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REVIEW ARTICLE

Multiple potential roles for B cells in atherosclerosis

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The development of atherosclerosis is the major etiological factor causing cardiovascular disease and constitutes a lipid-induced, chronic inflammatory and autoimmune disease of the large arteries. A long-standing view of the protective role of B cells in atherosclerosis has been challenged by recent studies using B cell depletion in animal models. Whereas complete B cell deficiency increases atherosclerosis, depletion of B2 but not B1 cells reduces atherosclerosis. This has led to a re-evaluation of the multiple potential pathways by which B cells can regulate atherosclerosis, and the apparent opposing roles of B1 and B2 cells. B cells, in addition to having the unique ability to produce antibodies, are now recognized to play a number of important roles in the immune system, including cytokine production and direct regulation of T cell responses. This review summarizes current knowledge on B cell subsets and functions, and how these could distinctly influence atherosclerosis development.

Key words: Atherosclerosis, immunity, lymphocytes

Key messages

- Selective B cell subsets play distinct roles in the development of atherosclerosis.
- B cell subsets influence atherosclerosis development through production of antibodies, cytokines, and direct regulation of other immune cell subsets.

Introduction

Cardiovascular disease remains a leading cause of death in the developed world and is growing in prominence worldwide, with 80% of cardiovascular deaths now occurring in low–middle-income countries (1). Atherosclerosis constitutes a lipid-induced, chronic inflammatory and autoimmune disease of the large arteries (2–5) and is the major underlying cause of heart disease and stroke. Control of cholesterol and blood pressure are effective preventive therapies; however, further demand remains to better target vulnerable, event-inducing plaques, or alternatively reduce development of vulnerability. Reducing the associated chronic inflammation sustaining the immune response is an important target for scientific and future therapeutic investigation. Despite a long-known association with atherosclerosis, B cells and antibodies have not always been a research focus. Current knowledge from the immunology field defines diverse functions for B cells beyond antibody secretion, with multiple subsets playing a role in both innate and adaptive immunity. This, in combination with results from mouse models suggesting different types of B cells can enhance as well as protect from atherosclerosis, has reignited interest in how these abundant and multifunctional cells impact all stages of atherosclerosis development. This review first describes B cell subsets and functions, and then summarizes current views on the potential roles of B cells in atherosclerosis and future targets for research that could enable therapeutic targeting.

B cell subsets and functions

B cell development and the different B cell subsets characterized are reviewed in detail elsewhere (6–8). Broadly, B1 and B2 cells constitute the major two groups of B cells and can be considered part of the innate and adaptive immune systems, respectively. B2 cells develop in the adult bone marrow from hematopoietic stem cells through a well-characterized series of precursor stages (7), whereas at least some B1 cells develop in the fetal liver and are self-sustaining in the adult independently of HSCs (Figure 1A) (9,10). B cells are primarily found in secondary lymphoid organs (spleen and lymph nodes), blood, and submucosa of the intestine and lung, but also in adipose tissue, aortic adventitia, and sites of inflammation (11). The primary and unique characteristic of B cells is the expression of surface antibody, or B cell receptor, and thus their major function as the source of antibody-producing plasma cells. Each B cell clonal lineage develops a unique B cell receptor through somatic genome rearrangement of the multi-gene immunoglobulin locus. The binding specificity and affinity of the B cell receptor to self or foreign antigen defines the fate of each B cell. In the steady state, a system has evolved that provides a repertoire depleted of highly autoreactive clones yet robust and diverse enough to target the multitude of potential antigens encountered. Thus, multiple

