Targeting B Cells in Atherosclerosis
Closing the Gap From Bench to Bedside

Dimitrios Tsiantoulas, Andrew P. Sage, Ziad Mallat, Christoph J. Binder

Abstract—Atherosclerotic plaque formation is strongly influenced by different arms of the immune system, including B lymphocytes. B cells are divided into 2 main families: the B1 and the B2 cells. B1 cells are atheroprotective mainly via the production of natural IgM antibodies that bind oxidized low-density lipoprotein and apoptotic cells. B2 cells, which include follicular and marginal zone B cells, are suggested to be proatherogenic. Antibody-mediated depletion of B cells has become a valuable treatment option for certain autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis that are also characterized by the development of premature atherosclerosis. Thus, B cells represent a novel interesting target for therapeutic modulation of the atherosclerotic disease process. Here, we discuss the effect of different B-cell subsets in experimental atherosclerosis, their mechanism of action as well as potential ways to exploit these findings for the treatment of human disease. (Arterioscler Thromb Vasc Biol. 2015;35:00-00.)

Key Words: antibodies ■ atherosclerosis ■ B lymphocytes ■ B-cell activating factor ■ belimumab ■ cardiovascular diseases ■ rituximab

Immunity and Atherosclerosis
Atherosclerosis is a multifactorial disease with multiple genetic and environmental risk factors and is characterized by the formation of a plaque in the artery wall. Plaque formation is initiated on trapping of low-density lipoproteins (LDL) in the intima where they undergo oxidation and acquire immunogenic properties. The oxidation of LDL results in the generation of many different immunogenic epitopes, termed oxidation-specific epitopes (OSEs), that are recognized by both innate and adaptive immune mechanisms. Monocytes that enter the intima differentiate to macrophages and take up oxidized LDL (oxLDL), which leads to their activation and results in the formation of foam cells. During this process, macrophages are stimulated by lipid-derived danger-associated molecular patterns such as oxidized phospholipids that promote cytokine secretion via scavenger receptor CD36 and TLR signaling and cholesterol crystals, which activate the inflammasome followed by interleukin-1β production.1,2 Plaque inflammation is further amplified and sustained as a result of recruitment/activation of the adaptive immune system and is an important and potentially central driving force in promoting vulnerable plaque features. Plaque rupture results in life-threatening manifestations, such as myocardial infarction and stroke. Surgery and reducing the risk of clotting are powerful end-stage solutions and lipid lowering is an effective preemptive treatment. However, significant risk remains and new strategies to target underlying causes of vulnerable plaque development and rupture are important future goals.3 Although an adaptive immune system is not essential for atherosclerosis to develop,4,5 many studies now demonstrate that it has a diverse range of important site-specific influences on plaque development and inflammation. (Auto)immune reactivity to a range of autoantigens, but most prominently modified LDL, is a mark of human cardiovascular disease and in experimental models plays a significant role in promoting atherosclerotic plaque progression. Atherosclerosis is a distinct case compared with typical autoimmune diseases because (1) the major autoantigen oxLDL is really a modified self-antigen or neo–self-antigen and (2) the oxLDL autoantigen, rather than playing a physiological function, is pathogenic and disease causing. There are also other autoantigens involved, such as heat shock protein 60,6,7 and the impact of other autoimmune diseases in promoting atherosclerosis such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) is well known.8 The role of T cells and interferon-γ–secreting Th1 cells, in particular, as key drivers of plaque inflammation is well documented, and experimental approaches to dampen these responses by enhancing the activity of regulatory T cells.
are being tested. More recently, it was found that B cells could also play both protective and pathogenic roles, and studies from animal models that have been reviewed extensively elsewhere\(^\text{10-11}\) are beginning to dissect the different pathogenic and protective B-cell responses. Here, we will discuss these insights in light of translational aspects (Figures 1 and 2).

**B-Cell Development, Subsets, and Functions**

B cells are defined by their unique expression of surface (B-cell receptors [BCRs]) and secreted (antibody) immunoglobulin, produced from multigenic loci somatically rearranged during B-cell development, giving each B-cell clone a BCR with a different specificity.\(^\text{12,13}\) Two major types of B cells, B1 and B2 cells, develop from hematopoietic stem cells. B1 cells develop from fetal liver hematopoietic stem cells and are subsequently maintained in the periphery via self-renewal, which is dependent on the spleen.\(^\text{14-16}\) B2 cells have a half-life of only a few days and are continually replaced from bone marrow hematopoiesis, developing from hematopoietic stem cells. B1 cells differentiate into antibody-secreting plasma cells, or alternatively resting memory B cells, that respond more rapidly to subsequent antigen encounters. B1 cells are further divided into B1a, which express the CD5 on their surface and B1b cells. Both B1a and B1b cells primarily patrol peritoneal and pleural niches, and form a major (50%) proportion of peritoneal B cells in mice,\(^\text{17}\) but only a minor population (<5%) in the spleen. B2 cells recirculate through the blood and lymphatics, encountering antigens in secondary lymphoid organs: the spleen, lymph nodes, and Peyer patches. Both B1- and B2-derived plasma cells are primarily found in the spleen and bone marrow,\(^\text{17}\) suggesting the existence of common plasma cell niches allowing antibodies quick access to the blood. In cases of chronic inflammation such as atherosclerosis, tertiary lymphoid organs develop adjacent to diseased tissue, the arterial adventitia in the case of atherosclerosis, and may become major sites of adaptive immune activation.\(^\text{18-20}\) It is likely that tertiary lymphoid organs accumulate B cells with relevant antigen specificity,\(^\text{21}\) or B-cell subsets that exhibit specific properties, for example, circulating capacity.\(^\text{22}\)

