Abstract: Atherosclerosis is a chronic inflammatory disease that is initiated by the retention and accumulation of cholesterol-containing lipoproteins, particularly low-density lipoprotein, in the artery wall. In the arterial intima, lipoprotein components that are generated through oxidative, lipolytic, and proteolytic activities lead to the formation of several danger-associated molecular patterns, which can activate innate immune cells as well as vascular cells. Moreover, self- and non-self-antigens, such as apolipoprotein B-100 and heat shock proteins, can contribute to vascular inflammation by triggering the response of T and B cells locally. This process can influence the initiation, progression, and stability of plaques. Substantial clinical and experimental data support that the modulation of adaptive immune system may be used for treating and preventing atherosclerosis. This may lead to the development of more selective and less harmful interventions, while keeping host defense mechanisms against infections and tumors intact. Approaches such as vaccination might become a realistic option for cardiovascular disease, especially if they can elicit regulatory T and B cells and the secretion of atheroprotective antibodies. Nevertheless, difficulties in translating certain experimental data into new clinical therapies remain a challenge. In this review, we discuss important studies on the function of T- and B-cell immunity in atherosclerosis and their manipulation to develop novel therapeutic strategies against cardiovascular disease. (Circ Res. 2016;118:668-678. DOI: 10.1161/CIRCRESAHA.115.306427.)

Key Words: atherosclerosis ■ B cells ■ immunomodulation ■ lipoproteins ■ T cells ■ therapy
Atherosclerosis is a chronic inflammatory disease that is initiated by the retention and accumulation of apolipoprotein B-containing lipoproteins, particularly low-density lipoprotein (LDL), in the intimal layer of the artery. In this process, extracellular matrix proteins in the innermost layer of the vessel, especially proteoglycans, are believed to play a significant role. LDL can bind to proteoglycans, for example, through ionic interactions between basic amino acids and negatively charged sulfate groups on proteoglycans, which keep LDL trapped in the artery.

In the early stages of atherosclerosis, the retention of LDL in the arterial intima triggers an inflammatory response in the vessel wall. It has been hypothesized that modifications to the trapped LDL, mediated by oxidative, lipolytic and proteolytic enzymes, and reactive oxygen species lead to the formation of several danger-associated molecular patterns (DAMPs), which can activate vascular and immune cells. In this context, other antigens in the plaque have been also suggested to trigger inflammation, including \( \beta-2 \) glycoprotein I, and heat shock proteins, both autologous and chlamydial heat shock protein 60 and mycobacterial heat shock protein 65.

Atherosclerotic plaques are preceded by the accumulation of lipid-laden cells that form fatty streaks in the intima. Although most cells in fatty streaks are macrophage-derived foam cells, T cells are also present in early disease. T cells and macrophages are found in all stages of disease, whereas B cells are only occasionally found in plaques. However, larger number of B cells may be found in the adventitial layer of the arterial wall, where tertiary lymphoid organs can be formed. T and B cells have been proposed to be key modulators of atherogenesis and plaque stability.

Stable plaques normally bear a fibrous cap, primarily composed of smooth muscle cells and collagen, formed as a response of the vessel wall to stabilize the lesion and prevent blood from making contact with prothrombotic molecules of the atherosclerotic plaque’s core. Hence, plaque growth and fibrous cap formation are associated with remodeling of the vessel wall, a complex process in which matrix metalloproteinases, cytokines, and lipid mediators are released by endothelial cells, smooth muscle cells, and leukocytes.

Elicited innate and adaptive immune responses may influence endothelial cell erosion, plaque stability, and thrombosis (Figure). T and B cells have been studied extensively, particularly in this century, due to advances in immune and molecular tools and genetic manipulation in animal models of atherosclerosis, which have allowed important disease mechanisms to be identified. In this review, we discuss key experimental studies that have increased our understanding of T and B cell immunity in atherosclerosis and the modulation of their responses in developing new potential treatment strategies against cardiovascular disease (CVD).