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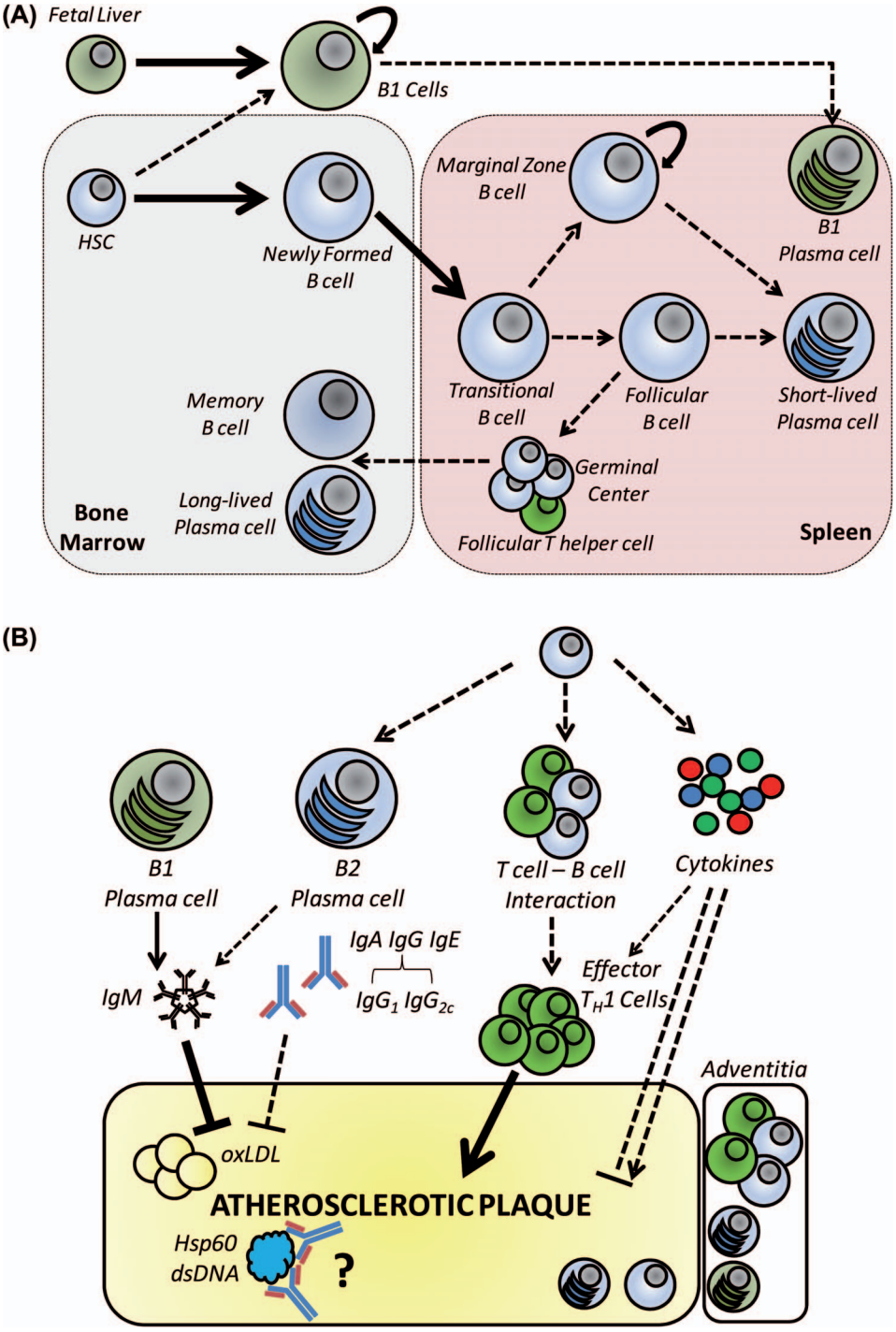

