T Cells in Atherogenesis  
For Better or For Worse?

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Abstract—The idea that atherosclerosis is an inflammatory disease is no longer controversial. Instead, much of the current research is now focused on understanding what drives this inflammation and how it is regulated. Adaptive immunity, in particular T cells, is highly involved in atherogenesis. It is well known that different subsets of T cells can drive or dampen inflammatory processes, but we still have much to learn about the regulation of this balance in the context of atherosclerosis. This review summarizes our knowledge of T cells in atherogenesis, their potential antigens, their contact-dependent activities, and their secretion of inflammatory and antiinflammatory mediators, aiming to illustrate how T cells can aggravate or attenuate this disease through cross-talk with other cells within or outside the atherosclerotic plaque. *(Arterioscler Thromb Vasc Biol. 2006;26:2421-2432.)*

Key Words: atherosclerosis pathophysiology ■ immune system ■ T cells ■ cytokines ■ macrophages

The presence of T cells in human atherosclerotic plaques was described in 1985.¹ Macrophage-derived lipid-laden foam cells were already known to exist, but antigen presentation and immune-effector responses were not considered in the context of atherosclerosis. A few years later, MHC class II and IFN-γ were found in lesions, and antibodies to oxidized low-density lipoprotein (LDL) were described.²,³ Whether we wanted to believe it, adaptive immunity had been established in the field of atherosclerosis.

Today, the concept that atherosclerosis is an inflammatory disease is no longer controversial,⁴ and instead of proving that point, we can focus on investigating how this inflammation is regulated. T cells are of particular interest, both because of their secretion of mediators that influence plaque development and because their activity depends on the triggering of specific antigens that are found within the disease site. T cells are regulated by soluble and membrane-bound molecules from many cell types and, in turn, they act on most other cells. This network of cell-to-cell interactions affects the development of many inflammatory and autoimmune diseases. Here we summarize the current knowledge of the diverse roles of T cells in atherogenesis and discuss obstacles that prevent us from learning more.

T Cells, Conductors of Adaptive Immunity, Are Involved in Atherogenesis

An efficient T cell response is generated when the naïve T cell encounters an antigen-presenting cell (APC) that presents an antigenic peptide for which the T cell is specific.⁵ CD8⁺ T cells recognize peptides presented on MHC class I, whereas CD4⁺ T cells recognize MHC class II. The primary activation takes place in secondary lymphoid organs such as the peripheral lymph nodes to which DCs that have ingested antigens migrate. The T cell then exits and migrates into
nonlymphoid tissues for a second activation by APCs that present the same antigen. Thus, naïve T cells are rarely found in nonlymphoid tissues, which is true also for T cells in atherosclerotic plaques; most T cells found in human lesions are effector or memory T cells and the proportion of activated T cells increase with severity of coronary syndrome. Both CD4 and CD8 T cells are found in human lesions, but CD4 T cells generally dominate in number. Most cells are TCRβ/α, although TCRγδ-positive T cells are also present. In the vicinity of the T cells, MHC class II-expressing macrophages and DCs are detected, indicating immune interactions between T cells and APCs (Figure, A).

**T Cells in Mouse Models of Atherosclerosis**

CD4 T cells are the predominant T cell subset in atherosclerotic lesions in apoE−/− and LDLR−/− mice. A global deficiency of adaptive immunity leads to reduced atherosclerosis in such mice, although the effect of the immune deficiency is less pronounced at extreme cholesterol levels. Reconstitution of immune-deficient scid/scid mice with CD4 T cells accelerates disease, indicating that CD4 T cells play a pathogenic role in atherosclerosis. Consistent with this concept, removal of CD4 T cells by depleting anti-CD4 antibodies reduced fatty streak development in C57BL/6 mice on an atherogenic diet. Similarly, CD4-deficient C57Bl/6 mice were protected against fatty streak formation, but conflicting data appear regarding the effect in CD4-deficient apoE−/− mice. T cell activation is important for early progression of atherosclerosis but not for its initiation, as shown using conditional ablation of dividing T cells in apoE−/− mice. The promoter that was used targets APC-T cell interactions in atherogenesis. A, Antigen-presenting cells within the atherosclerotic plaque and in regional LNs may present antigens to neighboring T cells and start a cascade of immunologic events. Depending on the cytokines, chemokines, growth factors, and hydrolytic enzymes secreted by the cells as a response to this inflammatory process, the plaque will gradually develop into an inflammatory, rupture-prone plaque, or to a stable plaque. B, Interactions between activated T cells and macrophages through soluble and surface-bound molecules lead to a cascade of macrophage-derived atherogenic events. C, APCs, such as DCs or macrophages, can activate T cells, which respond by proliferation and secretion of atherogenic or antiatherogenic cytokines.
both CD4+ and CD8+ T cells. Thus, the conclusion may be applicable to both cell subsets, although a role for CD4+ cells is more likely in this mouse model.

