B cell modulation in atherosclerosis

Douna, H.

Novel B cell subsets in atherosclerosis

Current Opinion in Lipidology; 2016, Volume 27 (5), p 493–498

[1]Hidde Douna
[1]Johan Kuiper

[1]*Division of Biotherapeutics, LACDR, Leiden University, Leiden, The Netherlands*

Abstract

Purpose of review

The number of deaths associated with cardiovascular disease remains high, despite great advances in treating the associated high levels of cholesterol. The main underlying pathology of cardiovascular disease is atherosclerosis, which is recognized as a chronic autoimmune-like inflammatory disease. Hence, there is a pressing need to shed light on the immune pathways associated with atherosclerosis. B cells have long been thought to have a general protective effect in atherosclerosis. However, findings in the last decade have challenged this paradigm, showing that it is crucial to differentiate between the various B cell subsets when assessing their effect on atherosclerosis.

Recent findings

It has become increasingly recognized that B cells can have significant effects on the immune system independent of antibody production. The understanding that B cells form a major source of cytokines and can directly influence T cell responses via surface markers, have led to the identification of novel B cell subsets. These subsets are important modulators of auto-immune disorders but have not yet been fully investigated in atherosclerosis.

Summary

Here we review the current known roles of B cell subsets and the putative effects of recently identified B cells on atherosclerosis.

Introduction

Atherosclerosis has long been established as a chronic autoimmune-like inflammatory disease. Substantial work in the last years has identified a broad spectrum of immune cells and immunomodulatory checkpoints that can have a significant contribution to the onset and/or progression of atherosclerosis. Immune cells such as macrophages, monocytes, neutrophils, dendritic cells and T cells have been extensively investigated and as such their impact on atherosclerosis has been illuminated to a great extent.

In addition, already in 2002 it was shown that B cells can also have a substantial impact on the pathophysiology of atherosclerosis[1,2]. Two independent studies showed a general atheroprotective effect on atherosclerosis. Since then, the number of studies on B cells in atherosclerosis has somewhat waned compared to the number of papers examining T cells. However, the discovery that different B cell subsets can have opposing effects on atherosclerosis has led to a resurge in interest in B cells and atherosclerosis in recent years. As a result, the two main B cell lineages and their effects on atherosclerosis are now understood to a certain extent. In contrast, novel B cell subsets have been recently identified which could have great potential in atherosclerosis and have not yet been fully explored.

In this review we briefly discuss the current understanding of the role of classical B cells and the putative contribution of novel B cell subtypes to atherosclerosis.

B1 and B2 and antibody production

Broadly, B cells can be divided into two lineages that differ greatly in ontogeny, function, location and surface marker expression: B1 and B2 cells (Fig. 1). B1 cells are the dominant type of B cell in neonates and originate mainly from fetal liver and omentum. In adult life, they reside mostly in pleural and peritoneal cavities and respond to self- and T-independent antigens by secreting polyreactive natural IgM antibodies. Based on the expression of the surface marker CD5, B1 cells can be further subdivided in B1a and B1b cells. Alternatively, the large majority of B cells in the adult is comprised of B2 cells that include the conventional follicular B cells and marginal zone B cells. The latter are often considered as part of the innate immune system, since they rapidly respond to blood-borne antigens, including bacterial cell wall components and self-antigens, with polyreactive antibodies. However, the largest population of splenic B cells consists of follicular B cells that undergo isotype

switching in germinal centers in response to T-cell dependent antigens and either give rise to memory B cells or antibody secreting plasma cells.

In 2002, two groups provided the first compelling evidence on the modulatory properties of B cells in atherosclerosis[1,2]. Initially, these two corroborating studies led to the idea of B cells having an overall atheroprotective effect. However, later studies