Figure 1. A: B cell development and subsets. B1 cells develop primarily from fetal liver and self-maintain in the adult. B1 cells are the source of natural IgM antibodies that target antigens such as oxidized phospholipids. B2 cells develop from adult bone marrow stem cells and undergo a series of differentiation steps in the bone marrow, then leave as immature/transitional B cells and further differentiate into either marginal zone or follicular B cells in the spleen. B2 cell activation leads to short-lived plasma cell differentiation or, in the case of T cell-dependent responses, germinal centre formation, which results in affinity maturation of the B cell clone and formation of memory B cells and long-lived plasma cells, which can persist in specific bone marrow niches until antigen re-encounter. B: B cell regulation of atherosclerosis. Natural antibodies and other IgM targeting oxLDL, primarily but not exclusively from B1 cells, are thought to be atheroprotective. The potential pro-atherogenic functions of B2 cells include production of IgG2 antibodies that activate macrophages via Fc receptors, activation of pro-atherogenic Th1 CD4 T cells, or production of pro-inflammatory T cells. B cells may act remotely from peripheral lymphoid organs or locally from the adventitia and atherosclerotic plaque.

checkpoints regulate developing B cells to hone the B cell repertoire through mechanisms including receptor editing, anergy, and apoptosis (12,13). In addition to antigen, important regulators of B cell development and activation include B cell activating factor (BAFF), and a proliferation-inducing ligand (APRIL) (14). BAFF is essential for B2 cell maturation beyond the transitional-1 (T1) stage in the spleen through interaction with BAFF receptor (BAFFR) (15–17). BAFF can also regulate survival, activation, and function of both B1 and B2 cells through BAFFR and the alternative receptor TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor). APRIL is a BAFF homologue but interacts only with TACI and a third receptor,

BCMA (B cell maturation antigen), but not BAFFR. TACI is up-regulated in activated B cells and plays complex roles in B cell proliferation, survival, and antibody class switching (18–20), whereas BCMA is up-regulated on plasma cells and is essential for plasma cell survival in the bone marrow by transducing APRIL signals in the plasma cell niche (21,22).

B1 cells play a major role in maintaining the barrier function of mucosal surfaces and possess B cell receptors with germline-encoded specificities for common pathogen-associated epitopes; they rarely undergo B cell receptor editing or maturation, thus are as much part of the innate as the adaptive immune system. B1 cells are the source of natural antibodies, which are produced independently of any external antigens. One major family of these epitopes is oxidation-associated epitopes such as phosphorylcholine (PC) and malondialdehyde (MDA) (23). Such epitopes are common in endogenous debris such as apoptotic and necrotic cells and modified lipoprotein particles, e.g. oxidized low-density lipoprotein (oxLDL). Thus, natural antibodies (and other antibodies targeting these antigens) play an important role in homeostatic clearance of dead cells and other debris, including oxLDL, and may contribute significantly to clearance pathways in atherosclerosis.

Mature B2 cells recirculate via the blood through secondary lymphoid organs where they may encounter antigens captured and displayed by macrophages and dendritic cells, in general as part of immune complexes with (presumably) low-affinity, natural, or previously produced antibodies. Depending on other signals and the precise location, B2 cells are then activated and proliferate, leading variously to short-lived plasma cells, long-lived plasma cells that migrate to the bone marrow, and memory B cells that enable lifelong immunological memory and more rapid responses upon subsequent antigen encounters (Figure 1A) (24). Bone marrow plasma cells can persist for years and continue producing antibodies, but re-exposure to the same antigens stimulates memory B cell activation and formation of new plasma cells. Marginal zone B2 cells bridge the functions of B1 and follicular B2 cells, possessing somatically rearranged B cell receptors but having a memory-like phenotype that allows a more rapid B1-like response that can be activated by innate signals like IL-5 or toll-like receptor ligands. In addition to developmental antibody receptor diversification, mature B2 cells selected and activated by the presence of cognate antigen undergo two further changes to their B cell receptor (antibody)—class switching of the Fc region and mutagenesis of the antigen-binding Fab region to enhance affinity. Affinity maturation occurs in proliferating activated B cells in the germinal centres (GC) of secondary lymphoid organs where higher affinity clones prevail through competition for antigen immobilized on follicular dendritic cells (25).

