

The immune system in atherosclerosis

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Cardiovascular disease, a leading cause of mortality worldwide, is caused mainly by atherosclerosis, a chronic inflammatory disease of blood vessels. Lesions of atherosclerosis contain macrophages, T cells and other cells of the immune response, together with cholesterol that infiltrates from the blood. Targeted deletion of genes encoding costimulatory factors and proinflammatory cytokines results in less disease in mouse models, whereas interference with regulatory immunity accelerates it. Innate as well as adaptive immune responses have been identified in atherosclerosis, with components of cholesterol-carrying low-density lipoprotein triggering inflammation, T cell activation and antibody production during the course of disease. Studies are now under way to develop new therapies based on these concepts of the involvement of the immune system in atherosclerosis.

Cardiovascular disease is the leading cause of mortality in many countries, accounting for 16.7 million deaths each year^{1,2}. Coronary artery disease (CAD) and cerebrovascular disease are the most common forms of cardiovascular disease, and they have severe consequences both for the individual person and society at large. Their underlying pathological process is atherosclerosis, a slowly progressing chronic disorder of large and medium-sized arteries that becomes clinically manifest when it causes thrombosis³. For many years it was believed that atherosclerosis was merely passive accumulation of cholesterol in the vessel wall. Today, the picture is much more complex, with atherosclerosis being thought of as a chronic inflammatory disease. This review provides an overview of the role of innate and adaptive immune mechanisms in atherosclerosis.

The atherosclerotic plaque is characterized by an accumulation of lipids in the artery wall, together with infiltration of immunocytes, such as macrophages, T cells and mast cells, and the formation by vascular smooth muscle cells of a fibrous cap composed mostly of collagen. Early lesions called 'fatty streaks' consist of subendothelial depositions of lipids, macrophage foam cells loaded with cholesterol and T cells (Fig. 1). Over time, a more complex lesion develops, with apoptotic as well as necrotic cells, cell debris and cholesterol crystals forming a necrotic core in the lesion. This structure is covered by a fibrous cap of variable thickness, and its 'shoulder' regions are infiltrated by activated T cells, macrophages and mast cells, which produce proinflammatory mediators and enzymes⁴. Plaque growth can cause stenosis (narrowing of the lumen) that can contribute to ischemia in the surrounding tissue.

Thrombosis is triggered at the surface as a plaque ruptures. This leads to exposure of thrombogenic material in the core and is fol-

lowed by platelet aggregation, humoral coagulation and formation of a thrombus that may either obliterate the lumen immediately or detach to become an embolus that can block blood flow distal to its point of origin. Atherothrombosis elicits ischemia, with myocardial infarction and brain infarction (ischemic stroke) as life-threatening consequences. Commonly used experimental mouse models, such as mice rendered hypercholesterolemic by targeted deletion of genes encoding molecules involved in cholesterol metabolism (such as apolipoprotein E (*ApoE*^{-/-} mice) or the receptor for low-density lipoprotein (LDL; *Ldlr*^{-/-} mice)), are very useful for delineating the mechanisms of disease initiation and early growth. However, they are not particularly helpful in studies of plaque rupture and thrombosis, which are still based mainly on histopathological and clinical studies. The field clearly needs reliable, quantitative models for this phase of the disease.

LDL initiates vascular inflammation

Animal experiments, epidemiological studies and clinical investigations have established that high circulating concentrations of cholesterol promote atherosclerotic cardiovascular disease. Cholesterol is transported in the blood by LDL. These particles contain esterified cholesterol and triglycerides surrounded by a shell of phospholipids, free cholesterol and apolipoprotein B100 (ApoB100). Circulating LDL particles can accumulate in the intima, the innermost layer of the artery. Here ApoB100 binds to proteoglycans of the extracellular matrix through ionic interactions⁵. This is an important initiating factor in early atherogenesis⁶. As a consequence of this subendothelial retention, LDL particles are trapped in the intima, where they are prone to oxidative modifications caused by enzymatic attack of myeloperoxidase and lipoxygenases, or by reactive oxygen species such as HOCl, phenoxyl radical intermediates or peroxynitrite generated in the intima during inflammation and atherosclerosis. The peroxidation of fatty acid residues in phospholipids, cholesteryl esters and triglycerides generates reactive aldehydes and truncated lipids. Among the latter, modified phospholipids such as lysophosphatidylcholine and oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phos-

