

1 **Targeting B cells in atherosclerosis: closing the gap from bench to**  
2 **bedside**

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4 Running title: Targeting B cells in atherosclerosis  
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30

31 Abbreviation list: Apoe: apolipoprotein E, LDL: low density lipoprotein, BAFF: B cell activating  
32 factor, BAFFR: B cell activating factor receptor, SLE: systemic lupus erythematosus, RA:  
33 rheumatoid arthritis  
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1 **Abstract**

2 Atherosclerotic plaque formation is strongly influenced by different arms of the immune  
3 system, including B lymphocytes. B cells are divided in two main families, the B1 and the B2  
4 cells. B1 cells are atheroprotective mainly via the production of natural IgM antibodies that  
5 bind oxidized LDL and apoptotic cells. B2 cells, which include follicular and marginal zone B  
6 cells, are suggested to be proatherogenic. Antibody-mediated depletion of B cells has  
7 become a valuable treatment option for certain autoimmune diseases, such as systemic  
8 lupus erythematosus (SLE) and rheumatoid arthritis (RA) that are also characterized by  
9 development of premature atherosclerosis. Thus, B cells represent a novel interesting target  
10 for therapeutic modulation of the atherosclerotic disease process. Here, we discuss the effect  
11 of different of B cell subsets in experimental atherosclerosis, their mechanism of action as  
12 well as potential ways to exploit these findings for the treatment of human disease.

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## 1 Immunity and atherosclerosis

2  
3 Atherosclerosis is a multifactorial disease with multiple genetic and environmental risk factors  
4 and is characterized by formation of a plaque in the artery wall. Plaque formation is initiated  
5 upon trapping of low density lipoproteins (LDL) in the intima where they undergo oxidation  
6 and acquire immunogenic properties. The oxidation of LDL results in the generation of many  
7 different immunogenic epitopes – termed oxidation-specific epitopes - that are recognized by  
8 both innate and adaptive immune mechanisms. Monocytes that enter the intima differentiate  
9 to macrophages and take up oxidized LDL (OxLDL), which leads to their activation and  
10 results in the formation of foam cells. During this process, macrophages are stimulated by  
11 lipid-derived danger associated molecular patterns (DAMPs) such as oxidized phospholipids  
12 that promote cytokine secretion via scavenger receptor CD36 and TLR signaling and  
13 cholesterol crystals, which activate the inflammasome followed by IL-1 $\beta$  production.<sup>1, 2</sup>  
14 Plaque inflammation is further amplified and sustained as a result of recruitment/activation of  
15 the adaptive immune system and is an important and potentially central driving force in  
16 promoting vulnerable plaque features. Plaque rupture results in life threatening  
17 manifestations such as myocardial infarction and stroke. Surgery and reducing the risk of  
18 clotting are powerful end-stage solutions and lipid lowering is an effective preemptive  
19 treatment. However, significant risk remains and new strategies to target underlying causes  
20 of vulnerable plaque development and rupture are important future goals.<sup>3</sup> Although an  
21 adaptive immune system is not essential for atherosclerosis to develop,<sup>4, 5</sup> many studies now  
22 demonstrate that it has a diverse range of important site-specific influences on plaque  
23 development and inflammation. (Auto)immune reactivity to a range of autoantigens, but most  
24 prominently modified low density lipoprotein (LDL), is a mark of human cardiovascular  
25 disease and in experimental models plays a significant role in promoting atherosclerotic  
26 plaque progression. Atherosclerosis is a distinct case compared to typical autoimmune  
27 diseases since 1) the major autoantigen OxLDL is really a modified self antigen or neo-self  
28 antigen, and 2) the OxLDL “auto”-antigen, rather than playing a physiological function, is  
29 pathogenic and disease-causing. There are also other autoantigens involved, such as heat  
30 shock protein 60<sup>6, 7</sup>, and the impact of other autoimmune diseases in promoting  
31 atherosclerosis such as RA and SLE is well known.<sup>8, 9</sup> The role of T cells and IFN $\gamma$ -secreting  
32 Th1 cells in particular as key drivers of plaque inflammation is well documented, and  
33 experimental approaches to dampen these responses by enhancing the activity of regulatory  
34 T cells are being tested. More recently, it was found that B cells could also play both  
35 protective and pathogenic roles, and studies from animal models that have been extensively  
36 reviewed elsewhere,<sup>10, 11</sup> are beginning to dissect the different pathogenic and protective B  
37 cell responses. Here, we will discuss these insights in light of translational aspects.