Little data exist regarding the precise role of CD8+ T cells in atherosclerosis. ApoE−/−CD8−/− mice exhibit no change in lesion formation compared with ApoE−/− mice,22 but CD8+ T cells are capable of promoting atherosclerosis, as shown by a model in which their stimulation was induced by the expression of a foreign antigen by vascular smooth muscle cells (SMCs).24 Thus, in a situation where an intracellular antigen triggers CD8+ T cells, as in the case of a viral infection, CD8+ T cells may promote the formation of an atherosclerotic lesion.

TCRαβ+ T cells are present in far greater numbers than TCRγδ+ cells in atherosclerotic lesions and may consequently play a more significant role in lesion development. Indeed, TCRαβ-deficient apoE−/− mice displayed reduced atherosclerosis, whereas mice deficient in TCRγδ+ T cells were only marginally affected.22 T cells are present within the adventitia of normal aortas along with B cells, DCs, and macrophages.25 In atherosclerotic mice, lymphocytes reside in ordered structures and the number of T cells increase at sites of lesions.23 The importance of these T cells for the initiation and/or progression of atherosclerosis remains to be shown. Importantly, studies in which RNA extracts from aortas are analyzed may study expression patterns from adventitial cells in combination of intimal cells.

What Do Plaque T Cells See?

Antigen Presentation in Atherosclerosis

The exact location for the initial antigen presentation to T cells in atherosclerosis is not known but is thought to occur in regional LNs.26 DCs are the most potent APCs and are responsible for the activation of naïve T cells. Immature DCs scavenge the body for antigens and may ingest atherosclerosis-related antigens from the vessel intima. DCs resembling Langerhans cells of the skin are present in human atherosclerotic lesions, implying that antigen capture may occur there.26,27 After antigen uptake, DCs mature and return to secondary lymphoid organs to present antigens to T cells. Hypercholesterolemia leads to activation and reduced migration of DCs from the periphery to LNs, which may aggravate local inflammation and progression of the atherosclerotic plaque, but may paradoxically impair T cell activating functions that require their migration to LNs.28 DCs are important in initial T cell activation as well as in T cell differentiation. Their influence on T cell polarization depends partially on the presence of factors such as microbes and cytokines in their microenvironment.29 Atherosclerosis-associated microbes can therefore play a role in the differentiation of the T cell population.

When effector or memory T cells home to inflamed tissues, they can be reactivated by different APCs. Mature DCs have been found in human atherosclerotic plaques in areas where activated T cells are present,13 suggesting in situ T cell reactivation. These clusters have been observed in rupture-prone regions of atherosclerotic plaques, implicating a role for T cell activation in plaque destabilization.13,27 Macrophages may also function as APCs to T cells during a secondary activation. B cells, which also can present antigen, are scarce in both human and murine lesions. Endothelial cells have been shown to function as APC in vitro,30 and both SMC and endothelial cells have been shown to express MHC class II in human atherosclerotic lesions.31 These data implicate additional possibilities for antigen-presentation by unconventional APCs.

Candidate T Cell Antigens in Atherosclerosis

Immunodominant T cell epitopes are peptides of a given protein that best fit in the groove of an MHC molecule and that are responsible for initiating disease through adaptive immunity. Such epitopes have not yet been identified for atherosclerosis. There are many candidate antigens in this disease, and the issue is further complicated by protein modifications as a result of lipid peroxidation. Most likely, many antigens drive the development of this disease.

Chlamydia, herpes simplex, and cytomegalovirus have been detected in atherosclerotic plaques,32 and patients with cardiovascular disease have high antibody titers to Chlamydia pneumoniae, Helicobacter pylori, and cytomegalovirus, indicating involvement of adaptive immunity system.33 Immune responses to microbial heat-shock protein (HSP) 65 can cross-react with human HSP60, which is detected in human atherosclerotic lesions.9 This process is called molecular mimicry. Immunization with HSP65/60 in mice and rabbits aggravates fatty streak formation,34,35 suggesting that adaptive immunity to HSP promotes atherogenesis. Consistent with this, patients with atherosclerosis display increased antibody titers to HSP65/60.36,37

Altered self-proteins may elicit immune responses, and the oxidation of LDL that takes place in the vascular wall and in circulation is thought to contribute to immunogenicity via this mechanism.38 Lipid peroxidation of polyunsaturated fatty acids in the phospholipids and cholesterol esters of lipoproteins results in the production of reactive aldehydes. They may bind to lysine and histidine residues in apolipoprotein B present in lipoproteins, or on other proteins, creating immunogenic neoepitopes. Oxidation of LDL also results in oxidized phospholipids. Increased antibody titers to oxidized LDL are observed in atherosclerotic mice39 and have been reported in some, but not all, studies of atherosclerotic patients.40 β2-glycoprotein I (β2GPI) is a phospholipid-binding protein that is present on platelets, on endothelial cells and in human atherosclerotic plaques.41 Autoantibodies against β2GPI have been found in patients with inflammatory disorders, including atherosclerosis, lupus, and antiphospholipid syndrome.42 Immunization with human β2GPI accelerates lesion development in LDLR−/− mice,43 an effect that may depend on antibodies to β2GPI that activate endothelial cells and promote uptake of oxidized LDL by macrophages.44