### Basic Concepts of T and B Cells in Immunity

T and B cells are the main constituents of the adaptive immune system. Unlike innate immunity, its reactions are typically highly specific and long-lasting, mainly through the generation of memory. Importantly, adaptive immunity has a key function in distinguishing foreign from self. In conjunction with innate responses, adaptive immune mechanisms are critical for the elimination of pathogens, to block toxic molecules and maintain homeostasis. If this system fails, it can react destructively against self-molecules, causing autoimmune disease.

Adaptive immunity is characterized by its exquisite specificity that permits recognition of millions of different molecular structures. Two structurally and functionally related surface receptors mediate specific recognition, the B-cell receptor (BCR) and the T-cell receptor (TCR). BCR and TCR, both members of the immunoglobulin superfamily of surface receptors, are generated by germ-line rearrangement during the differentiation of the B and T cells.

T cells can be categorized according to their expression of surface, intracellular proteins and function, which includes the release of cytokines and the capacity to help other cells, such as B cells and macrophages. T cells characteristically express an alpha/beta (\( \alpha \beta \)) or gamma/delta (\( \gamma \delta \)) TCR, express one of the mutually exclusive coreceptors CD4 or CD8, and CD3. In mammals, CD3 associates with TCR to form a protein complex that enables intracellular signal transduction on T cells upon the recognition of linear peptides, termed epitopes, bound to self major histocompatibility complex molecules (MHC or human leukocyte antigen [HLA] in humans) of an antigen presenting cell. CD4 and CD8+ T cells recognize an epitope in association with MHC class II or I molecules, respectively. During their interactions with epitope-MHC and costimulatory molecules on antigen presenting cells, T cells become activated and undergo a broad range of responses, including proliferation, secretion of cytokines and other soluble mediators, and the expression of surface molecules.

Other T cells, named natural killer T (NKT) cells, can recognize complex lipid antigens, such as glycolipids that are often presented in the context of CD1, and initiate similar responses as those of classical MHC-restricted T cells. A large proportion of NKT cells express a conserved TCR,
generated from the invariant rearrangement of variable and joining regions of TCR α, Vα14-Jα18 TCR in mice, or its homolog Vα24-JαQ TCR in humans. These cells are named invariant NKT (iNKT) cells.

The main role of B cells in immunity is to mount antibody responses and act as antigen presenting cells for T cells. Recent development of B cell biology has demonstrated that these cells may also modulate immunity through the release of cytokines. Similar to their secreted form, antibodies are also expressed on the surface of B cells, in which case they are called BCRs. Like T cells, B cells also go through several developmental stages—they mature, differentiate, and give rise to various cell subsets on antigen recognition. Different, B-cell subtypes can be defined by their expression patterns of surface and intracellular proteins, as well as their distinct BCRs, and antibody and cytokine secretion profiles. Nearly all cells of the B cell lineage express CD19. Notably, certain B cells, such as marginal zone B cells can function as innate-like cells that can become activated on ligation of Toll-like receptor instead of BCR.

**Figure. The role of adaptive immunity in atherosclerosis.** The adaptive immune system drives anti- and proatherogenic mechanisms. In the course of lesion development, the activation of different T- and B-cell subtypes can influence vascular inflammation and plaque stability, which may result in increased risk of clinical events. Th17 and IL-17A have been proposed to accelerate atherosclerosis as well as participating in plaque stabilization. Breg indicates regulatory B cell; CTLA4.Ig, cytotoxic T-lymphocyte-associated protein 4-Fc fusion protein; FoxP3, forkhead box P3; IFN-γ, interferon gamma; IL, interleukin; iTreg, inducible regulatory T cell; MHC, major histocompatibility complex molecules; MMP, matrix metalloproteinase; NKT, natural killer T; PD-1, programmed cell death protein 1; PDL-1, programmed death-ligand 1; SMOs, smooth muscle cells; Th1, T follicular helper; TGF-β, transforming growth factor beta; Th, T helper; TNF, tumor necrosis factor; Tr1, type 1 regulatory T cells; Treg, regulatory T cell; and Trp, tryptophan.