## 40 B cell development, subsets and functions

41  
42 B cells are defined by their unique expression of surface (B cell receptors) and  
43 secreted (antibody) immunoglobulin, produced from multigenic loci somatically rearranged  
44 during B cell development, giving each B cell clone a BCR with a different specificity<sup>12, 13</sup>.  
45 Two major types of B cells, B1 and B2 cells, develop from hematopoietic stem cells (HSCs).  
46 B1 cells develop from fetal liver HSCs and are subsequently maintained in the periphery via  
47 self-renewal, which is dependent on the spleen.<sup>14-16</sup> B2 cells have a half-life of only a few  
48 days and are continually replaced from bone marrow HSCs. Only B cell clones encountering  
49 antigens, or in some cases in response to innate signals, become activated and persist.  
50 These differentiate into antibody-secreting plasma cells, or alternatively resting memory B  
51 cells, that respond more rapidly to subsequent antigen encounters. B1 cells are further  
52 divided into B1a, which express the CD5 on their surface and B1b cells. Both B1a and B1b  
53 cells primarily patrol peritoneal and pleural niches, and form a major (50%) proportion of  
54 peritoneal B cells in mice,<sup>17</sup> but only a minor population (<5%) in the spleen. B2 cells  
55 recirculate through the blood and lymphatics, encountering antigens in secondary lymphoid

1 organs – the spleen, lymph nodes and Peyer’s patches. Both B1 and B2 derived plasma  
2 cells are primarily found in the spleen and bone marrow,<sup>17</sup> suggesting the existence of  
3 common plasma cell niches allowing antibodies quick access to the blood. In cases of  
4 chronic inflammation such as atherosclerosis, tertiary lymphoid organs (TLOs) develop  
5 adjacent to diseased tissue, the arterial adventitia in the case of atherosclerosis, and may  
6 become major sites of adaptive immune activation.<sup>18-20</sup> It is likely that TLOs accumulate B  
7 cells with relevant antigen specificity,<sup>21</sup> or B cell subsets that exhibit specific properties for  
8 example circulating capacity.<sup>22</sup>

9 The workload of responding to different antigens is divided between different B cell  
10 subsets. Responses are traditionally divided into T cell dependent, those requiring helper T  
11 cell signals (in addition to the antigen and antigen-specific B cell) and T cell independent  
12 responses, with several subtypes of responses within each group now recognized.<sup>23</sup> B1 cells  
13 produce natural antibodies to common microbial epitopes and (neo)self-determinants such  
14 as oxidation-specific epitopes independent of cognate T cell help.<sup>24</sup> Multiple types of T cell-  
15 independent responses are now recognized, including those to TLR ligands such as bacterial  
16 polysaccharides. Marginal zone (MZ) B cells, which differ from other B2 cells in only the final  
17 stages of their development, also contribute to innate antibody production. They can respond  
18 to multiple antigen types and their location in the marginal zone of the spleen provides them  
19 with the ability to respond rapidly to blood-borne antigens.<sup>25</sup> Follicular B2 cells, which form  
20 the majority of recirculating mature B cells, respond to protein antigens presented to them  
21 complexed with Ig or complement and often immobilized on the surface of innate immune  
22 cells. These responses are T cell-dependent. Upon interaction with an antigen-specific T  
23 helper cell at the follicular-T cell zone border of SLOs, B2 cells migrate along with the T cell  
24 into the follicle and proliferate, forming germinal centers, where they undergo antibody  
25 isotype class- switching i.e. from IgM to IgG, IgA or IgE, and affinity maturation through  
26 natural selection by competition for antigen and T cell help.<sup>26</sup> In addition to antibody  
27 secretion, B cells can also be key sources of cytokines and chemokines. Production of GM-  
28 CSF by innate response activator B cells (IRA), a subset related to B1 cells, is important for  
29 dendritic cell activation<sup>27</sup> and B cell secretion of Ccl7 (MCP-3) is a key regulator of monocyte  
30 mobilization after acute myocardial infarction.<sup>28</sup>