T Cell Specificity in Atherosclerosis

The presence and the role of specific T cells in atherosclerosis can be studied in humans or in animal models using different approaches. In humans, T cell responses to specific antigens in atherosclerosis have been shown indirectly by findings of circulating T cell–dependent antibodies to antigens implicated in the pathogenesis. Direct approaches include isolation of T cells from human atherosclerotic lesions to study their specificity. Using such methods, 10% of the clones derived from plaque T cells responded to oxidized LDL in an MHC class II-dependent manner.45 Several studies have reported
Chlamydia-reactive T cells and T cells reactive to HSP60 of Porphyromonas gingivalis, a pathogen involved in periodontitis.46,47

Animal models can be used directly to investigate the importance of T cell specificity because we can transfer specific T cells into a disease-prone host and assess their effect. In this way, T cells from β2GPI-immunized mice increased the development of fatty streaks.48 Similarly, mononuclear cells from HSP-immunized mice accelerated fatty streak formation, an effect that may be attributed to both T cells and antibody-producing B cells.49 When CD4+ T cells from donors immunized with oxidized LDL or an irrelevant protein antigen were transferred to apoE–/– scid/scid mice, reactivity to oxidized LDL accelerated T cell atherogenicity.50

Clonality and Expansion of T Cell Populations Within the Plaques

Several studies have demonstrated polyclonal distribution of T cells in human atherosclerotic plaques,51 utilizing the knowledge that TCR diversity is caused by rearrangement of TCR genes and that analysis of their expression will provide information about clonality within a tissue. Inflamed tissues recruit T cells in an antigen-nonspecific manner. Thus, not all lesion-infiltrating T cells are relevant in atherogenesis. Antigen-induced activation of T cells that meet APCs that are presenting “their” antigen leads to proliferation and clonal expansion, which can be detected by spectratyping the peptide-binding complementarity-determining region 3 (CDR3) of the TCR. Because clonal expansion of T cells in lesions from both apoE–/– mice and humans has been demonstrated,52,53 antigen presentation, T cell activation, and T cell expansion probably occur within atherosclerotic lesions. TCR usage was more limited in murine mature plaques compared with earlier plaques,53 which may imply a nonselective and heterogeneous recruitment of T cells in early phases of the disease, whereas a selective expansion of T cell clones with specificity for antigens in the plaques may occur at later stages. This hypothesis is supported by the scattered distribution of T cells within fatty streaks and T cell clustering close to MHC class II-expressing APC at later stages. In addition, unstable plaques from patients with acute coronary syndromes (ACS) were demonstrated to contain specific T cell clonotype expansions, whereas only minor derangements were seen in some plaques from patients with chronic stable angina.54 Expansions of T cell clones in the plaques were not accompanied by a similar expansion in the peripheral blood, indicating site-specific T cell expansions.

The Many Roles of Costimulation

Costimulation is crucial during the primary activation of T cells,55 but the second activation can take place without costimulation.56 During atherogenesis, however, costimulation during the second activation is still important because T cells may provide important signals to other cells through costimulatory molecules. T cells can activate macrophages in the lesions, resulting in increased secretion of inflammatory cytokines, extracellular matrix-degrading proteases (MMPs), and tissue factor, an initiator of blood coagulation57 (Figure, B). Because costimulatory signals play important roles at multiple stages, it is difficult to establish the principal reason why abrogation of costimulatory pathways affects atherosclerosis in experimental models. Until animal models exist in which these effects can be separated, the most likely scenario is a combination of effects in secondary lymphoid organs and in the lesion itself. Furthermore, the discovery that regulatory T cells are dependent on certain costimulatory molecules obfuscates the issue further; consequently, costimulation may also play an antiatherogenic role.

CD28 and CD80/CD86

The primary costimulatory pair is CD28, which is expressed on T cells, and CD80/CD86, also known as B7.1/B7.2, which are expressed on APCs. LDLR–/– mice deficient in CD80 and CD86 exhibited reduced early atherosclerotic lesion development concomitant with reduced MHC class II expression and IFN-γ secretion.58 As a result, this costimulatory pathway was suggested to be atherogenic. Surprisingly, a recent study showed that irradiated LDLR–/– mice reconstituted with CD80/CD86-deficient or CD28-deficient bone marrow displayed increased lesion development compared with controls.59 This effect was explained by the absence of regulatory T cells in the CD28–/– and CD80/CD86–/– mice, because the generation and function of regulatory T cells are dependent on these costimulatory molecules, but why the two models yielded different results is currently unclear.