Antibodies play a primary effector function in neutralizing and clearing pathogens, pathogen-associated molecules, and infected cells through a range of mechanisms, including antibody-dependent cellular cytotoxicity, recruitment of the complement system, and promoting phagocytosis (26–28). Antibodies also influence innate immune function and phenotype, particularly macrophage inflammatory activation through the Fc region binding to Fc receptors on the cell surface (26).

B cells, like most immune cells, produce a range of cytokines with potent and diverse effects. For B cells, these include most prominently IL-6, IL-10, and TNF (29). B cells also respond to many cytokines including IL-6, TNF, IFN-α, IL-4, IL-5, and IFN-γ. More recently, a distinct subset of spleen B cells has been shown to be capable of GM-CSF production and of significantly influencing innate immune functions (30). Alternatively, B cell-derived MCP-3 (Ccl7) is critical to monocyte mobilization in response to myocardial infarction, with B cell depletion leading to reduced infarct area and improved heart function (31).

B cells also regulate their adaptive immune counterparts, T cells, through antigen presentation and co-stimulation (32). In general, B cells are not phagocytic like myeloid antigen presenting cells, and only antigen endocytosed while bound to the B cell receptor (surface antibody) is processed and presented on B cell MHCII molecules to helper CD4 + T cells. Although dendritic cells act as primary activators of helper T cells, in many cases B cell–helper T cell interactions are critical in sustaining and regulating the nature of both T cell and B cell responses, for example in response to lower antigen levels (33).

Not all B cells require the presence of cognate antigen or the presence of helper T cells for their activation and differentiation into plasma cells. B1 cells and certain B2 cell subsets, such as marginal zone B cells, can be activated directly by pathogen-associated molecular patterns, for example toll-like receptor ligands, or cytokines such as IFN-α, IL-1β, and IL-5 (34,35). In contrast, responses to protein antigens are commonly T cell-dependent and require ongoing contact with antigen, co-stimulation, and cytokines, the last-mentioned two signals received from specialized follicular helper T cells. BAFF and APRIL also play prominent roles in B cell activation in addition to being critical to homeostatic maintenance (16,17,36). Thus, both innate and adaptive immune pathways could lead to B cell activation in atherosclerosis (Figure 1B).

Association of B cells and autoantibodies with atherosclerosis

The humoral nature of B cell antibody responses suggests a remote regulation of atherosclerosis is most likely and most important. Furthermore, a number of other remote pathways negate a need for the presence of B cells within or adjacent to plaques for them to be important. These include T cell responses in spleen and lymph node, regulation of innate cell differentiation, monocyte mobilization from bone marrow, and their presence at alternative sites of cardiovascular disease-associated inflammation such as adipose tissue. Nevertheless, B cells can be found in plaques but are more often found at adventitial sites close to plaques (37–39). Initial reports suggested these cells may be plasma cells, whereas a recent report concluded that these B cells are most likely B2-derived plasmablasts, with evidence of local affinity maturation occurring in both adventitia and plaque, and the presence of a limited number of class-switched clones (40). The adventitia is a well-recognized site for immune responses (41), and advanced human and mouse atherosclerosis leads to the development of tertiary lymphoid organs in the adjacent adventitia (38,42). This suggests a potential for local B cell responses in advanced human atherosclerosis and thus ongoing regulation of plaque status. Whether adventitial immune responses and cell accumulation also occur in symptomatic coronary and carotid plaques is unclear; however, it is easy to envisage aorta-originating B cell and T cell responses regulating plaques at other sites. Very recently, a study examining whole-blood global gene co-expression identified a strong indication of B cell dysregulation in coronary heart disease patients compared to controls (43), and another showed that activated CD86 + B cells associate with a higher risk of stroke (44).

The presence of antibodies within atherosclerotic plaques is much more prominent than B cells themselves, with abundant IgM and IgG detected in human and mouse plaques (39,45,46).