31 B1 and B2 cells also display different survival properties. Mature B2 cell survival is  
32 dependent on B cell activating factor receptor (BAFFR) signaling. BAFFR signaling prevents  
33 B2 cell apoptosis by binding the BAFF ligand. BAFF is mainly produced by stromal cells as  
34 well as by macrophages, monocytes, dendritic cells and activated T cells<sup>29, 30</sup> BAFF is  
35 recognized by two other receptors named transmembrane activator and calcium modulator  
36 and cyclophilin ligand interactor receptor (TACI) and B cell maturation antigen (BCMA), both  
37 of which also bind a second ligand of the so called BAFF system, named a proliferation  
38 inducing ligand (APRIL), which has been shown to facilitate IgA class switching<sup>31</sup>. TACI  
39 receptor has been shown to mediate antibody class switching in mature B cells, whereas  
40 BCMA is essential for plasma cell survival. Disruption of BAFFR signaling, for example by  
41 genetic deletion of BAFF or BAFFR, leads to mature B2 cell apoptosis, whereas B1 cell  
42 numbers remain unaltered. Interestingly, IRA B cells are also depleted in BAFFR deficient  
43 mice, despite the fact that they are B1 cell derived.<sup>32</sup>  
44 Our increased understanding of the diversity of B cell functions has reignited research into B  
45 cell regulation of atherosclerosis.

## 46 47 48 **B cells are modulators of atherosclerosis**

49  
50 The role of B cells in murine atherosclerosis was first investigated by Caligiuri et al,  
51 who showed that accelerated atherosclerosis upon splenectomy was reversed by adoptive  
52 transfer of splenic B cells isolated from either wild type or apolipoprotein E deficient mice  
53 (*Apoe*<sup>-/-</sup>). Notably, the latter had a stronger atheroprotective effect (below the sham operated  
54 mice) indicating that B cells acquire increased or even novel atheroprotective properties in  
55 hypercholesterolemic conditions.<sup>33</sup> The results of this study were supported by Major et al

1 who performed B cell deficient ( $\mu$ MT) bone marrow transfer into lethally irradiated low density  
2 lipoprotein receptor deficient mice ( $Ldlr^{-/-}$ ) that led to enhanced atherosclerotic plaque  
3 formation upon atherogenic diet feeding.<sup>34</sup> Collectively these data suggested an overall  
4 protective role of B cells in atherosclerosis. However, as described above, B cells are very  
5 heterogeneous and consist of several cell subsets with different localization properties,  
6 activation requirements, survival characteristics and immunoglobulin secretion profile. Thus,  
7 different B cell subsets may have different or even opposing roles in atherogenesis, and the  
8 understanding of this is critical for the optimal development of B cell targeting therapies.