A surprisingly low portion (5% to 10%) of CD3+ T cells in human atherosclerotic plaques expresses CD28.60 CD4+ CD28null T cells may represent prematurely senescent cells resulting from persistent immune activation of CD28+ cells.61 These cells are potent secretors of IFN-γ and tumor necrosis factor (TNF)-α and may contribute to disease progression in autoimmune and inflammatory diseases. The low expression of CD28 on T cells in atherosclerotic lesions is similar to the situation in rheumatoid arthritis and other inflammatory syndromes,62 in which levels of CD28null T cells correlate with severity of disease.62 Levels of circulating CD4+CD28null T cells are increased in patients with ACS.63 These cells have cytolytic function and can lyse endothelial cells without the need for antigen recognition.64 They can also establish an immunologic synapse with vascular SMCs and trigger apoptotic death, perhaps attributed to a reduced T cell activation threshold, and may therefore promote plaque instability.65

CD4+CD28null T cells from peripheral blood and plaques of patients with ACS spontaneously express IL-12 receptors.66 IL-12 appears to be connected to lesion recruitment of these T cells, because IL-12 enhanced the expression of factors that are involved in tissue recruitment of effector T cells, and CD4+CD28null T cell infiltration into a human atheroma engrafted into a scid/scid mouse was enhanced when the T cells had been cultured with IL-12.

CD40 and CD40L

Interaction between CD40 on the APC and CD40L on T cells results in priming and expansion of antigen-specific CD4+ T cells.67 This further activates the APC, leading to upregulated co-stimulatory activity and production of proinflammatory cytokines (IL-1, IL-6, and TNF-α) and IL-12, all of which are atherogenic. Consequently, anti-CD40L antibody-treated or CD40L-deficient atherosclerosis-prone mice exhibit ameliorated disease, illustrated by reduced lesions and plaques with
a less vulnerable phenotype. Surprisingly, CD40L deficiency only on leukocytes did not affect atherosclerosis. Because macrophages, platelets, endothelial cells and SMC in atherosclerotic lesions can also express CD40L, T cell-independent routes for inflammatory activation are possible. Likewise, CD40 is expressed on a variety of cell types: lesional macrophages, endothelial cells, SMCs, and platelets, creating many possible interactions between CD40 and CD40L within the atherosclerotic plaque. CD40–CD40L ligation leads to increased expression of chemokines and adhesion molecules, contributing to leukocyte recruitment. The formation of a vulnerable plaque phenotype may therefore be promoted by CD40–CD40L signaling and, furthermore, CD40 ligation induces the production of extracellular MMPs and tissue factor. Unfortunately, CD40L ligation can lead to platelet activation, which may contribute to thrombotic complications, an unfortunate obstacle for use of the CD40–CD40L pathway for therapeutic approaches.

The CD40–CD40L and CD28–CD80/86 pathways are closely connected; ligation between CD40 and CD40L increases CD80 and CD86 expression, and stimulation by CD80 and CD86 upregulates CD40L expression. The findings of decreased atherosclerosis by inhibition of one pathway may hence be linked to the attenuation of the other.

**Ox40 and Ox40L**

Ox40 is present on T cells and Ox40L on a wide array of cells. This costimulatory pair is induced by IL-12, it is important for development and survival of memory CD4+ T cells and is implicated in several autoimmune diseases including diabetes and multiple sclerosis, and also in atherosclerosis. Overexpression of Ox40L in fat-fed mice increased fatty streak formation, whereas Ox40L−/− mice exhibited smaller lesions than did controls. Furthermore, a polymorphism in the Ox40L gene, Tnfsf4, was identified as a genetic risk factor for myocardial infarction in humans, suggesting that the Ox40–Ox40L pathway may be an interesting target for therapeutic intervention of atherosclerosis.

**Soluble Mediators Are Secreted by Cell–Cell Interactions**

Activation of T cells and APCs involves not only contact-dependent mechanisms but also the secretion of soluble mediators, many of which affect the development of atherosclerosis. Again, we can count on effects both at the level of the secondary lymphoid tissues, where the primary T cell activation takes place, and in the lesion itself, where cross-talk between APCs and T cells probably drives local inflammation. APCs activate T cells and, in turn, T cells activate macrophages, which secrete proinflammatory cytokines. As a result of this cellular dialogue, cytokines from both T cells and macrophages increase the expression of adhesion molecules, chemokines, scavenger receptors, and extracellular matrix-degrading proteases (Figure, B and C).