Circulating levels of modified LDL-reactive antibodies also associate with atherosclerosis (47,48), as do those to heat shock protein 65 (49). Levels of both natural IgM antibodies and adaptive IgG antibodies reacting with moieties found in modified LDL and on apoptotic cells, e.g. phosphocholine or malondialdehyde, are associated with atherosclerotic burden in many studies, although not all (50–52). Interestingly, IgM levels decline with age (53), consistent with studies showing an inverse correlation with disease and extensive experimental evidence of its protective influence (46,54). Overall, the prevailing view is that anti-oxLDL IgG is positively correlated whereas IgM is negatively correlated; however, doubt still remains, particularly in the case of IgG. Equally, prospective studies have shown mixed results (52). For example, anti-modified LDL antibodies predicted myocardial infarction in type 2 diabetes patients in the VADT study (55), but there was no prognostic value in the EPIC-Norfolk study (56). A further impact of B cells on atherosclerosis is the association of other autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and Sjogren's syndrome (57,58) with an increased risk of cardiovascular disease. How the presence of these autoimmune diseases influences cardiovascular disease is complex, but cross-reactivity of autoantibodies is one contributory mechanism as well as systemic pro-inflammatory cytokines that may be induced in part dependently on autoantibodies. In RA, the correlation between oxLDL IgG antibodies and atherosclerosis seems more consistent (59–61). Recently, in SLE patients, levels of autoantibodies to dsDNA and cardiolipin were associated with higher levels of non-calcified plaques, potentially more prone to rupture (62), and a previously unappreciated pro-atherosclerotic role for IgE antibodies was demonstrated in ApoE$^{-/-}$ mice (63). The functional roles and overall influence of antibodies in atherosclerosis are further discussed below.

B cells regulate atherosclerosis—evidence from mouse models

Given the diverse functions and wide-ranging impact of B cells on immune responses, it is unsurprising that B cell regulation of atherosclerosis is complex to dissect. Past studies suggesting a solely protective role for B cells in atherosclerosis must now be reinterpreted based on the divergent roles of different B cell subsets and their antibodies. The increased atherosclerosis in B cell-deficient (μMT), atherosclerotic-prone ApoE$^{-/-}$ mice (64) and splenectomized mice (65) could be primarily due to the lack of B1 cells and natural IgM (46,54), which plays a prominent role in opsonization and non-inflammatory removal of oxLDL as well as apoptotic cells. In contrast to genetic deficiency of all B cells, anti-CD20 antibody treatment, which preferentially depletes B2 cells, leaving B1 cells largely intact, reduces atherosclerosis (66,67). Corroborating these studies, the specific lack of mature B2 cells in ldlr$^{-/-}$ mice transplanted with B cell activating factor receptor (BAFFR)-deficient bone marrow reduces atherosclerosis (68). The same is true for BAFFR$^{-/-}$ mice on the ApoE$^{-/-}$ background (69) and B cell-specific BAFFR deficiency (68). Resupplementation of B cell-deficient mice with spleen B2 cells reverses the reduction of atherosclerosis (67). However, other groups have shown protective effects of transferred spleen B cells (65,70), suggesting as yet unclear complexities and multiple potential mechanisms for B2 cell regulation of atherosclerosis. Doran et al. (70) showed that the ability to home to the aorta through CCL20-CCR6 signalling was key for atheroprotection. It is not clear if this property was important to recruit a certain subset or to the localization of B cells in general to the adventitia. Further fractionation of B2 (or spleen) subsets, or transfer of B cells from appropriate knock-out mice, will be necessary to determine these remaining questions.

Mechanisms for B cell regulation of atherosclerosis

There are three major modalities by which B cells could influence atherosclerosis: 1) through antibodies, 2) through regulation of T cell responses via cell–cell contact, and 3) through production of cytokines. These different modalities may act through a variety of pathways, and each has both anti- and pro-atherogenic potential.