9 We and others have investigated the effect of anti-CD20 antibody treatment in  
10 experimental atherosclerosis. Anti-CD20 treatment, which preferentially leads to B2 cell  
11 depletion, while B1a cells remain nearly intact, reduced atherosclerosis in atherogenic diet  
12 fed  $Apoe^{-/-}$  and  $Ldlr^{-/-}$  mice.<sup>35, 36</sup> In agreement with an effect that depends on B2 cell depletion,  
13 adoptive transfer of splenic B2 cells into lymphocyte-deficient  $Rag2^{-/-}\gamma$ -chain $^{-/-}Apoe^{-/-}$  or B cell  
14 deficient  $\mu$ MT/ $Apoe^{-/-}$  mice aggravated atherosclerosis in two studies from one group,  
15 whereas another showed a protective effect<sup>22, 36</sup> emphasizing the need for alternative  
16 models. Further evidence on the proatherogenic role of B2 cells, came from studies on the  
17 role of BAFFR deletion in atherosclerosis prone mice. BAFFR deficient  $Apoe^{-/-}$  mice as well  
18 as BAFFR deficient  $Ldlr^{-/-}$  bone marrow chimeric mice, which lack mature B2 cells, developed  
19 decreased atherosclerosis.<sup>37, 38</sup> Similar data were obtained by Kyaw et al who treated  
20 atherogenic diet fed  $Apoe^{-/-}$  mice with a blocking anti-BAFFR antibody.<sup>39</sup> The mechanism by  
21 which B2 cell depletion protects mice from atherosclerosis is not entirely clear. Of note, anti-  
22 CD20 treatment failed to protect western diet fed  $Apoe^{-/-}$  mice that were co-treated with a  
23 neutralizing antibody against IL-17 suggesting that Th17 responses may be involved in the  
24 protective mechanism of anti-CD20 treatment. Moreover, though in anti-CD20 treated mice  
25 the prototypic natural IgM antibody T15/E06 that binds OxLDL was largely unaffected, both  
26 total and anti-OxLDL specific IgG titers were dramatically reduced.<sup>35</sup> This is particularly  
27 interesting, as previous epidemiological and experimental data point to proatherogenic role of  
28 IgG antibodies.<sup>40</sup> For example, IgG antibodies to ApoB100 have been suggested to promote  
29 atherosclerosis in mice.<sup>41</sup> Alternatively, the proatherogenic role of B2 cells may be due to  
30 their capacity for IgE antibody production. IgE antibodies have been shown to be elevated in  
31 CHD patients compared to healthy individuals<sup>42</sup> and to be a prognostic maker for myocardial  
32 infarction in the Helsinki Heart study.<sup>43</sup> Supporting experimental evidence on the  
33 proatherogenic role of IgE antibodies comes from Wang et al who investigated the role of the  
34 high affinity receptor of IgE (Fc $\epsilon$ RI) in atherogenic diet fed  $Apoe^{-/-}$  mice. Fc $\epsilon$ RI deficient  $Apoe^{-/-}$   
35 mice developed reduced atherosclerosis and plaque complexity.<sup>42</sup> Whether these might  
36 constitute an underlying mechanism by which B2 cells promote atherosclerotic plaque  
37 formation remains to be shown.

38 In contrast to Kyaw et al,<sup>36</sup> Doran et al found that adoptive transfer of splenic B2 cells  
39 from  $Apoe^{-/-}$  mice reduced atherosclerosis in cholesterol-fed  $\mu$ MT/ $Apoe^{-/-}$ .<sup>22</sup> Possible  
40 explanations for this discrepancy may include different cell ratio of follicular and MZ B cells or  
41 B2 cell purity of these cell preparations. In addition, Kyaw et al transferred  $5 \times 10^6$  whereas  
42 Doran et al transferred  $3 \times 10^7$  or  $6 \times 10^7$  B2 cells into B cell deficient mice. Based on the fact  
43 that BCR interaction with self antigens strongly controls the developmental fate and survival  
44 of B cells,<sup>44</sup> one may hypothesize that difference in the number of transferred B2 cells into B  
45 cell deficient mice, impacts the way the cells interact with the endogenous self antigen pool.  
46 Thus, dependent on their numbers, transferred B cells may acquire distinct phenotypes and  
47 undergo distinct responses to those normally occurring in B cell-sufficient mice. Notably,  
48 Doran et al suggest that local B cell responses in the adventitia of affected arteries, at least  
49 after B cell transfer, may be protective, whereas the localization of the pathogenic transferred  
50 B cells in two other studies was not defined. In conclusion B2 cells seem to be  
51 proatherogenic though additional studies on the role of each B2 cell subset would provide  
52 more conclusive evidence on their role in atherosclerosis.