**The Atherogenic Th1 Cells**

**IFN-γ**

The principal Th1 cytokine, IFN-γ, is produced by most T cells in the human atherosclerotic plaque. IFN-γ−/− mice, as well as IFN-γR−/− deficient mice both exhibit attenuated atherosclerosis (Table 1), and injections of recombinant IFN-γ increase lesion size. NK cells and NKT cells, which are implicated in atherogenesis, also express IFN-γ. IFN-γ injections lead to a decrease of serum cholesterol, but...
apparently this cannot protect against the atherogenic effect of IFN-γ.88

Its effects on immune cells and other cells suggest that IFN-γ potentiates most stages that lead to inflammation in atherosclerosis.91,92 Its atherogenic properties include enhanced recruitment of T cells and macrophages to the plaques, increased macropage uptake of lipids leading to the formation of foam cells, increased activation of APC, and enhanced secretion of Th1-promoting cytokines, which subsequently continues to drive these processes. In addition, the destabilizing effect of IFN-γ may lead to the thinning or inhibition of fibrous cap formation, resulting in vulnerable, rupture-prone plaques. These effects are manifested by prevention of smooth muscle cell infiltration and proliferation, reduction of collagen synthesis, and augmentation of the production of extracellular matrix-degrading proteins.

Surprisingly, LDLR−/− mice transplanted with bone marrow from IFN-γ−/− mice exhibited larger atherosclerotic lesions than mice that received bone marrow from IFN-γ−/− mice,93 implicating a protective role of IFN-γ, which also possesses certain potentially antiatherosclerotic properties in vitro.91,92 It decreases macrophage expression of LDLR-related protein, scavenger receptor A and CD36, and inhibits lipoprotein lipase. IFN-γ may also inhibit lipoprotein oxidation and downregulate the expression of MMP9. Thus, the in vivo effects of IFN-γ are complex and may depend on experimental approaches.

The transcription factor T-bet is indispensable for Th1 differentiation.94 Consequentially, T-bet−/−deficient LDLR−/− mice display reduced lesion development.95 A combination of factors probably lead to this phenotype, because in addition to abrogated Th1 cell differentiation, the T-bet−/−deficient mouse model has impaired CD4+ T cell migration to inflammatory sites caused by deficient expression of certain selectins and chemokines.96

**IL-12 and IL-18**

Being the principal cytokine to promote Th1 development, IL-12 is likely to be a key mediator in atherogenic inflammation. IL-12 is primarily produced by monocytes, macrophages, and DC, and further promotes an efficient immune response through induction of MHC class II, CD80, and CD86 in APC.97 Indeed, treatment of apoE−/− mice with recombinant IL-12-aggravated disease, concomitant with increased IFN-γ expression in the aorta.98 Correspondingly, apoE−/−IL-12p40−/− mice exhibited reduced plaque area.99 The IL-12p40−/− model is not specific for IL-12 since the IL-12p40 subunit is shared by the related cytokine IL-23 (see below). Effects seen in IL-12p40−/−apoE−/− mice may therefore not only reflect the outcome of IL-12 deficiency but a combined effect of IL-12 and IL-23 deficiency.

IL-18, which is produced by monocytes/macrophages, dendritic cells, and several nonhematopoietic cell types, acts in synergy with IL-12 to induce IFN-γ production in NK cells, T cells, and macrophages, and can induce IFN-γ in SMCs.100 In addition, IL-18 increases the expression of certain inflammatory cytokines and MMPs in endothelial cells, SMCs, and macrophages.100,101 Thus, IL-18 was hypothesized to have atherogenic effects, which was confirmed using 3 different approaches: ApoE−/− mice treated with plasmid DNA encoding for IL-18 binding protein102 and ApoE−/−IL-18−/− mice103 exhibited reduced lesion development, whereas IL-18 treatment of apoE−/− mice accelerated atherosclerosis development.104 IL-18 administration did not affect lesion development in apoE−/− IFN-γ−/− mice,105 elegantly showing that IL-18 exerts its main effect in atherosclerosis through the induction of IFN-γ.

Atherogenic properties of IL-18 in non-T cells were demonstrated using apoE−/− scid/scid mice injected with IL-18.106 Administration of IL-18 induced IFN-γ production in NK cells and macrophages and aggravated atherosclerosis even without the presence of T cells.

**TNF Family Members**

Th1 cells produce TNF-α as well as lymphotoxin (LT, TNF-β). Whereas LT/TNF-β is primarily a T cell cytokine, TNF-α is also produced by macrophages and other cell types. Therefore, Th1 activation leads to TNF-α secretion indirectly, via macrophage activation, as well as directly from the T cell. Both these cytokines are proinflammatory and promote several autoimmune diseases. In addition, TNF-α inhibits lipoprotein lipase, leading to hypertriglyceridemia and reduced fatty acid oxidation, and stimulates production of oxygen and nitrogen radicals. Many potentially proatherogenic effects have been identified in cell culture studies of TNF-α, and compound knockout experiments have shown reduced atherosclerosis in the absence of functional TNF-α.106

The large superfamilies of TNF-like molecules and TNF receptors include several members implicated in atherosclerosis, including CD40,CD40L and OX40/OX40L, which are discussed elsewhere in this review. Another family member, LIGHT (lymphotoxin-like inducible protein that competes with glycoprotein D for binding herpesvirus entry mediator on T cells), is expressed by activated T cells, macrophages, and several other cell types, and contributes to activation of T cells, macrophages, and dendritic cells. Similar to CD40L, LIGHT is also expressed by platelets, which can induce endothelial activation.107

