings further highlight the intricate relationship between B cells, other immune cells, and vascular disease.

4.6. B Cells and Human Vasculature

A large body of evidence suggests that B cells play an important role in vascular disease in humans. Histological examination of AAAs has revealed that there is a substantial number of B lymphocytes present (78). Patients with the autoimmune disease systemic lupus erythematosus have increased risk of developing cardiovascular disease in comparison to healthy subjects, and this risk has been associated with the production of autoantibodies and immune cell dysfunction (120). Anti-ox-LDL antibodies have been found in human atherosclerotic lesions (91). Moreover, Ketelhuth et al. (121) reported a significant increase in the levels of autoantibodies to ox-LDL in chromatographic fractions of oxidized LDL from healthy controls when compared to patients with unstable angina. CD19^{+} B cells have been identified by immunofluorescence in arterial wall samples obtained from patients with advanced atherosclerosis. Atherosclerotic tissues from patients undergoing carotid endarterectomy revealed that adventitial B cells were mainly mature B2 CD20^{-} plasmablasts lacking markers of terminal differentiation to plasma cells (i.e., CD138 and Blimp-1) (122). This study also examined the expression of immunoglobulin transcripts by reverse transcriptase PCR and found that IgA, IgG, and IgM are present in the arterial walls of patients with atherosclerosis. Notably, the authors reported that B cells in the vascular wall express mainly proinflammatory cytokines such as TNF, IL-6, and GM-CSF. Interestingly, GM-CSF^{+} IgM^{+} IRA B cells were found in the spleen of humans with cardiovascular disease (109). Hilgendorf et al. (109) also showed that the number of IRA B cells in the spleen is increased in cardiovascular disease patients when compared to patients without cardiovascular disease. In the context of vasculitis in patients, activation of B cells as determined by immunohistochemistry on histological sections has been shown to correlate with disease activity in human specimens from patients with Wegener granulomatosis (123).

4.7. B Cell Subsets in Human Atherosclerosis

Thus far, it has been challenging to apply our knowledge with respect to the role of subsets of murine B cells to atherosclerosis in humans. Many human studies have focused on investigating a relationship between distinct B cell subsets and the risk of vascular events. Meeuwsen et al. (124) investigated the association between circulating B cell populations and the risk of secondary cardiovascular events in patients with severe cardiovascular disease. The B cell subpopulations identified in the study were (*a*) B1-like B cells (CD27^{+} CD43^{+}), (*b*) unswitched memory B cells (CD27^{+} CD43^{-} IgD^{+}), (*c*) switched memory B cells (CD27^{+} CD43^{-} IgD^{-}), and (*d*) naive B cells

(CD27$^-$ CD43$^-$ IgD$^+$). The authors found that the total number of B cells and the number of (un)switched memory B cells had an inverse relationship to the number of secondary events. In a different study, Mantani et al. (125) assessed the association between B cell subsets, carotid disease, and stroke in a large prospective cohort. The authors identified two subsets of circulating CD19$^+$ cells according to expression of CD40 and CD86 and found that high levels of CD19$^+$ CD40$^+$ B cells are associated with a decreased risk of stroke during 15 years of follow-up. On the contrary, subjects with high numbers of CD19$^+$CD86$^+$ cells show an increased risk of stroke (126). Although there is much to be learned about the role of B cells in vascular disease, the literature on humans suggests that the findings from animal models are relevant to human atherosclerotic and vascular disease and that B cells might be a relevant target for the development of novel therapeutic options.

5. CONCLUDING REMARKS

B cells have been traditionally studied for their role in the context of mediating adaptive immune responses. Indeed, the studies that identified the ability of B lymphocytes to confer a long-lasting immunological memory provided the biological basis for developing vaccines, which have had a tremendous impact on human health worldwide. However, there is also a growing appreciation that B cells do far more than produce antibodies in the context of adaptive immune responses. Today we recognize that B lymphocytes are key modulators of both the innate and adaptive immune responses through a variety of mechanisms. Here we have reviewed the emerging evidence that suggests that B cells play a critical role in the context of the cardiac adaptation to injury and likely heart failure. We have also discussed the abundant evidence demonstrating that B cells play an important role in the context of atherosclerosis and vascular disease. Although there are many areas of uncertainty that remain to be explored, the basic and clinical data suggest that B cells play an important role in the cardiovascular system in health and disease and that B cells are a potential therapeutic target in a variety of different cardiovascular diseases.

SUMMARY POINTS

1. B cells have many effector functions in the context of the immune response, including production of specific antibodies, production of natural antibodies with low specificity, cytokine secretion, and antigen presentation.
2. B cells are present in the heart and vasculature, both in the naive state and in the context of disease.
3. A growing body of evidence from animal models suggests that B cells play an important role in the context of myocardial adaptation to injury and myocardial adverse remodeling.
4. Data from animal models and human observations suggest that B cells play a role in the development and progression of atherosclerosis and other vascular diseases.
5. Different subsets of B cells have different, competing functions in the context of atherosclerosis in rodents.
6. The limited evidence available from human studies of B cells' involvement in cardiovascular disease suggests that findings from animal models might be generalizable to humans.

FUTURE ISSUES

1. The identity, dynamics, and function of B cells in the naive heart and vasculature are unclear.
2. Different subsets of B cells have been shown to have competing roles in the context of atherosclerosis. It is unknown whether this is true in the context of cardiac disease or in the context of other vascular diseases.
3. The role of different B cell effector functions in the context of cardiovascular disease remains underexplored.
4. It is unclear whether cardiovascular injury is associated with long-lasting changes in the functions of B cells other than antibody production.
5. The mechanism underpinning the recruitment of B cells to sites of cardiac and vascular injury remains poorly explored.
6. The role of Bregs in cardiovascular disease needs further clarification.

DISCLOSURE STATEMENT

L.A. and D.L.M. have a startup company focused on the development of PEGylated Pyridones for B cell modulation and treatment of cardiac disease.

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