53 On the other hand, the data on B1a cells are more robust and suggest a strong  
54 atheroprotective role. Kyaw et al showed that splenectomy of  $Apoe^{-/-}$  mice - which results in  
55 accelerated atherosclerosis - leads to nearly 50% reduction of B1a cells in the peritoneum

1 followed by a strong decrease in plasma IgM titers. Moreover, adoptive transfer of peritoneal  
2 B1a cells into splenectomized *ApoE*<sup>-/-</sup> recipients fed an atherogenic diet, reduced  
3 atherosclerosis even beyond the disease-accelerating effect resulting from splenectomy.  
4 This was dependent on the capacity of B1a cells to secrete natural IgM antibodies as there  
5 was no protective effect when the splenectomized *ApoE*<sup>-/-</sup> mice received B1a cells isolated  
6 from secreted IgM deficient donor mice.<sup>16</sup> Natural IgM antibodies (NAbs) have been shown to  
7 be atheroprotective.<sup>40</sup> Lewis et al, demonstrated that *slgM*<sup>-/-</sup> crossed onto *Ldlr*<sup>-/-</sup> background  
8 develop strongly accelerated atherosclerosis when fed regular chow or atherogenic diet.<sup>45</sup>  
9 The atheroprotective capacity of natural IgM may be to a large extent mediated by the IgM  
10 with specificity for oxidation-specific epitopes (OSE). We have previously shown that a large  
11 part of B1 cell derived natural IgM antibodies is directed against OSE, which are major  
12 antigenic determinants on the surface of apoptotic cells and on OxLDL. OSE-specific natural  
13 IgM have the potential to neutralize proinflammatory effects of OxLDL, inhibit foam cell  
14 formation and promote clearance of apoptotic cells. A protective role for OSE-specific IgM is  
15 also supported by epidemiological data, which show that anti-OxLDL specific IgM antibodies  
16 are inversely associated with CVD adverse effects.<sup>40</sup> Thus strategies that would promote the  
17 expansion of atheroprotective natural IgM antibodies may be beneficial in human  
18 atherosclerosis.

19 The recently identified IRA B cells<sup>32</sup> also play a role in atherosclerosis. IRA B cell  
20 deficient *Ldlr*<sup>-/-</sup> mice, which were generated by reconstitution with GM-CSF and B cell  
21 deficient bone marrow, developed reduced atherosclerosis in the entire aorta. These mice  
22 had a strong reduction in IFN $\gamma$  secreting CD4<sup>+</sup> T cells and anti-OxLDL IgG2c specific  
23 antibodies.<sup>27</sup> Because IRA B cells are depleted in BAFFR deficient mice,<sup>32</sup> this could be an  
24 alternative mechanism by which neutralization of BAFFR signaling protects from  
25 atherosclerosis.

26 A critical role of B cells in human atherosclerosis has been suggested by the finding  
27 that several critical genes involved in survival, proliferation or activation status of B cells,  
28 were identified as key drivers of CHD based on an integrated analysis of whole blood gene  
29 expression profiles from Framingham Heart Study participants and data from genome wide  
30 association studies (GWAS).<sup>46</sup> In line with this it has recently been shown that increased  
31 numbers of a B cell subset identified as CD19<sup>+</sup>CD86<sup>+</sup> associate with increased risk for stroke  
32 but not with coronary artery disease.<sup>47</sup> Thus, developing or exploiting existing therapeutic  
33 approaches that modulate the survival or activation status of B cells may provide a novel line  
34 of treatment in atherosclerosis.