The LIGHT receptor, TNFRSF14, is expressed in atherosclerotic plaques, where it may promote metalloproteinase secretion and expression of tissue factor, scavenger receptors, and TNF.108,109 Circulating levels of soluble LIGHT protein are elevated in patients with unstable angina.109 Another TNF superfamily member, TRAIL (TNF-related apoptosis-inducing ligand) is expressed by plaque T cells and can also be detected, in its soluble form, in peripheral blood.110 TRAIL-expressing T cells can induce apoptosis in vascular SMCs within atherosclerotic plaques.111 Interestingly, patients with ACS have reduced levels of soluble TRAIL,112 but T cells that express TRAIL on stimulation are expanded.111 Whether soluble LIGHT, TRAIL, CD40L, or other TNF family members will be useful markers of atherosclerotic disease remains unclear.112

**The Multifaceted Th2 Cells**

**IL-5**

IL-5, which together with IL-4 and IL-13 defines the Th2 subset, promotes the development of B-1 cells, the B cells that produce so-called natural antibodies. Some of these IgM antibodies cross-react with oxidized LDL and may inhibit cholesterol uptake and subsequent foam cell formation by macrophages.113 IL-5 deficiency in LDLR−/− mice led to enhanced lesion formation in a bone marrow transplantation model.114 Thus, IL-5 may act in an antiatherogenic fashion by stimulating the production of protective antibodies.
IL-4 and IL-13

Because of its ability to inhibit Th1 differentiation, IL-4 is protective against disease in many Th1-mediated conditions, but the effects of IL-4 in atherosclerosis seem more complex. Two independent studies, one using transferred bone marrow cells from IL-4−/− to irradiated LDLR−/− mice, and the other using apoE−/−IL-4−/− mice, have shown that IL-4 deficiency leads to reduced lesion development in atherosclerosis prone mice, demonstrating an atherogenic role of IL-4. Indeed, IL-4 has effects on the non-T cell population that may explain this, leading to increased lipid oxidation, enhanced leukocyte adhesion and attraction, and increased uptake of modified lipoproteins and foam-cell formation. IL-4 can activate mast cells, which may lead to apoptosis of SMCs, reduced collagen production, and increased production of proteases, resulting in destabilization and plaque rupture. Finally, IL-4 induces MMP-12, a potent elastase that can digest structural elements of the artery wall and promote aneurysm formation. Thus, Th1 polarization in the IL-4−/− mouse may be insufficient to accelerate the development of atherosclerosis when other functions of IL-4 are abrogated.

IL-4 injections, however, decrease fatty streak formation in C57Bl/6 mice, and stat6 deletion in fat-fed BALB/c mice, which normally are resistant to atherosclerosis, results in the development of fatty streak lesions.

Several properties of IL-4 are shared by IL-13, which is induced by IL-4–driven Th2 differentiation. The direct role of IL-13 in the context of atherosclerosis has not been investigated; such studies will be valuable for the continued discussion on the role of Th2 responses in atherogenesis.

Th-17 Cells: A New Player on the Scene

Recently, a novel lineage of CD4+ T cells was identified: Th17 cells. The Th-17 subset, which produces the inflammatory cytokine IL-17, is induced by transforming growth factor (TGF)-β in combination with IL-6. IL-12 or by IL-23, IL-17, also produced by memory CD8+ T cells, and neutrophils, acts as a potent proinflammatory mediator and synergizes with TNF-α and IL-1.

IL-17 has been linked to many autoimmune and inflammatory diseases, but no evidence currently exists for the role of IL-17 or IL-23 in atherosclerosis. As mentioned earlier, studies of apoE−/−IL-12 p40−/− mice may also reflect IL-23 function.

IL-6−/− deficient mice have a deficit in IL-17–producing cells and are resistant to the induction of EAE, a disease model that is dependent on IL-23 and to a certain extent IL-17. IL-6−/− deficient LDLR−/− mice, however, exhibit only a small and insignificant reduction in lesion development. Thus, this pathway may be more important in EAE than in atherogenesis.

Antiinflammatory Cytokines and Regulatory T Cells

A balance is required to have an effective immune system that can combat aggressors from the outside without attacking self-tissues. The antiinflammatory cytokines IL-10 and TGF-β are important components in this balance, as are regulatory T cells, different T cell populations with the same function, ie, to suppress the effector function of other immune cells.