Multiple roles of antibodies in atherosclerosis

Past studies have provided many useful insights into the potential roles for autoantibodies in atherosclerosis. However, the lack of complete discrimination between natural or innate-derived and adaptive antibodies, the use of immunization strategies that also stimulate natural antibody and regulatory T cell-mediated immune responses (71), and a focus on oxLDL targeting antibodies (72) mean that the overall influence of B cell antibodies is still unclear. The most recognized and studied autoantigen in atherosclerosis is modified LDL, with epitopes from various forms targeted both by natural antibodies and B2 cell-derived, class-switched IgG antibodies. Hsp60 and 65, as well as β2 glycoprotein I, are further autoantigens (73). Passive Ig transfer (intravenous immunoglobulin; IVIg) suppresses atherosclerosis (74). IVIg, used in other autoimmune diseases and transplant patients, is hypothesized to work through differential sialylation of the Fc portion, leading to immunosuppressive pathways via IL-33, IL-4, and induction of macrophage FcγRIIb (75) as well as through induction of regulatory T cells (76). Although a number of modified LDL or ApoB peptide-based active immunization strategies consistently reduce atherosclerosis (77,78), their effects are primarily due to increased regulatory T cell responses, enhanced natural antibody production, and a shift to Th2 IgG responses, thus may not represent endogenous humoral responses (71,79). Indeed, both immunogenic adjuvant-containing (77,78) and tolerogenic (e.g. mucosal or oral) formulations (79,80) lead to enhanced Treg levels and reduced atherosclerosis. In contrast, immunization with Hsp65 can enhance atherosclerosis (81–83), whereas tolerogenic strategies targeting Hsp65 reduce atherosclerosis (84–86). Immunization against β2-GPI can also enhance atherosclerosis (87). The consequences of endogenous antibody responses are far better understood for IgM antibodies than IgG and other isotypes, as exemplified by the E06 monoclonal phospholipid-reactive IgM (88). It is likely that enhanced clearance of oxLDL, preventing its accumulation in plaque and foam cell formation, is a major mechanism for the action of B1 cell-derived IgM (89). Interestingly, very recent data suggest that excessive IgM accumulation in plaque bound to necrotic and lipid debris could in fact be pro-inflammatory (90), emphasizing the need for further investigation. A major effector pathway for IgG antibodies linked to atherosclerosis are the Fcγ receptors, of which there are both pro-inflammatory activating receptors (I, IIa, III, and IV) and an inhibitory receptor (IIb) (26,91). A pathogenic role for IgG antibodies/immune complexes in atherosclerosis is supported by findings that mice lacking activating, pro-inflammatory Fcγ (IgG) receptors have reduced atherosclerosis (92,93), whereas mice lacking the inhibitory FcγRIIb have enhanced atherosclerosis (94). Immune complexes are known to activate macrophages (95), a central mediator of plaque formation via Fc receptors, and oxLDL immune complexes can promote foam cell formation. Different IgG isotypes have varying affinities for Fc receptors and thus different activatory capacity; for example, IgG2a/c (different strains express a or c isoforms) in mice is highly activatory (26). IgG2c

is mainly induced in association with Th1 type responses such as those which dominate atherosclerosis; accordingly anti-oxLDL IgG2c is commonly found in atherosclerotic mice (46,66,68,92). In addition, the pathogenic or protective role of the targeted antigen (e.g. oxLDL versus heat-shock proteins) could strongly influence the effects of humoral responses against it, i.e. targeting the clearly pro-atherogenic oxLDL autoantigen may well be protective, whereas endogenous responses to distinct autoantigens may in contrast be pathogenic. Thus, the consequences of endogenous IgG responses in atherosclerosis remain ill-defined, and methods to target them specifically must be developed to improve our understanding.