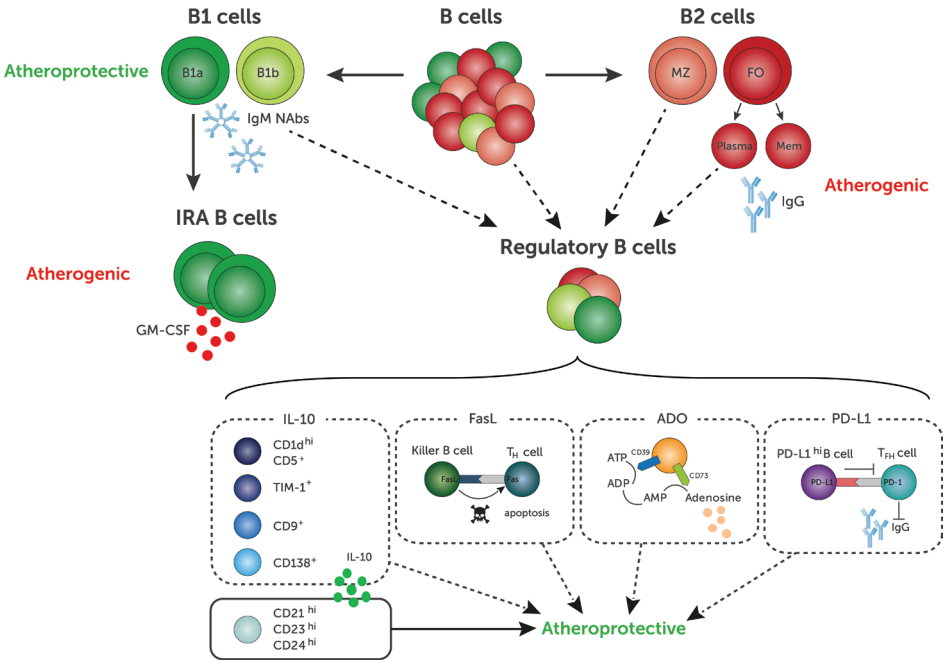

Figure 1. Overview of the main B cell lineages and novel B cell subtypes with their known and putative effects on atherosclerosis. B cells can be divided into two main lineages; B1 and B2 cells. B1 cells can be further divided into B1b and B1a B cells that both attenuate atherosclerosis by the secretion of natural IgM antibodies (IgM NAbs). IRA B cells are derived from B1a cells and are characterized by GM-CSF production by which they promote inflammation in the spleen, which results in increased atherosclerosis. Conventional B2 cells, including marginal zone (MZ) and follicular (FO) B cells, are generally believed to have an atherogenic effect, which may be caused by their IgG secretion. Regulatory B cells (B_{regs}) negatively influence the immune response and hence could have potential atheroprotective effects. They share markers with many other B cell subsets and it remains unknown if they develop from a specific type of B cell or if all B cells can become regulatory when provided with the right immunological context. The majority of B_{regs} are dependent on IL10 production and many different markers have been ascribed to these cells, such as $CD1d^{hi}CD5^{+}$, $TIM\text{-}1^{+}$, $CD9^{+}$, $CD138^{+}$ and $CD21^{hi}CD23^{hi}CD24^{hi}$ B cells. Only the latter have already been reported to protect against atherosclerosis. Killer B cells can induce apoptosis in T_H cells by their expression of FASL. B cells expressing CD39 and CD73 can drive the purinergic response from the ATP to ADP and adenosine that is associated with atheroprotection. B cells expressing high levels of PD-L1 can inhibit the T_{FH}-germinal center axis and decrease the secretion of high-affinity IgG antibodies. *Dotted lines/arrows depict not yet determined roles in atherosclerosis.

challenged this paradigm by pointing out major differences between B1 and B2 cells in modulating atherosclerosis[3–5]. Currently, the evidence for a protective function of B1a cells is firmly established[5–8]. There is a widespread agreement that B1 cells protect against atherosclerosis due to the production of natural IgM antibodies that induce the clearance of oxidized low-density lipoproteins (oxLDL) and apoptotic cells[9–11]. In addition, it was recently shown that B1b cells contain similar atheroprotective characteristics by secreting natural antibodies[12]. On the other hand, there is an abundance of data claiming an atherogenic effect of B2 cells[3,4,13,14]. The most important evidence for this statement is provided by depletion studies, which show that selective depletion of B2 cells limits the development of atherosclerosis[3,4,13,14]. One reason for this is that the antibodies secreted by B2 cells are usually of the IgG isotype which are, in contrast to IgM antibodies, often associated with aggravated atherosclerosis. However, the understanding about the effects of different isoforms of IgG is still very limited[15]. In addition, the exact influence of B2 cells on atherosclerosis remains debatable, since conflicting evidence shows that adoptively transferred B2 cells can also reduce lesion size[16,17]. The main difference between these apparent contradictory studies is the number and genotype of adoptively transferred B cells, $5x10^6$ B cells from C57BL/6J (BL/6) mice versus $30x10^6$ B cells from apolipoprotein E knockout (*apoE*$^{-/-}$) mice[4,16]. This discrepancy points out the importance of taking into consideration that not all B2 cells are similar and that the source and number of cells used can have a major influence on the development of atherosclerosis.