The workload of responding to different antigens is divided between different B-cell subsets. Responses are traditionally divided into T-cell dependent, those requiring helper T-cell signals (in addition to the antigen and antigen-specific B cell) and T-cell independent responses, with several subtypes of responses within each group now recognized.\(^\text{23}\) B1 cells produce natural antibodies to common microbial epitopes and (neo)self-determinants such as OSEs independent of cognate T-cell help.\(^\text{24}\) Multiple types of T-cell-independent responses are now recognized, including those to TLR ligands such as bacterial polysaccharides. Marginal zone B cells, which differ from other B2 cells in only the final stages of their development also contribute to innate antibody production. They can respond to multiple antigen types and their location in the marginal zone of the spleen provides them with the ability to respond rapidly to...
B Cells Are Modulators of Atherosclerosis

The role of B cells in murine atherosclerosis was first investigated by Caligiuri et al., who showed that accelerated atherosclerosis on splenectomy was reversed by adoptive transfer of splenic B cells isolated from either wild-type or apolipoprotein E-deficient mice (ApoE−/−). Notably, the latter had a stronger atheroprotective effect (below the sham operated mice) indicating that B cells acquire increased or even novel atheroprotective properties in hypercholesterolemic conditions. The results of this study were supported by Major et al., who performed B-cell-deficient (µMT) bone marrow transfer into lethally irradiated LDL receptor-deficient mice (Ldlr−/−) that led to enhanced atherosclerotic plaque formation on atherogenic diet feeding. Collectively these data suggested an overall protective role of B cells in atherosclerosis. However, as described above, B cells are heterogeneous and consist of several cell subsets with different localization properties, activation requirements, survival characteristics, and immunoglobulin secretion profile. Thus, different B-cell subsets may have different or even opposing roles in atherogenesis, and the understanding of this is critical for the optimal development of B-cell–targeting therapies.

We and others have investigated the effect of anti-CD20 antibody treatment in experimental atherosclerosis. Anti-CD20 treatment, which preferentially leads to B2-cell depletion, whereas B1a cells remain nearly intact, reduced
atherosclerosis in atherogenic diet–fed Apoe−/− and Ldlr−/− mice.35,36 In agreement with an effect that depends on B2-cell depletion, adoptive transfer of splenic B2 cells into lymphocyte-deficient Rag2−/−γ-chain−/−ApoE−/− or B-cell-deficient μMT/ApoE−/− mice aggravated atherosclerosis in 2 studies from 1 group, whereas another showed a protective effect37,36 emphasizing the need for alternative models. Further evidence on the proatherogenenic role of B2 cells came from studies on the role of BAFFR deletion in atherosclerosis-prone mice. BAFFR-deficient Apoe−/− mice as well as BAFFR-deficient Ldlr−/− bone marrow chimeric mice, which lack mature B2 cells, developed decreased atherosclerosis.37,38 Similar data were obtained by Kyaw et al39 who treated atherogenic diet–fed Apoe−/− mice with a blocking anti-BAFFR antibody. The mechanism by which B2-cell depletion protects mice from atherosclerosis is not entirely clear. Of note, anti-CD20 treatment failed to protect Western diet–fed Apoe−/− mice that were cotreated with a neutralizing antibody against interleukin-17 suggesting that Th17 responses may be involved in the protective mechanism of anti-CD20 treatment. Moreover, although in anti-CD20-treated mice the prototypic natural IgM antibody T15/E06 that binds oxLDL was largely unaffected, both total and anti–oxLDL-specific IgG titers were dramatically reduced.34 This is particularly interesting, as previous epidemiological and experimental data point to proatherogenenic role of IgG antibodies.40 For example, IgG antibodies to ApoB100 have been suggested to promote atherosclerosis in mice.41 Alternatively, the proatherogenenic role of B2 cells may be because of their capacity for IgE antibody production. IgE antibodies have been shown to be elevated in patients with CHD compared with healthy individuals42 and may be because of their capacity for IgE antibody production.43 The protective role of natural IgM may be to a large extent mediated by the IgM with specificity for OSEs. We have shown previously that a large part of B1–cell–derived natural IgM antibodies is directed against OSE, which are major antigenic determinants on the surface of apoptotic cells and on oxLDL. OSE-specific natural IgM have the potential to neutralize proinflammatory effects of oxLDL, inhibit foam cell formation, and promote clearance of apoptotic cells. A protective role for OSE-specific IgM is also supported by epidemiological data, which show that anti–oxLDL-specific IgM antibodies are inversely associated with CVD adverse effects.44 Thus, strategies that would promote the expansion of atheroprotective natural IgM antibodies may be beneficial in human atherosclerosis.45