## 37 Targeting B cells in atherosclerosis and myocardial infarction

38  
39 B cells along with the antibodies they produce promote the pathology of several  
40 autoimmune disorders such as rheumatoid arthritis (RA) and systemic lupus erythematosus  
41 (SLE).<sup>48</sup> Interestingly, both RA and SLE patients are characterized by increased risk of CVD  
42 complications, mainly ischemic heart disease, which is associated with the development of  
43 premature atherosclerosis.<sup>49</sup> Accelerated atherosclerosis in SLE and RA patients seems to  
44 be independent of classical Framingham risk factors such as age, total cholesterol, HDL and  
45 systolic blood pressure. This suggests that aggravated atherosclerosis in these patients may  
46 be a result of increased inflammation and altered immune responses, such as autoantibody  
47 production. For example, SLE patients have been found to develop autoantibodies against  
48 apolipoprotein A-I, which have been associated with acute coronary syndromes.<sup>9</sup>

49 The development of B cell targeting therapeutics for RA and SLE has gained a lot of  
50 attention in the last years. The first B cell therapeutic agent that has been approved for  
51 clinical use in RA patients is the anti-CD20 antibody (Rituximab). Rituximab cross links the  
52 CD20 receptor present on all B cells, leading to Fc $\gamma$  mediated cell depletion and  
53 consequently to decreased immunoglobulin/autoantibody titers.<sup>50</sup> Another B cell depleting  
54 agent, a blocking antibody against BAFF (Belimumab) has been approved by the FDA in  
55 2011 for clinical use in SLE patients, who have been shown to have increased plasma BAFF

1 levels.<sup>30</sup> Belimumab, which is the first drug approved for SLE in 50 years, blocks soluble  
2 BAFF from binding to its receptor (BAFFR) resulting in apoptosis of mature B cells. SLE  
3 patients treated with Belimumab show an improvement of clinical score<sup>51</sup>, which was  
4 associated with reduced B cell numbers as well as reduced total immunoglobulins and  
5 autoantibody titers against dsDNA.<sup>52</sup> As mentioned above, anti-CD20 mediated depletion of  
6 B cells as well as BAFFR deficiency or treatment with an anti-BAFFR antibody has been  
7 found to reduce plaque burden in atherosclerosis prone mice.

8 We have recently also shown that B cell derived CCL7 (MCP-3) drives monocyte  
9 mobilization leading to enhanced tissue injury in a mouse model of myocardial infarction.  
10 Treatment with an anti-CD20 or an anti-BAFF antibody which lead to B cell depletion and B  
11 cell derived CCL7 reduction, reduced infarct size and improved cardiac remodeling.<sup>28</sup> Thus, it  
12 can be speculated that Rituximab or Belimumab treated patients may also have a better  
13 outcome upon myocardial infarction.

14 Besides anti-CD20 and anti-BAFF antibodies, additional B cell targeting agents are  
15 being developed that may have the potential to modulate atherosclerotic lesion formation as  
16 well. In line with this, a decoy form of the TACI receptor (TACI-Ig/Atacicept) has been tested  
17 in clinical phase II/III trial as treatment for SLE patients. The results suggest a protective  
18 effect of Atacicept treatment in SLE at a high dose, though the recruitment of patients and  
19 treatment in this group was terminated prematurely due to two sudden deaths.<sup>53</sup> Combined  
20 neutralization of BAFF and APRIL upon TACI-Ig treatment, results in depletion of plasma  
21 cells and mature B cells as well as strong antibody level reduction in mice.<sup>54, 55</sup> Although  
22 TACI-Ig could be considered as a therapeutic option in atherosclerosis, given its B cell  
23 depleting properties, one should keep in mind that this treatment also strongly reduces IgM  
24 titers<sup>54</sup>, which have a protective effect in atherosclerosis.