In recent years, it has been shown that within the B1 and B2 lineages additional B cell subsets can be identified (Fig. 1). While most papers discussing B1 and B2 cells often emphasize their antibody producing capacities, the majority of these novel B cell subsets are either identified based on secretion of specific cytokines or surface marker expression. These B cells can modulate immune responses by altering the cytokine network or directly altering T cell responses via co-receptor expression. These emerging concepts play major roles in auto-immune disorders, but are not yet fully appreciated in the context of atherosclerosis.

IL-10 dependent regulatory B cells

One major group of B cells that has seen a lot of attention in the last decade is that of regulatory B cells ($B_{regs,}$ Fig. 1). In general, B cells that negatively modulate the immune response are considered B_{regs} and the majority of these B cells depend in one way or another on the secretion of interleukin-10 (IL-10)[18]. IL-10 is an extensively studied

anti-inflammatory cytokine with well-known anti-atherogenic properties[19–21]. It is primarily secreted by macrophages and T cells and protects against atherosclerosis in several ways, including inhibition of macrophage and T cell activation but it also has impact on lipid metabolism[21,22]. It is now known that B cells are also capable in regulating auto-immune disorders via secretion of IL-10[23], but this concept has only recently been investigated in atherosclerosis[24,25]. Using a chimeric system to generate *Ldlr*$^{-/-}$ mice with a B cell specific deficiency in IL-10, Sage et al. showed that a loss of B cell-derived IL10 unexpectedly did not result in a significant effect on plaque size[24]. A second study, however, showed that B-cell derived IL-10 is actually capable of reducing atherosclerosis development in *apoE*$^{-/-}$ mice[25]. Strom et al. reported a significant increase in IL10^{+} B cells in the lymph nodes of female *apoE*$^{-/-}$ mice compared to BL/6 mice. Subsequent adoptive transfer of these lymph node B cells into *apoE*$^{-/-}$ mice protected them against collar-induced neo-intima formation. This effect was IL10-dependent since concurrent injection with an IL-10 neutralizing antibody abrogated the effects. Similarly, adoptive transfer of IL-10$^{-/-}$ B cells failed to protect against atherosclerosis[25]. The apparent discrepancy between these two studies can in part be explained by their different experimental approaches (e.g. *apoE*$^{-/-}$ vs. *Ldlr*$^{-/-}$ or chow diet vs. high-fat diet). However, another possibility is that IL10-producing B cells or the CD21^{hi}CD23^{hi}CD24^{hi} B cell population as used by Strom et al. does not exclusively secrete IL-10. Two recent papers elegantly show that IL-35 is able to induce IL-10-producing B cells which in turn also secrete large amounts of IL-35 that can suppress autoimmune disease[26,27]. As suggested by others, there could be a complex interplay between IL-35 and IL-10 in mediating immunosuppression[28]. Thus, silencing only one of these cytokines could lead to compensatory mechanisms resulting in upregulation of the cytokine by other cell types or the induction of other anti-inflammatory cytokines. For instance, in the aorta of mice deficient for B cell derived IL-10 a significant increase in IL-10 gene expression was found, while IL-35 or transforming growth factor β were not measured[24]. Moreover, the IL-10 blocking antibody used by Strom et al. did not neutralize the decrease in lesional macrophages, again displaying only a partial response of IL-10-mediated immune suppression[25].

Adding to the complexity is the lack of a unifying surface marker or transcription factor to identify IL-10 producing B cells. Currently, there is a growing collection of literature that identifies different B cell subsets in which IL10-producing B cells are enriched[29–33]. Many of these subsets are frequently used as biomarker for IL10-producing B cells or for adoptive transfer experiments. Since these subsets differ largely in their surface marker expression and also in the actual number of IL-10 producing B cells, the research into the exact contribution of B cell derived IL-10 to

atherosclerosis and other auto-immune disorders remains a challenge. It will thus be interesting to examine the effects of adoptive transfer studies using pure IL-10 producing B cells. In addition, studies that shed light on the complex network of multiple immune-suppressive B cell derived cytokines are warranted to fully understand the effects of IL-10 secreting B cells in atherosclerosis.

Other regulatory B cells

While typically regulatory B cells are associated with IL-10 producing B cells, the family of regulatory B cells has greatly expanded since the term was claimed[18]. It now also includes B cells expressing specific surface markers that can negatively regulate the immune response independent of IL-10 (Fig. 1).