**Global Deficiency of T and B Cells and Plaque Development**

The role of T and B cells in atherosclerosis has been examined experimentally using hypercholesterolemic mice that lack important elements of adaptive immunity, ie, recombination-activating genes (RAGs). RAG1 and RAG2 encode key enzymes for the rearrangement of the genes encoding the TCR and BCR that are essential for the generation and maturation of T and B cells. In the Apoe and Ldlr knockout strains (Ldlr<sup>−/−</sup> and Apoe<sup>−/−</sup>, respectively), global deficiency of T and B cells, for example, by being combined with Rag1<sup>−/−</sup> or
Role of Different T-Cell Subtypes in Atherosclerosis

Previous studies showed that CD4+ T cell deficiency in Apoe−/− leads to atherosclerosis,23 whereas the adoptive transfer of CD8+ T cells from old Apoe−/− mice or CD4+ T cells that are reactive to modified LDL into immunodeficient mice (Apoe−/−, nude/scid/−/−) accelerates disease. The latter of these findings were mirrored by increased peripheral blood levels of IFN-γ.21

The role of CD8+ T cells seems not as clear, both because these cells are less frequent in murine lesions and the fact that CD4 deficiency leads to compensatory changes in the CD8+ T cell population.24 Increased atherosclerosis and more CD8+ T cells have been found in plaques of mice fed high-fat diet,25 or injected with anti-CD137, a CD8+ T cell stimulating antibody.26 Surprisingly, CD8 deficiency had no effect in plaque size in Apoe−/− mice.24 Yet, the adoptive transfer of CD8+ T cells from mice immunized with the P210 peptide of ApoB100 was atheroprotective, suggesting that certain CD8+ T cells may even have a completely different function in atherosclerosis.27

The function of CD4+ T-cell subtypes, Th1 and Th2, in atherosclerosis continue to be studied since the initial reports were published over a decade ago. Depletion of the Th1 lineage-specific transcription factor T-bet, as well as interference with IFN-γ signaling, and absence of the Th1-promoting cytokines interleukin (IL)-12 and IL-18 have been shown to reduce the burden of atherosclerosis in hypercholesterolemic mice.28–35 Consistent with these findings, ablation of TGF-β signaling in CD4+ T cells of Apoe−/− mice, which aggravates Th1 responses, exacerbates the disease.36 Thus, there is a general consensus about CD4+ Th1 cells being proatherogenic.

Similar to Th1 cells, an unusual subset of T cells that lack CD28 and secrete high levels of IFN-γ and TNF were suggested to modulate atherosclerosis in humans.37–39 Unfortunately, CD4+CD28−/− T cells are not present in mice, and their contribution to disease cannot, therefore, be assessed by depletion or genetic experiments.

On the contrary to the Th1 cells, Th2 cells were initially considered to be antiatherogenic although this hypothesis remains debated. Targeted deletion of two Th2 cytokines, IL-5 and IL-13, accelerates disease progression, which suggests that Th2 cells are protective.40,41 Additionally, the induction of Th2 responses against ApoB100 through vaccination has been suggested to be the main mechanism mitigating atherosclerosis.42 However, hypercholesterolemic mice with a concomitant deficiency in IL-4, the signature Th2 cytokine, developed less severe disease,43,44 implying a proatherogenic function of these cells. The conflicting findings regarding the role of Th2 cells in atherogenesis could be attributed to various factors, including the capability of Th2 cytokines to induce immunoglobulin class switching, eg, IL-4 induction of IgE production. Notably, Fc epsilon receptor I (IgE receptor) deficiency reduces atherosclerosis in Apoe−/− mice.44 Th2 cytokines are known modulators of eosinophil and mast cell responses, both cells that could as well influence disease.45,46