TGF-β

TGF-β is gaining much interest as an antiatherosclerotic mediator. Because TGF-β neutralizing antibodies and soluble TGF-β receptors were first used, demonstrating an antiatherosclerotic effect of TGF-β in apoE−/− mice and, recently, atherogenesis in LDLR−/− mice was suppressed by adenovirus-mediated delivery of activated TGF-β1. The availability of mice with T cell-specific abrogation of TGF-β signaling made it possible to establish T cells as key targets of the antiatherosclerotic property of TGF-β. These studies showed a potent lesion-stabilizing effect of TGF-β and, in some cases, an effect on lesion size, depending on the extent of TGF-β neutralization or TGF-β signaling ablation or on other differences between the models. In addition to dampening atherogenic T cells, TGF-β may target endothelial cells, DCs, macrophages, and SMCs. Recruitment of leukocytes into the lesion, foam cell formation, and disease-promoting adaptive immune responses are therefore all likely to be inhibited by TGF-β. Moreover, the formation of a stable lesion may be promoted by TGF-β through the induction of collagen and smooth muscle cell synthesis of tissue inhibitors of MMPs.

If the immune dampening effect of TGF-β is crucial in atherosclerosis, which cells are the key producers of TGF-β within the atherosclerotic lesion? Regulatory T cell subsets that express TGF-β are likely to be important, but in principle every cell type within an atherosclerotic lesion is capable of TGF-β secretion. At sites of plaque rupture, TGF-β can also be released from degranulated platelets in the thrombus.

IL-10

IL-10 is mainly produced by macrophages, DCs, and T cells, and its antiatherogenic property has been demonstrated using gain and loss of function strategies in atherosclerosis-prone mouse models. IL-10 has an inhibitory effect on lesion size and promotes plaque stabilization.

When IL-10 was first shown to inhibit atherogenesis, the prevailing interpretation was that of a protective Th2 response, because Th2 cells can produce IL-10. But today, IL-10 is usually not counted as one of the Th2 cytokines, and there is furthermore no proof for a polarization to a Th2 phenotype by IL-10 in the aforementioned studies. So how does IL-10 protect? IL-10 reduces cytokine secretion by macrophages, Th1 and Th2 cells and, in particular, overexpression of IL-10 leads to reduced IFN-γ production. Furthermore, IL-10 reduces the expression of certain MMPs and stimulates expression of TIMPs in macrophages. It also inhibits apoptosis, contributing to the formation of a stable plaque phenotype.

Some possibilities remain that have not been addressed in these studies: First, both IL-10 and TGF-β induce differentiation of regulatory cells. Overexpression of IL-10 may therefore lead to increased populations of Tr1 cells (see below). IL-10 transgenic mice also have expanded populations of tolerogenic DCs that may induce the development of regulatory T cells. The regulatory
cells may alleviate atherosclerotic disease through the inhibition of inflammation in plaques. Second, because IL-10 enhances the production of TGF-β (and vice versa), overexpression of IL-10 may result in increased expression of TGF-β, further inhibiting the activity of T cells and APC and promoting stable plaque formation. Because IL-10 increases TGF-βRII expression, IL-10 deficiency may lead to insufficient TGF-βR expression and decreased TGF-β sensitivity. Finally, IL-10 may reduce the expression of adhesion molecules on the endothelial surface, thus affecting leukocyte migration.

**Regulatory T Cells as the Panacea?**

CD4+CD25+ regulatory T cells, so-called natural Tregs, are implicated in the maintenance of self-tolerance and control of autoimmunity. They express the transcription factor FoxP3, and their suppressive effect appears to be contact-dependent, perhaps mediated by TGF-β.141 FoxP3-expressing T cells are present in human atherosclerotic plaques,142 and Foxp3 mRNA is expressed in the aorta of ApoE−/− mice.143 CD4+CD25+ Treg cells play a protective role in atherosclerosis, which was demonstrated using injections of anti-CD25 antibodies in apoE−/− mice.59 Such antibodies do not deplete FoxP3+ Tregs but appear to inhibit their function.144 Anti-CD25 antibodies failed to influence lesion development in apoE−/− mice with abrogated TGF-β signaling in T cells, indicating that such cells are refractory to the inhibitory effect of CD4+CD25+ T cells. Treatment of LDLR−/− mice with nonmitogenic anti-CD3 antibodies that stimulate the activation and proliferation of Tregs resulted in regression of established lesions (F. Mach, personal communication). Thus, such a regimen may have a therapeutic benefit even at later stages of the disease. The role of Tregs was further demonstrated by using CD80/CD86-deficient and CD28-deficient mouse models in which Treg numbers and function are reduced.59 Atherosclerosis-prone mice that were reconstituted with such cells displayed increased lesion formation.

Tr1 cells develop under stimulation with IL-10 and suppress through secretion of IL-10.145 Ovalbumin-specific Tr1 cells reduced lesion size and elevated IL-10 levels in apoE−/− mice compared with mice that had not received Tr1 cells,146 implying that Tr1 cells may be used to modulate the development of atherosclerosis. The complex experimental set-up should, however, raise some caution in interpretation because it involved transfer of cells to histoincompatible recipients. Yet the study demonstrated that the effector arm of the atherogenic immunity can be dampened by Tr1 cells.