The recently identified IRA B cells also play a role in atherosclerosis. IRA B–cell-deficient Ldlr−/− mice, which were generated by reconstitution with GM-CSF and B–cell–deficient bone marrow, developed reduced atherosclerosis in the entire aorta. These mice had a strong reduction in interferon-γ–secreting CD4+ T cells and anti-oxLDL IgG2c-specific antibodies.27 Because IRA B cells are depleted in BAFFR-deficient mice, this could be an alternative mechanism by which neutralization of BAFFR signaling protects from atherosclerosis.

A critical role of B cells in human atherosclerosis has been suggested by the finding that several critical genes involved in survival, proliferation, or activation status of B cells were identified as key drivers of CHD based on an integrated analysis of whole blood gene expression profiles from Framingham Heart Study participants and data from genome-wide association studies.46 In line with this, it has been recently shown that increased numbers of a B-cell subset identified as CD19−CD86+ associate with increased risk for stroke but not with coronary artery disease.47 Thus, developing or exploiting existing therapeutic approaches that modulate the survival or activation status of B cells may provide a novel line of treatment in atherosclerosis.
Targeting B Cells in Atherosclerosis and Myocardial Infarction

B cells along with the antibodies they produce promote the pathology of several autoimmune disorders such as RA and SLE. Interestingly, patients with both RA and SLE are characterized by increased risk of CVD complications, mainly ischemic heart disease, which is associated with the development of premature atherosclerosis. Accelerated atherosclerosis in patients with SLE and RA seems to be independent of classical Framingham risk factors such as age, total cholesterol, high-density lipoprotein, and systolic blood pressure. This suggests that aggravated atherosclerosis in these patients may be a result of increased inflammation and altered immune responses, such as autoantibody production. For example, patients with SLE have been found to develop autoantibodies against ApoAI, which have been associated with acute coronary syndromes.

The development of B-cell–targeting therapeutic agents for RA and SLE has gained a lot of attention in the past years. The first B-cell therapeutic agent that has been approved for clinical use in patients with RA is the anti-CD20 antibody (rituximab). Rituximab cross links the CD20 receptor present on all B cells, leading to Fcy-mediated cell depletion and consequently to decreased immunoglobulin/autoantibody titers. Another B-cell–depleting agent, a blocking antibody against BAFF (belimumab) has been approved by the Food and Drug Administration in 2011 for clinical use in patients with SLE, who have been shown to have increased plasma BAFF levels. Belimumab, which is the first drug approved for SLE in 50 years, blocks soluble BAFF from binding to its receptor (BAFF-R) resulting in apoptosis of mature B cells. Patients with SLE treated with belimumab show an improvement of clinical score, which was associated with reduced B-cell numbers as well as reduced total immunoglobulins and autoantibody titers against dsDNA. As mentioned above, anti-CD20–mediated depletion of B cells as well as BAFFR deficiency or treatment with an anti-BAFFR antibody has been shown to reduce plaque burden in atherosclerosis-prone mice.

We have recently also shown that B-cell–derived CCL7 (MCP-3) drives monocyte mobilization leading to enhanced tissue injury in a mouse model of myocardial infarction. Treatment with an anti-CD20 or an anti-BAFF antibody, which leads to B-cell depletion and B-cell–derived CCL7 reduction, reduced infarct size and improved cardiac remodeling. Thus, it can be speculated that rituximab- or belimumab–treated patients may also have a better outcome on myocardial infarction.

Besides anti-CD20 and anti-BAFF antibodies, additional B-cell–targeting agents are being developed that may have the potential to modulate atherosclerotic lesion formation as well. In line with this, a decoy form of the TACI receptor (TACI-immunoglobulin/Atacicept) has been tested in clinical phase II/III trial as treatment for patients with SLE. The results suggest a protective effect of Atacicept treatment in SLE at a high dose, although the recruitment of patients and treatment in this group was terminated prematurely because of 2 sudden deaths. Combined neutralization of BAFF and APRIL on TACI-Ig treatment results in depletion of plasma cells and mature B cells as well as strong antibody level reduction in mice. Although TACI-Ig could be considered as a therapeutic option in atherosclerosis, given its B-cell depleting properties, one should keep in mind that this treatment also strongly reduces IgM titers, which have a protective effect in atherosclerosis.