Th17 cells have been implicated in autoimmune diseases, such as multiple sclerosis, and have been recently raising attention in atherosclerosis. Deficiency or depletion of IL-17, the signature Th17 cytokine, or its receptor was reported to be atheroprotective.47–51 Paradoxically, other work has suggested that IL-17 slows the development of atherosclerosis.52,53 IL-17A was found to be profibrotic, increasing collagen production and fibrous cap formation in Ldlr−/− mice. Although their role in plaque growth may remain unclear, data suggest that Th17 cells could be atheroprotective by promoting plaque stability.54

The role of NKT cells in atherosclerosis has been examined by different approaches experimentally, ie, CD1d-deficient hypercholesterolemic mice, administration of the prototypical CD1 ligand α-galactosylceramide (α-Galcer), and by adoptive transfer of iNKT cell-enriched splenocytes into immunodeficient Rag−/−/ ApoB100 mice.55–59 In general, these studies indicate that NKT cells are proatherogenic. Notably, some studies indicated that NKT-dependent atherogenic mechanisms are pronounced only in the early stages of disease.57,59

An atheroprotective role to NKT has also been proposed. Injection of α-Galcer into Ldlr−/− mice fed western diet, and receiving collar placement around the carotid arteries, had antiatherogenic effects.60 The same study reported significantly more IL-10-producing T cells in the spleen and mediastinal lymph nodes of α-Galcer–injected mice, which mechanistically could be linked to anti-inflammatory mechanisms and the phenotype observed. Little is known about the natural ligands of NKT cells, and whether they could determine pro- and anti-inflammatory subtypes of NKTs, warranting further investigation on them.

Foxp3+ natural and inducible Tregs, and type 1 regulatory T cells (Tr1) exert immunoregulatory and suppressive activity through various mechanisms, the most common of which are the production of anti-inflammatory cytokines, such as TGF-β and IL-10, and contact-dependent cell-mediated inhibition (reviewed by Jäger et al).61 Although cellular immunity in atherosclerosis has been suggested to be generally deleterious, all Treg subtypes have been implicated in atheroprotection. Indeed, the balance between T-cell subsets might be crucial in the development of this disease. Depletion of CD25+CD4+ Tregs significantly exacerbates atherosclerosis.62 In line with these data, TGF-β blockade or loss of TGF-β signaling through disruption of TGF-β receptor II signaling in T cells, or IL-10 deficiency has been shown to accelerate plaque formation.63,64 Otherwise, the induction of Tr1 cells significantly reduced atherosclerotic lesions.65
In order to directly assess the function of Tregs in atherosclerosis, Klingenberg et al. used the depletion of regulatory T cell (DEREG) mouse model to study their role. The DEREG mouse expresses a fusion protein of the human diphtheria toxin receptor under control of the Foxp3 promoter and permits conditional depletion of Tregs in hyperlipidemic mice. Supporting previous findings, depletion of Foxp3+ Tregs induced a robust increase in disease. Surprisingly, plasma cholesterol increased substantially because of impaired clearance of very-low-density lipoprotein on the depletion of Tregs. This study indicates that in addition to its importance in immunoregulation, Tregs are key players in the cross talk between immunity and lipoprotein metabolism, both of which can influence atherosclerosis and CVD.

A less common Treg, the CD8+ regulatory T cell, was recently described in the context of atherosclerosis. It has been proposed that these cells can regulate follicular T helper cell–germinal center B cell interactions and germinal center deleterious reactions, which result in atheroprotection. These data propose a unique mechanism by which Tregs could be atheroprotective.

The activity of indoleamine 2,3-dioxygenase (IDO) and the production of tryptophan metabolites from the kynurenine pathway have been proposed as important checkpoints influencing T-cell responses, particularly Tregs, inducing their stabilization and enhancing their regulatory phenotype. In experimental atherosclerosis, exogenous treatment with the antibody response to oxidized LDL (oxLDL) and T cell activation, whereas B1a cells are not depleted completely by the treatment. Consistent with the proposition that some B-cell subtypes are deleterious in atherosclerosis, the overreactivation of T follicular helper–germinal center B cell axis has been suggested to be atheroprotective. Although several questions remain about the role of B cell responses in atherogenesis, data suggest that B2 cells are atheroprotective while B1a cells atheroprotective.