For instance, Khan et al. identified a unique B cell subset that highly expresses programmed death ligand 1 (PD-L1)[34]. These PD-L1^{hi} B cells are potent regulators of T cell responses both *in vitro* and *in vivo*. In particular the expansion of follicular helper T cells (T_{FH}) that express the receptor for PD-L1 (PD-1) is negatively regulated by PD-L1^{hi} B cells[34]. T_{FH} cells are important modulators of the humoral immune response since they trigger and maintain the development of germinal centers. It has recently been demonstrated that this T_{FH}-germinal center axis develops during atherosclerosis, including isotype switching of antibodies from IgM to IgG[35]. Blockade or promotion of this axis results in attenuated or enhanced atherosclerosis in *apoE*$^{-/-}$ mice, respectively[35]. Since the PD-1/PD-L1 pathway has also shown its potential in modulating atherosclerosis, it will be very interesting to investigate the effects of the PD-L1^{hi} B cells in this context [36–38].

Another B cell subtype that has been shown to negatively regulate T cell responses is the so called killer B cell[39]. This type of B cell expresses Fas ligand (FasL, CD178) which is usually found on cytotoxic CD8$^+$ T cells and natural killer (NK) cells. Binding of FasL to its receptor Fas (CD95) leads to apoptosis in target cells. FasL$^+$ B cells are mainly found within the splenic CD5$^+$ population and can be induced by CD40 stimulation and IL-5[40]. Killer B cells are able to induce T_H cell apoptosis and promote tolerance[39,41], which are both crucial processes in treating atherosclerosis. In addition, soluble levels of FasL are clinically associated with a lower risk of cardiovascular disease[42,43]. Although it has not been directly shown that killer B cells secrete FasL, it remains of interest to study their influence on the pathophysiology of atherosclerosis.

A third type of B cell that has been reported to exert immune suppression depends on the ectoenzymes CD39 and CD73[44]. Both enzymes work towards the removal of extracellular ATP and increasing the level of extracellular adenosine (ADO), which is associated with atheroprotection[45]. CD39 is expressed on all B cells, but the expression of CD73 is restricted to a specific subset of B1 cells. Sorting of $CD73^-$ and $CD73^+$ B cells showed that only the latter were able to protect against experimental inflammatory colitis[44]. Ablation of CD73 results in increased neo-intima formation in a wire injury model[46] and increased atherosclerosis in *apoE*$^{-/-}$ mice[47]. Since these studies used total knockouts of CD73, the role of CD73 and/or CD39 on B cells in atherosclerosis has not yet been elucidated.

Innate response activator (IRA) B cells

In contrast to B_{regs}, additional B cell subsets have been identified that predominantly exert pro-inflammatory functions (Fig. 1). IRA B cells were identified as a unique B cell subset regarding their ontology, development and function. They are particularly characterized by the secretion of granulocyte macrophage colony stimulating factor (GM-CSF)[48]. GM-CSF is a pleiotropic cytokine that was first thought to be exclusively secreted by non-hematopoietic cells, macrophages and T cells. However, in response to LPS or sepsis a small population of peritoneal B1a cells is able to relocate to the spleen, secrete GM-CSF and hence protect against bacterial infection[48]. Several studies have been performed using total GM-CSF knockout mice on an apoE- or Ldlr-deficient background to examine the contribution of GM-CSF to atherosclerosis, but the results remain somewhat ambiguous. Gm-csf/apoE double knockout mice displayed an increase in atherosclerosis[49], while gm-csf/Ldlr double knockout mice showed no difference[50] or a sex-related reduction in plaque size (i.e. only in female mice)[51]. These conflicting results highlight the complex immune pathways of this cytokine and suggest that the context, timing and cellular source of GM-CSF might be important for the regulation of atherosclerosis.

Recently, it was shown by Hilgendorf et al. that during atherosclerosis progression, a small population of IRA B cells develops in the spleen[52]. Notably, they did not find any IRA B cells within the aorta, suggesting that B cells do not contribute to aortic GM-CSF and that they exert their effects indirectly. This was confirmed by showing that an increased expression of GM-CSF in the spleen during atherosclerosis progression resulted in an expansion of conventional $CD11c^+$ dendritic cells in the spleen. These in turn stimulated the proliferation of effector T cells and skewed them towards a Th1-dominant phenotype with a concomitant increase in antibodies