25 Additional B cell modulating agents that are tested as treatment for SLE and RA  
26 patients include anti-CD19 and anti-CD22 antibodies. CD19 is a B cell specific surface  
27 marker and is involved in the formation of the B cell receptor complex as well as in its  
28 activation.<sup>56</sup> In contrast to CD20, a subset of plasma cells expresses CD19. Thus, targeting  
29 CD19 could also result in depletion of CD19<sup>+</sup> antibody producing plasma cells and in more  
30 efficient plasma IgG reduction but -similar to TACI-Ig-, anti-CD19 treatment may result in  
31 decrease of atheroprotective IgM titers as well. An antibody against CD19 named MDX1342  
32 is in clinical trial as treatment of RA patients.<sup>57</sup> CD22 is a transmembrane sialoglycoprotein  
33 and is expressed by the majority of mature B cells and is a negative modulator of B cell  
34 receptor signaling. Epratuzumab is a humanized antibody (clinical phase III trial for SLE  
35 patients) that binds CD22 induces its internalization and phosphorylation. Apart from the  
36 moderate B cell depleting capacity (mainly CD27<sup>-</sup> B cells), Epratuzumab exhibits  
37 immunomodulatory properties such as inhibition of B cell proliferation, *in vitro*.<sup>57, 58</sup> In mice,  
38 CD22 deficiency results in strongly reduced MZ B cells,<sup>59</sup> thus investigation of the impact of  
39 CD22 deficiency could help to elucidate the role of different B2 cells in atherosclerosis.  
40 Finally, neutralizing IgE antibodies for example by using Omalizumab (an FDA approved  
41 human anti-IgE antibody that neutralizes free IgE antibodies) may be an alternative more  
42 specific approach of limiting a B cell mediated pro-atherogenic mechanism in selective  
43 settings. Interestingly, IgE antibodies have been recently shown to be involved in the  
44 pathogenesis of SLE<sup>60</sup>.

45 All above mentioned B cell depleting therapeutic approaches are also characterized  
46 by the risk of compromising immunity in general with an increased risk of infections and  
47 presumably cancer development as well as decreased responsiveness to vaccination.  
48 Moreover, different B cell depletion strategies have also been found to result in different  
49 therapeutic efficacy. For example, treatment of SLE patients with Rituximab showed no  
50 clinical benefit in two double blind phase II/III clinical trials, despite the fact that it is a very  
51 efficient B cell depleting agent that should be beneficial in SLE patients given the protective  
52 effect of Belimumab. One may speculate that interfering with the BAFF-BAFFR signaling  
53 results in additional effects on top of B cell depletion. For example, BAFF stimulation of  
54 human monocytes, induces surface expression of TACI and promotes cell survival.<sup>61</sup> The  
55 effects of anti-CD20 treatment or the consequences of interfering with BAFF-BAFFR

1 signaling on CVD in humans are not known, and only detailed understanding of the role of B  
2 cells and the BAFF system will help the identification of the best therapeutic option for CVD.

### 4 **Summary and Outlook**

6 In addition to the use of genetic models resulting in B cell deficiencies, the treatment  
7 of mouse models of atherosclerosis with B cell depleting agents has provided more  
8 information on the role of different B cells in plaque formation. For example, anti-CD20  
9 treatment or blockage of the BAFFR signaling pathway that results in B2 cell depletion  
10 protects mice from atherosclerosis. It is particularly interesting that similar B cell depletion  
11 strategies are approved as treatments in autoimmune diseases such as SLE and RA that are  
12 associated with increased risk of cardiovascular disease due to the development of  
13 accelerated atherosclerosis. Therefore, studies monitoring the effects of Rituximab and  
14 Belimumab treatment (and of other B cell targeting agents that are being developed) on CVD  
15 would be highly important for the understanding of the role of B cells in human atherogenesis  
16 and the potential of B cell targeting therapeutic strategies.