CD4+ T cells expressing the platelet-endothelial cell adhesion molecule, CD31 (PECAM) has been reported to downregulate activation of human T cells. In the circulation of patients with atherosclerotic abdominal aortic aneurysms and in mice with plaque thrombosis, this T cell subset was reduced.147,148 Further studies will be needed to address the importance of CD31+ T cells.

**Why Have We Not Come Further?**

If we critically evaluate the progress of T cell research in experimental models of atherosclerosis compared with that in disease models like diabetes, EAE, IBD, and arthritis, we are clearly lagging behind. Why is that? A major obstacle is the deficiency of adequate methods to isolate these cells from the disease site; the lesions in the mouse are small and, although important, the numbers of T cells are not large enough to permit analysis with current methods of tissue

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**TABLE 2. Experimental Models Showing Antiatherogenic Effects of Cytokines That Are Related to T Cell Function**

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<th>Cytokine</th>
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<td>IL-10</td>
<td>IL-10−/− B6</td>
<td>Pinderski Oslund et al 1999</td>
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<td>IL-10 Tg (IL-2 promoter) B6</td>
<td>Systemic and local adenovirus-mediated transfer of IL-10 in LDLR−/−</td>
<td>Von der Thüsen et al 2001</td>
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<tr>
<td>BMT of IL-10 Tg (IL-2 promoter) to LDLR−/−</td>
<td>Pinderski Oslund et al 2002</td>
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<td>ApoE−/−IL-10−/−</td>
<td>Caligiuri et al 2003</td>
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<td>IM gene transfer of IL-10 cDNA in apoE−/−</td>
<td>Namik et al 2004</td>
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<td>IL-10−/− BMT to LDLR−/−</td>
<td>Potteaux et al 2004</td>
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<tr>
<td>Transfer of IL-10−/− splenocytes to LDLR−/−</td>
<td>Potteaux et al 2004</td>
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<td>TGF-β</td>
<td>Neutralizing antibodies in apoE−/−</td>
<td>Mallat et al 2001</td>
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<td>Recombinant soluble TGFβRII in apoE−/−</td>
<td>Luft et al 2002</td>
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<td>BMT of CD2-dnTGFβRII Tg to LDLR−/−</td>
<td>Gojova et al 2003</td>
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<td>CD4-dnTGFβRII Tg × apoE−/−</td>
<td>Robertson et al 2003</td>
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<td>Adenovirus-mediated transfer of activated TGF-β in LDLR−/−</td>
<td>Li et al 2006</td>
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<td>IL-5</td>
<td>BMT of IL-5−/− to LDLR−/−</td>
<td>Binder et al 2004</td>
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</table>

B6 indicates wild-type C57BL/6 mice; BMT, bone marrow transfer; IM, intramuscular; Tg, transgene.

Transgenic constructs under the IL-2 promoter are expressed in activated T cells.

Transgenic constructs under the CD2 promoter are expressed in T cells.

Transgenic constructs under this particular CD4 promoter are expressed in CD4+ and CD8+ T cells.
digestion. As a consequence, we are unable to study the phenotype of single cells from the lesions by fluorescence-activated cell sorter but are directed instead to immunohistochemistry or immunofluorescence, which are often less informative. Because we cannot isolate T cells from lesions, we are also prevented from performing cell transfers to study their function. Instead, recent studies using straightforward yet informative techniques such as anti-CD25 antibody injection in an atherosclerotic mouse highlight the importance of T cell regulation in atherosclerosis.\(^{29}\)

Another hurdle is the limited knowledge regarding secondary lymphoid tissue where the priming of the atherogenic T cells takes place, such as the draining LN. Furthermore, we know too little about the antigens responsible for triggering T cells in human atherosclerosis or in our experimental models.

New methods offer new possibilities for advancing our knowledge of “athero-immunology.” Laser microdissection and single-cell polymerase chain reaction (PCR) may be useful to phenotype T cells in atherosclerotic plaques. Xenograft models, in which human lesions are implanted into immunodeficient mice,\(^ {131}\) is another under-utilized technique, albeit limited by the differences between the human and murine immune system. Perhaps, in the future, humanized mouse models will provide novel opportunities for studying T cells from human plaques.

**Conclusion**

The fine-tuning between pro-inflammatory and anti-inflammatory factors may determine whether an atherosclerotic lesion will develop into a silent stable plaque or if a cascade of activating events will lead to immune activation, loss of EC matrix, weakening of the fibrous cap, and, finally, a rupture that leads to myocardial infarction or a stroke. T cells play important roles on both sides of this balance.

We are still missing crucial information such as the importance of antigen-specific versus unspecific T cell activations, and which the immunodominant epitopes are in the T cell antigens that are implicated in atherosclerosis. Thus, we need new techniques and approaches in the field of atheroimmunology to learn more about the specific events on cell and molecular levels that lead to or prevent the formation of a vulnerable life-threatening atherosclerotic plaque.

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**Disclosures**

None.

**References**


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