of the IgG2c isotype. This unfavorable immune response initiated by IRA B cells eventually culminated in an increase in atherosclerotic lesion size in *Ldlr*$^{-/-}$ mice[52]. Although this study elegantly showed that IRA B cells harbor atherogenic properties in the spleen of *Ldlr*$^{-/-}$ mice, it does not account for the differences found in the complete GM-CSF knock-out models between *Ldlr*$^{-/-}$ and *apoE*$^{-/-}$ mice. Since the increase in IRA B cells was most prominent in the *apoE*$^{-/-}$ mouse model, it would be expected that the contribution of B cells to the total levels of GM-CSF would be maximal in these mice. Along that line, *gm-csf*$^{-/-}$*apoE*$^{-/-}$ mice would most likely demonstrate a decrease in lesion size, however the opposite has been reported[49]. On the other hand, it could be suggested that since women are better protected against sepsis than men[53], they naturally contain more IRA B cells, which could at least partially explain the sex-difference found between females and males in *gm-csf*$^{-/-}$ *Ldlr*$^{-/-}$ mice[51].

Taken together, the study by Hilgendorf et al. was one of the first to report the effects of specific cytokine producing B cells on atherosclerosis, underscoring the immune-modulating effect B cells can have during atherosclerosis besides antibody production[52].

Concluding remarks

B cells can have a tremendous impact on atherosclerosis, however it is crucial to differentiate between the different B cell subsets regarding their effects on atherosclerosis. The increased knowledge on other B cell subtypes than B1/B2 and the additional roles besides antibody production in recent years, has also improved the understanding of B cells in cardiovascular disease. However, one drawback is that most subsets share surface markers, proving it difficult to pinpoint the individual effects to a specific subtype of B cells. In terms of therapeutic potential, the frequently used option of global B cell depletion with CD20 antibodies might not be the most favorable option. Ideally, treatments that either selectively induce protective B cells or limit the effects of atherogenic B cells could offer more potential.

In conclusion, there still is a lot to gain in exploring and harvesting the potential of B cells in the field of atherosclerosis.

References

1. Caligiuri G, Nicoletti A, Poirier B, Hansson GK. Protective immunity against atherosclerosis carried by B cells of hypercholesterolemic mice. *J Clin Invest* 2002;**109**:745–753.
2. Major AS, Fazio S, Linton MF. B-Lymphocyte Deficiency Increases Atherosclerosis in LDL Receptor–Null Mice. *Arterioscler Thromb Vasc Biol* 2002;**22**:1892–1898.
3. Ait-Oufella H, Herbin O, Bouaziz J-D, Binder CJ, Uyttenhove C, Laurans L, Taleb S, Vré EV, Esposito B, Vilar J, Sirvent J, Snick JV, Tedgui A, Tedder TF, Mallat Z. B cell depletion reduces the development of atherosclerosis in mice. *J Exp Med* 2010;**207**:1579–1587.