B cell regulation of atherogenic T cells

Results from B2 cell depletion models (66–68) support a role for B2 cell potentiation of pro-atherogenic CD4 + T cell effector responses. Each of these studies was associated with significantly reduced numbers of T cells in plaques as well as systemic decreases in pro-atherosclerotic IFN-γ-producing T cells, whereas complete B cell deficiency results in enhanced plaque T cell levels (65). These B cell-deficient (μMT) mice have defective Th2 (IL-4) differentiation capacity due to an effect on dendritic cells (DCs) (96), which may partly be due to lack of antibody-mediated uptake and lysosomal targeting (97). B cell depletion therapy is also effective in reducing other T cell-dependent chronic diseases such as multiple sclerosis and rheumatoid arthritis (reviewed in (98)). Conventional $CD11c^{hi}$ dendritic cells are now recognized as the primary and non-redundant antigen presenting cells activating, as well as maintaining, naïve T cells. However, macrophages and B cells can also play prominent roles in presenting antigen to effector T cells and influencing the nature (Th1, Th2, Th17, Treg), power, and longevity of effector T cell responses (98). It has not yet been investigated whether MHCII-mediated antigen presentation by B cells is important for their stimulation of pro-atherogenic CD4 + T cell responses. Co-stimulatory molecules, such as CD40 and OX40, or cytokines like TNF or IL-6 are also likely candidates for future investigation. A recent study investigating RP105, a TLR receptor regulator, also supports an important role for TLR-mediated regulation of B cells in atherosclerosis (99).

B cell-derived cytokines and atherosclerosis

Kyaw et al. (67) showed that transferred B2 cells promoted atherosclerosis in T cell (and B cell)-deficient $RAG2^{-/-}/ApoE^{-/-}$ mice, although to a lesser extent than in T cell-sufficient mice. This suggests there are both T cell-dependent and -independent mechanisms involved. Beyond antibodies, B cells are now recognized to modulate immune responses, particularly those initiating in the spleen, through cytokines. For example, B cells are a major source of MCP-3 (Ccl7) in response to myocardial infarction and promote heart injury through monocyte mobilization into the infarct site (31). GM-CSF+ B cells have very recently been reported to be pro-atherogenic by promoting TH1 cells (100). In contrast to these potentially pro-atherogenic B cells, IL-10-producing B cells are likely protective. Although rare 'regulatory' B cell subsets characterized by high CD1d, CD19, and CD5 expression are important IL-10 producers (also called B10 cells) (32), other groups suggest that more common B cell subsets including marginal zone and B1 cells may potentially produce IL-10 given the correct environmental stimuli, such as TLR ligands (35). Indeed, switching B cells between IL-10 and IL-6 production is associated with functional regulation of experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (29). B cells are also reported to express further cytokines such as TNF, which may be important in regulating T cell responses (101). Thus B cell cytokine-mediated regulation of atherosclerosis merits further attention in future studies.

Conclusion

The characterization of heterogeneity within B cells has led to a better appreciation that B cells can play multiple roles in atherosclerosis, with different subsets playing potentially opposing roles. Recent studies in mice have revealed the source of B cell protective immunity as primarily IgM, strongly supported by numerous clinical studies assessing human circulating anti-oxLDL IgM levels, whereas despite multiple lines of indirect evidence the jury is still out on the functional roles of IgG and the nature of pro-atherogenic B cell immunity. Current research is focused on determining through which of the distinct functional pathways B cells regulate atherosclerosis development and whether these can be translated into therapeutic strategies. An important question will be how closely the roles of murine B cell subsets match their human counterparts, given various differences in cell and antibody isotype subsets. Therapeutically, the BAFF/APRIL system may be a fruitful area of research; establishing their role in endogenous atherosclerotic B cell (and potentially other immune cell) responses could be important in both understanding the underlying causes and mechanisms, and in therapeutic interventions, since a number of BAFF and APRIL targeting monoclonal antibodies have been developed and are already in clinical trials for cancer and autoimmune diseases. This is further supported by data showing up-regulated BAFF and APRIL in human plaques (102) and the association of increased BAFF with myocardial infarction (31).

Declaration of interest: The authors report no conflicts of interest.

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