Both pro- and anti-atherosclerotic mechanisms of B cells could be driven by antibodies against oxidation-specific epitopes of LDL that are found in the circulation and in plaques of animals and humans. Nevertheless, active immunization against LDL and other atherosclerosis-related antigens that are involved in atherosclerosis protect against disease in hypercholesterolemic animals. In addition to inducing B-cell responses, several of these protocols have been shown to also deviate deleterious effector T cell responses toward beneficial Treg activity. Therefore, immunotherapies that induce Tregs are under investigation by several groups worldwide.

Several immunization and tolerization protocols against antigens that are involved in atherosclerosis protect against disease in hypercholesterolemic animals. In addition to inducing B-cell responses, several of these protocols have been shown to also deviate deleterious effector T cell responses toward beneficial Treg activity. Therefore, immunotherapies that induce Tregs are under investigation by several groups worldwide.

In experimental atherosclerosis, the induction and expansion of Tregs by anti-CD3, CD31 receptor globulin treatment, administration of an active form of vitamin D3 (calcitriol), or II-2 and anti-IL-2 complexes have shown atheroprotective effects. Subcutaneous and mucosal administration of ApoB100 peptides, injection of ApoB100-loaded IL-10–induced
Immunomodulation of T- and B-Cell Responses in Human Disease

Despite the improvement in the efficacy of therapeutic agents against CVD, such as statins,138 two thirds of CVD cases cannot be prevented, and at least 10% of healthy individuals develop CVD in the absence of classical risk factors.139 None of the currently available clinical therapies directly target the immune reactions involved in atherosclerosis, although statins may also exert certain immunomodulatory effects.140

Immunosuppressants and anti-inflammatory drugs, such as corticosteroids, cyclosporine, sirolimus, tacrolimus, azathioprine, cyclophosphamide, and methotrexate, remain valuable options for treating and preventing undesirable response of T and B cells in the context of autoimmune diseases and transplant rejection.142 Modulation of T- and B-cell responses is an attractive target considering their specificity and long-lasting characteristics. Although certain immunosuppressants are used to prevent and treat atherosclerotic CVDs, such as by local delivery using drug-eluting stents,143 their systemic use may result in undesirable side effects, including thrombosis, dyslipidemia, hypertension, and diabetes mellitus.144,145

Methotrexate, which is a safe first-line treatment for some autoimmune conditions, decreases the risk of CVD in rheumatoid arthritis and psoriatic patients146 and is currently being examined with regard to its activity against CVD.147,148 Interestingly, methotrexate showed high activity against atherosclerosis in animal models,149,150 suggesting that it may induce clinical benefits in the general human population. Similarly, TNF blockade, of which is also a standard therapy for autoimmune diseases,151 has been linked to decreased CVD risk in rheumatoid arthritis.152,153

T-cells, particularly the naïve, depend highly on costimulation to be activated. Thus, targeting surface costimulatory molecules has been considered as a method of controlling T-cell–mediated responses, which has been taken to humans. CTLA4-Ig is being examined as a first-line biological for the treatment of rheumatoid arthritis,154-156 where it has been shown to reduce disease activity and improve plasma lipoprotein profiles in patients.157 Whether CTLA4-Ig can influence effector T cell responses in the vessel wall is unknown. Although it may be early to make conclusions about the advantages of biologics—drugs that are not chemically synthesized, eg, anticytokines, and fusion proteins—in preventing and treating CVD, the fact that some of these compounds may improve classical risk factors for CVD may facilitate that they can be used against atherosclerotic diseases.

With regard to anti-CD20–based depletion of B cells and its potential activity against CVD, there is a discussion over whether statins may reduce its efficacy.158,159 Hence, undesired drug–drug interactions, particularly those involving current first-line drugs for CVD, might limit their use.