### 18 **Significance**

19 It is clear that some B cell responses promote atherosclerosis whereas others are  
20 protective. Natural antibody secretion from the B1a cell subset is a major protective pathway,  
21 but which types of B cell responses or functions are most pathogenic is unclear. To study this  
22 in more detail is critical since:

- 23 • Understanding the critical components specific to pathogenic B cell responses will inform  
24 future therapeutic strategies against atherosclerosis.
- 25 • B cell responses are complex and it is important to understand which specific pathways  
26 and components are pathogenic rather than protective.
- 27 • Many autoimmune disease patients at high risk for cardiovascular disease are being  
28 treated by B cell-targeting therapies.
- 29 • There are diverse opportunities to target B cells and many existing therapies used in  
30 autoimmune diseases and cancer could be translated for use in cardiovascular disease,  
31 and the previous successes of this mode of intervention bode well for future therapeutic  
32 developments

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## Figure Legends

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20 **Figure 1. Role of different B cell subsets in murine atherosclerosis.** B cells consist of  
21 two major cell subsets, named B1 and B2 cells, which are characterized by different  
22 localization properties and activation requirements. B1 cells reside predominately in the  
23 peritoneum and are sub-divided into B1a and B1b. B1a cells protect from atherosclerosis.  
24 Their atheroprotective properties depend mainly on the capacity to produce OxLDL specific  
25 natural IgM antibodies – which can be enhanced by IL-5 stimulation - that block OxLDL  
26 uptake and foam cell formation. In addition natural IgM have also been shown to promote  
27 apoptotic cell clearance. The role of B1b in atherosclerosis remains still elusive. B2 cells are  
28 mainly found in the spleen and consist of marginal (MZ) and follicular (FO) B cells. B2 cells  
29 seem to be proatherogenic. Although the underlying mechanism is yet to be identified, this  
30 may include production of proatherogenic IgG and IgE antibodies. Innate response activator  
31 B cells (IRA), which are characterized by GM-SCF secretion, promote the expansion of  
32 IgG2c anti-OxLDL antibodies and aggravate atherosclerosis. Mph: macrophage, BCR: B cell  
33 receptor, TCR: T cell receptor, BAFFR: B cell activating factor receptor, DC: dendritic cell,  
34 MHC: major histocompatibility complex.

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36 **Figure 2. Biologicals that target B cells and their effect on murine atherosclerosis.**  
37 Anti-CD20 antibodies cross-link the surface CD20 receptor on B cells, resulting in Fcγ  
38 mediated depletion of predominantly B2 cells. Plasma IgM titers are moderately reduced  
39 whereas IgG titers are strongly decreased following anti-CD20 treatment. Atherosclerosis-  
40 prone mice injected with anti-CD20 antibody develop reduced atherosclerotic plaque size. In  
41 contrast to B1 cells, B cell activating factor (BAFF) signaling via the B cell activating factor  
42 receptor (BAFFR) is essential for B2 cell survival. Treatment with a neutralizing antibody  
43 against BAFF leads to B2 cell apoptosis followed by strong reduction in plasma IgG titers.  
44 Combined neutralization of BAFF and a proliferation inducing ligand (APRIL) upon treatment  
45 with the extracellular domain of the transmembrane activator and calcium modulator and  
46 cyclophilin ligand interactor receptor (TACI) fused to an Ig backbone (TACI-Ig) results in  
47 reduced numbers of B2 and to lesser extent of B1 cells. Moreover, TACI-Ig treatment inhibits  
48 plasma cell survival followed by reduction in total Ig plasma titers. The effect of both anti-  
49 BAFF and TACI-Ig treatment in atherosclerosis remains to be shown. Finally, IgE antibodies  
50 have been suggested to promote atherosclerotic plaque development and to correlate with  
51 CVD risk. The effect of IgE antibody blockage via anti-IgE treatment in atherosclerosis is yet  
52 to be investigated. IRA: innate response activator B cells.

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