4. Kyaw T, Tay C, Khan A, Dumouchel V, Cao A, To K, Kehry M, Dunn R, Agrotis A, Tipping P, Bobik A, Toh B-H. Conventional B2 B Cell Depletion Ameliorates whereas Its Adoptive Transfer Aggravates Atherosclerosis. *J Immunol* 2010;**185**:4410–4419.
5. Kyaw T, Tay C, Krishnamurthi S, Kanellakis P, Agrotis A, Tipping P, Bobik A, Toh B-H. B1a B lymphocytes are atheroprotective by secreting natural IgM that increases IgM deposits and reduces necrotic cores in atherosclerotic lesions. *Circ Res* 2011;**109**:830–840.
6. Hosseini H, Li Y, Kanellakis P, Tay C, Cao A, Tipping P, Bobik A, Toh B-H, Kyaw T. Phosphatidylserine liposomes mimic apoptotic cells to attenuate atherosclerosis by expanding polyreactive IgM producing B1a lymphocytes. *Cardiovasc Res* 2015;**106**:443–452.
7. Grimm M, Tischner D, Troidl K, Albarrán Juárez J, Sivaraj KK, Ferreirós Bouzas N, Geisslinger G, Binder CJ, Wettschureck N. S1P2/G12/13 Signaling Negatively Regulates Macrophage Activation and Indirectly Shapes the Atheroprotective B1-Cell Population. *Arterioscler Thromb Vasc Biol* 2016;**36**:37–48.
8. Gruber S, Hendrikx T, Tsiantoulas D, Ozsvar-Kozma M, Göderle L, Mallat Z, Witztum JL, Shiri-Sverdlov R, Nitschke L, Binder CJ. Sialic Acid-Binding Immunoglobulin-like Lectin G Promotes Atherosclerosis and Liver Inflammation by Suppressing the Protective Functions of B-1 Cells. *Cell Rep* 2016;**14**:2348–2361.
9. Perry HM, Bender TP, McNamara CA. B cell subsets in atherosclerosis. *B Cell Biol* 2012;**3**:373.
10. Tsiantoulas D, Gruber S, Binder CJ. B-1 cell immunoglobulin directed against oxidation-specific epitopes. *B Cell Biol* 2013;**3**:415.
11. Kyaw T, Tipping P, Bobik A, Toh B-H. Protective Role of Natural IgM-Producing B1a Cells in Atherosclerosis. *Trends Cardiovasc Med* 2012;**22**:48–53.
12. Rosenfeld SM, Perry HM, Gonen A, Prohaska TA, Srikakulapu P, Grewal S, Das D, McSkimming C, Taylor AM, Tsimikas S, Bender TP, Witztum JL, McNamara CA. B-1b Cells Secrete Atheroprotective IgM and Attenuate Atherosclerosis. *Circ Res* 2015;**117**:e28-39.
13. Kyaw T, Tay C, Hosseini H, Kanellakis P, Gadowski T, MacKay F, Tipping P, Bobik A, Toh B-H. Depletion of B2 but not B1a B cells in BAFF receptor-deficient ApoE mice attenuates atherosclerosis by potently ameliorating arterial inflammation. *PloS One* 2012;**7**:e29371.
14. Sage AP, Tsiantoulas D, Baker L, Harrison J, Masters L, Murphy D, Loinard C, Binder CJ, Mallat Z. BAFF receptor deficiency reduces the development of atherosclerosis in mice--brief report. *Arterioscler Thromb Vasc Biol* 2012;**32**:1573–1576.
15. Tsiantoulas D, Diehl CJ, Witztum JL, Binder CJ. B Cells and Humoral Immunity in Atherosclerosis. *Circ Res* 2014;**114**:1743–1756.
16. Doran AC, Lipinski MJ, Oldham SN, Garmey JC, Campbell KA, Skaflen MD, Cutchins A, Lee DJ, Glover DK, Kelly KA, Galkina EV, Ley K, Witztum JL, Tsimikas S, Bender TP, McNamara CA. B-Cell Aortic Homing and Atheroprotection Depend on Id3. *Circ Res* 2012;**110**:e1–e12.
17. Lipinski MJ, Perry HM, Doran AC, Oldham SN, McNamara CA. Comment on "Conventional B2 B Cell Depletion Ameliorates whereas Its