There is compelling experimental data that support the use of anti-oxLDL to reduce vascular inflammation and to decrease and stabilize atherosclerotic plaques. The Goal of Oxidized LDL and Activated Macrophage Inhibition by Exposure to a Recombinant Antibody trial (GLACIER—NCT01258907) was a randomized, double-blind study that for the first time evaluated an anti-oxLDL–targeted monoclonal antibody in

tolerogenic DCs116; and subcutaneous and mucosal tolerization against oxLDL.137 Hsp65,105,118 and β2-glycoprotein I109 have all been shown to reduce plaque formation, which could be at least in part due to the induction of antigen-specific Tregs.

Harmful T-cell responses in autoimmunity can be targeted by modulation of costimulation pathways. Targeting CD28–CD80/86 with cytotoxic T-lymphocyte–associated protein 4–Fc fusion protein (CTLA4.Ig),120 CD40–CD40L, 121–123 CD28–CD80/86 with cytotoxic T-lymphocyte–associated proteins 4–Fc fusion protein (CTLA4.Ig),120 CD40–CD40L, 121–123 and the unexpected fatal thrombotic events that occurred in systemic lupus erythematosus (SLE) patients who were administered with intravenous immunoglobulin treatment (IVIg), which could affect antigen-specific Tregs.

As discussed earlier, B-cell responses can be efficiently modulated by depletion, ie, using anti-CD20, or by immunization, which concomitantly to the induction of Tregs can induce atheroprotective antibody responses. Particularly, humoral immunity to oxLDL has been proposed to play an important role in CVD in several studies. It has been shown that plaque size correlates negatively with levels of IgG anti-oxLDL that are induced by immunization with this antigen.99,100 It has been proposed that antibodies against oxidized epitopes of LDL might exert their protective effects by blocking ox-LDL uptake132 or by inducing inhibitory signaling through Fc gamma receptor II B (FcγRIIB) inhibitory receptors on macrophages.134

Consistent with the protective mechanisms of antibodies that have been proposed, the lack of IgG receptor FcγRIIB on leukocytes is associated with increased activation of B and T cells, an imbalance between T effector and Treg cells, and increased atherosclerosis.135 Further, intravenous immunoglobulin treatment (IVIg), which could affect antigen-specific clearance of oxLDL as well as the induction of inhibitory signals via FcγRIIB, reduced atherosclerosis in hypercholesterolemic mice.136–138

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humans. Although it was well tolerated, the treatment did not significantly reduced 18F-fluorodeoxyglucose (FDG) uptake in the arterial wall, which was used as a biomarker for plaque inflammation, versus placebo. Moreover, no changes in plasma lipids or high-sensitivity C-reactive protein levels were observed. Although these results were disappointing, there may be several reasons for the failure of the GLACIER trial to meet its end point, including the selected study population, which had stable CVD on background statins, and the use of FDG-PET to assess plaque inflammation, as this method has several limitations and is not a standardized method between medical centers. New and better studies will be needed to properly access the potential of anti-oxLDL therapies for CVD in humans.

Conclusions
There is compelling evidence that T- and B-cell immune responses are involved in all phases of atherosclerosis, from the initiation to its end point complications. Despite their limitations, animal models have been instrumental in determining the molecular mechanisms that link these cells to CVD. However, translating the results into novel and more effective therapies remains challenging. More investments from the pharmaceutical industry, rather than academic support alone, should be made in the early stages of research and drug development to further develop this field. The success of certain immunomodulatory drugs in decreasing CVD risk in autoimmune disease patients indicate they could be used in a relatively large segment of the population. Yet, it remains unclear which patients could benefit most from these therapies, considering the chronic aspect of atherosclerosis, and the risk that long-term use of some drugs could result in immunosuppression-related complications. Vaccination is an attractive option because it specifically targets disease-associated antigens, while leaving the general host defense unhampered. It is encouraging that vaccine studies are being pursued with the aim of reaching the clinical trial phase in the near future.

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