Additional B-cell–modulating agents that are tested as treatment for patients with SLE and RA include anti-CD19 and anti-CD22 antibodies. CD19 is a B-cell–specific surface marker and is involved in the formation of the BCR complex as well as in its activation. In contrast to CD20, a subset of plasma cells expresses CD19. Thus, targeting CD19 could also result in depletion of CD19+ antibody-producing plasma cells and in more efficient plasma IgG reduction but, similar to TACI-Ig, anti-CD19 treatment may result in decrease of atheroprotective IgM titers as well. An antibody against CD19 named MDX1342 is in clinical trial as treatment of patients with RA. CD22 is a transmembrane sialglycoprotein and is expressed by the majority of mature B cells and in a negative modulator of BCR signaling. Epratuzumab is a humanized antibody (clinical phase III trial for patients with SLE) that binds CD22 induces its internalization and phosphorylation. Apart from the moderate B-cell–depleting capacity (mainly CD27+ B cells), epratuzumab exhibits immunomodulatory properties such as inhibition of B-cell proliferation, in vitro. In mice, CD22 deficiency results in strongly reduced marginal zone B cells, thus investigation of the impact of CD22 deficiency could help to elucidate the role of different B2 cells in atherosclerosis. Finally, neutralizing IgE antibodies, for example, using omalizumab (an FDA-approved human anti-IgE antibody that neutralizes free IgE antibodies) may be an alternative more specific approach of limiting a B-cell–mediated proatherogenic mechanism in selective settings. Interestingly, IgE antibodies have been shown recently to be involved in the pathogenesis of SLE.

All above-mentioned B-cell–depleting therapeutic approaches are also characterized by the risk of compromising immunity in general with an increased risk of infections and presumably cancer development as well as decreased responsiveness to vaccination. Moreover, different B-cell depletion strategies have also been found to result in different therapeutic efficacy. For example, treatment of patients with SLE with rituximab showed no clinical benefit in 2 double-blind phase II/III clinical trials, despite the fact that it is a efficient B-cell–depleting agent that should be beneficial in patients with SLE given the protective effect of belimumab. One may speculate that interfering with the BAFF-BAFFR signaling results in additional effects on top of B-cell depletion. For example, BAFF stimulation of human monocytes induces surface expression of TACI and promotes cell survival. The effects of anti-CD20 treatment or the consequences of interfering with BAFF-BAFFR signaling on CVD in humans are not known, and only detailed understanding of the role of B cells and the BAFF system will help the identification of the best therapeutic option for CVD.
Summary and Outlook
In addition to the use of genetic models resulting in B-cell deficiencies, the treatment of mouse models of atherosclerosis with B-cell–depleting agents has provided more information on the role of different B cells in plaque formation. For example, anti-CD20 treatment or blockage of the BAFFR signaling pathway that results in B2-cell depletion protects mice from atherosclerosis. It is particularly interesting that similar B-cell depletion strategies are approved as treatments in autoimmune diseases such as SLE and RA that are associated with increased risk of cardiovascular disease because of the accelerated atherosclerosis. Therefore, studies monitoring the effects of rituximab and belimumab treatment in patients with lupus and rheumatoid arthritis contribute to in vivo and adaptive immune responses in advanced mouse atherosclerosis. Circ Res. 2014;114:1772–1787.


Sources of Funding
C.J. Binder is supported by grants of the Austrian Science Fund (SFB 1030 and F54) and the European Union (FP7). A.P. Sage and Z. Mallat are supported by grants from the British Heart Foundation.

Disclosures
None.

References
Significance

It is clear that some B-cell responses promote atherosclerosis, whereas others are protective. Natural antibody secretion from the B1a cell subset is a major protective pathway, but which types of B-cell responses or functions are most pathogenic is unclear. To study this in more detail is critical because (1) understanding the critical components specific to pathogenic B-cell responses will inform future therapeutic strategies against atherosclerosis; (2) B-cell responses are complex and it is important to understand which specific pathways and components are pathogenic rather than protective; (3) many autoimmune disease patients at high risk for cardiovascular disease are being treated by B-cell-targeting therapies; and (4) there are diverse opportunities to target B cells and many existing therapies used in autoimmune diseases and cancer could be translated for use in cardiovascular disease, and the previous successes of this mode of intervention bode well for future therapeutic developments.
Targeting B Cells in Atherosclerosis: Closing the Gap From Bench to Bedside
Dimitrios Tsiantoulas, Andrew P. Sage, Ziad Mallat and Christoph J. Binder

Arterioscler Thromb Vasc Biol. published online October 30, 2014;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/early/2014/10/30/ATVBAHA.114.303569

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/