Adoptive Transfer Aggravates Atherosclerosis". *J Immunol* 2011;**186**:4–4.

18. Mauri C, Menon M. The expanding family of regulatory B cells. *Int Immunol* 2015;**27**: 479–486.
19. Potteaux S, Esposito B, Oostrom O van, Brun V, Ardouin P, Groux H, Tedgui A, Mallat Z. Leukocyte-Derived Interleukin 10 Is Required for Protection Against Atherosclerosis in Low-Density Lipoprotein Receptor Knockout Mice. *Arterioscler Thromb Vasc Biol* 2004;**24**: 1474–1478.
20. Mallat Z, Besnard S, Duriez M, Deleuze V, Emmanuel F, Bureau MF, Soubrier F, Esposito B, Duez H, Fievet C, Staels B, Duverger N, Scherman D, Tedgui A. Protective Role of Interleukin-10 in Atherosclerosis. *Circ Res* 1999; **85**:e17–e24.
21. Thüsen JHV der, Kuiper J, Berkel TJCV, Biessen EAL. Interleukins in Atherosclerosis: Molecular Pathways and Therapeutic Potential. *Pharmacol Rev* 2003;**55**:133–166.
22. Han X, Boisvert WA. Interleukin-10 protects against atherosclerosis by modulating multiple atherogenic macrophage function. *Thromb Haemost* 2015;**113**:505–512.
23. Fillatreau S, Sweenie CH, McGeachy MJ, Gray D, Anderton SM. B cells regulate autoimmunity by provision of IL-10. *Nat Immunol* 2002; **3**:944–950.
24. Sage AP, Nus M, Baker LL, Finigan AJ, Masters LM, Mallat Z. Regulatory B cell-specific interleukin-10 is dispensable for atherosclerosis development in mice. *Arterioscler Thromb Vasc Biol* 2015;**35**:1770–1773.
25. Strom AC, Cross AJ, Cole JE, Blair PA, Leib C, Goddard ME, Rosser EC, Park I, Hultgårdh Nilsson A, Nilsson J, Mauri C, Monaco C. B regulatory cells are increased in hypercholesterolaemic mice and protect from lesion development via IL-10. *Thromb Haemost* 2015;**114**:835–847.
26. Wang R-X, Yu C-R, Dambuza IM, Mahdi RM, Dolinska MB, Sergeev YV, Wingfield PT, Kim S-H, Egwuagu CE. Interleukin-35 induces regulatory B cells that suppress autoimmune disease. *Nat Med* 2014;**20**:633–641.
27. Shen P, Roch T, Lampropoulou V, O'Connor RA, Stervbo U, Hilgenberg E, Ries S, Dang VD, Jaimes Y, Daridon C, Li R, Jouneau L, Boudinot P, Wilantri S, Sakwa I, Miyazaki Y, Leech MD, McPherson RC, Wirtz S, Neurath M, Hoehlig K, Meinl E, Grützkau A, Grün JR, Horn K, Kühl AA, Dörner T, Bar-Or A, Kaufmann SHE, Anderton SM, et al. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. *Nature* 2014;**507**:366–370.
28. Mauri C, Nistala K. Interleukin-35 takes the 'B' line. *Nat Med* 2014;**20**:580–581.
29. Yanaba K, Bouaziz J-D, Haas KM, Poe JC, Fujimoto M, Tedder TF. A Regulatory B Cell Subset with a Unique CD1dhiCD5+ Phenotype Controls T Cell-Dependent Inflammatory Responses. *Immunity* 2008;**28**:639–650.
30. Evans JG, Chavez-Rueda KA, Eddaoudi A, Meyer-Bahlburg A, Rawlings DJ, Ehrenstein MR, Mauri C. Novel Suppressive Function of Transitional 2 B Cells in Experimental Arthritis. *J Immunol* 2007;**178**:7868–7878.
31. Sun J, Wang J, Pefanis E, Chao J, Rothschild G, Tachibana I, Chen JK, Ivanov II, Rabadan R, Takeda Y, Basu U. Transcriptomics Identify CD9 as a Marker of Murine IL-10-Competent Regulatory B Cells. *Cell Rep* 2015;**13**:1110–1117.
32. Matsumoto M, Baba A, Yokota T, Nishikawa H, Ohkawa Y, Kayama H, Kallies A, Nutt SL, Sakaguchi S, Takeda K, Kurosaki T, Baba Y. Interleukin-10-Producing Plasmablasts Exert Regulatory Function in Autoimmune Inflammation. *Immunity* 2014;**41**:1040–1051.
33. Xiao S, Brooks CR, Sobel RA, Kuchroo VK. Tim-1 Is Essential for Induction and Maintenance of IL-10 in Regulatory B Cells and Their Regulation of Tissue Inflammation. *J Immunol* 2015;**194**:1602–1608.
34. Khan AR, Hams E, Floudas A, Sparwasser T, Weaver CT, Fallon PG. PD-L1hi B cells are critical regulators of humoral immunity. *Nat Commun* 2015;**6**.

35. Clement M, Guedj K, Andreata F, Morvan M, Bey L, Khallou-Laschet J, Gaston A-T, Delbosc S, Alsac J-M, Bruneval P, Deschildre C, Le Borgne M, Castier Y, Kim H-J, Cantor H, Michel J-B, Caligiuri G, Nicoletti A. Control of the T follicular helper-germinal center B-cell axis by $CD8^+$ regulatory T cells limits atherosclerosis and tertiary lymphoid organ development. *Circulation* 2015;**131**:560–570.
36. Bu D, Tarrio M, Maganto-Garcia E, Stavrakis G, Tajima G, Lederer J, Jarolim P, Freeman GJ, Sharpe AH, Lichtman AH. Impairment of the PD-1 pathway increases atherosclerotic lesion development and inflammation. *Arterioscler Thromb Vasc Biol* 2011;**31**:1100–1107.
37. Gotsman I, Grabie N, Dacosta R, Sukhova G, Sharpe A, Lichtman AH. Proatherogenic immune responses are regulated by the PD-1/PD-L pathway in mice. *J Clin Invest* 2007;**117**:2974–2982.
38. Cochain C, Chaudhari SM, Koch M, Wiendl H, Eckstein H-H, Zernecke A. Programmed cell death-1 deficiency exacerbates T cell activation and atherogenesis despite expansion of regulatory T cells in atherosclerosis-prone mice. *PloS One* 2014;**9**:e93280.
39. Lundy SK, Fox DA. Reduced Fas ligand-expressing splenic CD5+B lymphocytes in severe collagen-induced arthritis. *Arthritis Res Ther* 2009;**11**:R128.
40. Klinker MW, Reed TJ, Fox DA, Lundy SK. Interleukin-5 supports the expansion of fas ligand-expressing killer B cells that induce antigen-specific apoptosis of CD4(+) T cells and secrete interleukin-10. *PloS One* 2013;**8**:e70131.
41. Minagawa R, Okano S, Tomita Y, Kishihara K, Yamada H, Nomoto K, Shimada M, Maehara Y, Sugimachi K, Yoshikai Y, Nomoto K. The critical role of Fas-Fas ligand interaction in donor-specific transfusion-induced tolerance to H-Y antigen. *Transplantation* 2004;**78**:799–806.
42. Blanco-Colio LM, Martín-Ventura JL, Sol JM, Díaz C, Hernández G, Egido J. Decreased circulating Fas ligand in patients with familial combined hyperlipidemia or carotid atherosclerosis: normalization by atorvastatin. *J Am Coll Cardiol* 2004;**43**:1188–1194.
43. Blanco-Colio LM, Martín-Ventura JL, Teresa E de, Farsang C, Gaw A, Gensini G, Leiter LA, Langer A, Martineau P, Hérnandez G, Egido J, ACTFAST investigators. Increased soluble Fas plasma levels in subjects at high cardiovascular risk: Atorvastatin on Inflammatory Markers (AIM) study, a substudy of ACTFAST. *Arterioscler Thromb Vasc Biol* 2007;**27**:168–174.
44. Kaku H, Cheng KF, Al-Abed Y, Rothstein TL. A Novel Mechanism of B Cell–Mediated Immune Suppression through CD73 Expression and Adenosine Production. *J Immunol* 2014;**193**:5904–5913.
45. Ferrari D, Vitiello L, Idzko M, Sala A la. Purinergic signaling in atherosclerosis. *Trends Mol Med* 2015;**21**:184–192.
46. Zernecke A, Bidzhekov K, Özüyaman B, Fraemohs L, Liehn EA, Lüscher-Firzlaff JM, Lüscher B, Schrader J, Weber C. CD73/Ecto-5′-Nucleotidase Protects Against Vascular Inflammation and Neointima Formation. *Circulation* 2006;**113**:2120–2127.
47. Buchheiser A, Ebner A, Burghoff S, Ding Z, Romio M, Viethen C, Lindecke A, Köhrer K, Fischer JW, Schrader J. Inactivation of CD73 promotes atherogenesis in apolipoprotein E-deficient mice. *Cardiovasc Res* 2011;**92**:338–347.
48. Rauch PJ, Chudnovskiy A, Robbins CS, Weber GF, Etzrodt M, Hilgendorf I, Tiglao E, Figueiredo J-L, Iwamoto Y, Theurl I, Gorbatov R, Waring MT, Chicoine AT, Mouded M, Pittet MJ, Nahrendorf M, Weissleder R, Swirski FK. Innate Response Activator B Cells Protect Against Microbial Sepsis. *Science* 2012;**335**:597–601.
49. Ditiatkovski M, Toh B-H, Bobik A. GM-CSF Deficiency Reduces Macrophage PPAR-γ Expression and Aggravates Atherosclerosis in ApoE-Deficient Mice. *Arterioscler Thromb Vasc Biol* 2006;**26**:2337–2344.
50. Subramanian M, Thorp E, Tabas I. Identification of a Non-Growth Factor Role for GM-CSF in Advanced Atherosclerosis Promotion of

Macrophage Apoptosis and Plaque Necrosis Through IL-23 Signaling. *Circ Res* 2015;**116**: e13–e24.

51. Shaposhnik Z, Wang X, Weinstein M, Bennett BJ, Lusis AJ. Granulocyte Macrophage Colony-Stimulating Factor Regulates Dendritic Cell Content of Atherosclerotic Lesions. *Arterioscler Thromb Vasc Biol* 2007;**27**:621–627.
52. Hilgendorf I, Theurl I, Gerhardt LMS, Robbins CS, Weber GF, Gonen A, Iwamoto Y, Degousee N, Holderried TAW, Winter C, Zirlik A, Lin HY, Sukhova GK, Butany J, Rubin BB, Witztum JL, Libby P, Nahrendorf M, Weissleder R, Swirski FK. Innate Response Activator B Cells Aggravate Atherosclerosis by Stimulating T Helper-1 Adaptive Immunity. *Circulation* 2014;**129**: 1677–1687.
53. Angele MK, Pratschke S, Hubbard WJ, Chaudry IH. Gender differences in sepsis. *Virulence* 2014;**5**:12–19.