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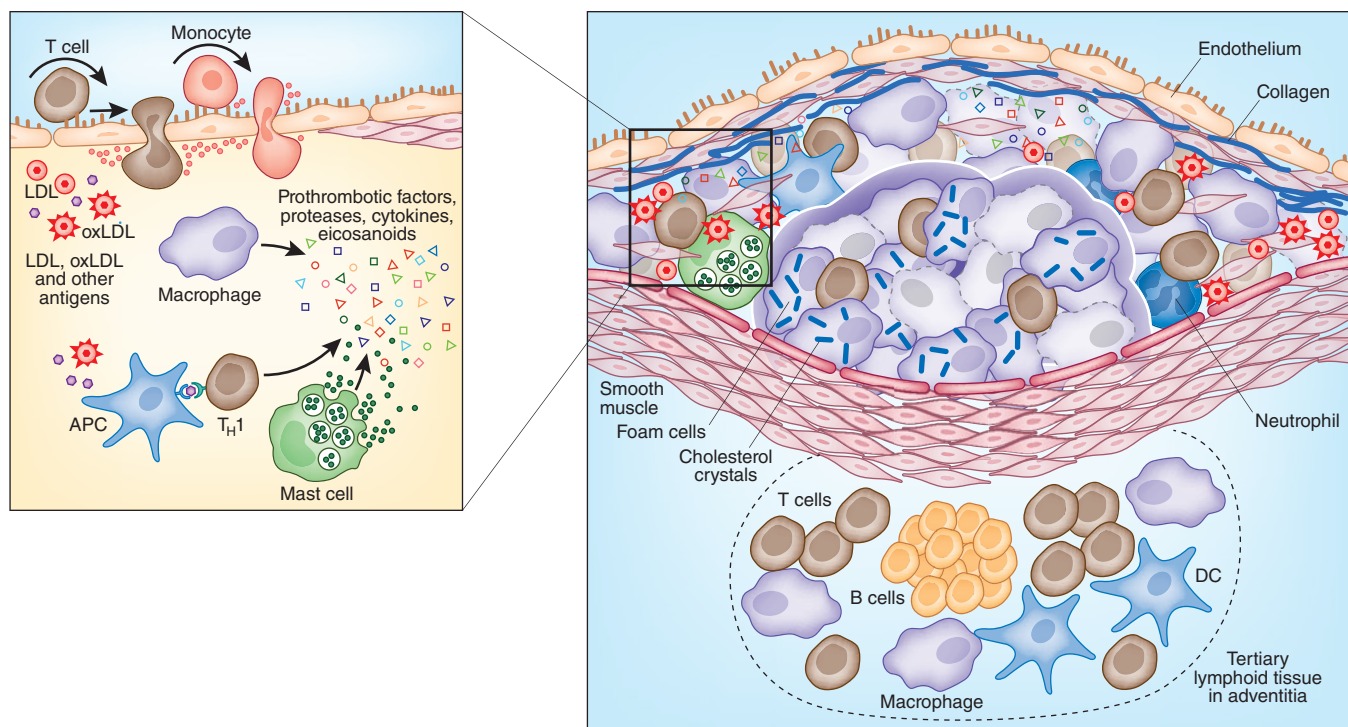


Figure 1 Immune components of the atherosclerotic plaque. The atheroma has a core of lipids, including cholesterol crystals, living and apoptotic cells and a fibrous cap with smooth muscle cells and collagen. Plasma lipoproteins accumulate in the subendothelial region. Several types of cells of the immune response are present throughout the atheroma including macrophages, T cells, mast cells and DCs. The atheroma builds up in the intima, the innermost layer of the artery. Outside the intima, the media contains smooth muscle cells that regulate blood pressure and regional perfusion, and further abuminally, the adventitia continues into the surrounding connective tissue. Here, cells of the immune response accumulate outside advanced atheroma and may develop into tertiary lymphoid structures with germinal centers. APC, antigen-presenting cell.

phocholine can initiate innate inflammatory responses. These lipids activate endothelial cells and macrophages to produce adhesion molecules and chemokines. The mechanisms that mediate this response are not fully understood but seem to involve the early growth response 1 pathway⁷ and Jak kinase–STAT transcription factor pathway⁸ and the unfolded protein response⁹. Oxidized LDL (oxLDL) and components thereof have also been reported to activate innate immunity by binding to Toll-like receptors (TLRs), although this is controversial (as will be discussed below).

Oxidation not only leads to release of bioactive lipids, it also causes modification of the remaining LDL particle. With ongoing oxidation, the physicochemical properties gradually change, including alterations in charge, particle size, lipid content and other features. The precise nature of each of these alterations obviously depends on the oxidizing agent. For all these reasons, 'oxidized LDL' is not a defined molecular species but is instead a spectrum of LDL particles that have undergone a variety of physicochemical changes.

Malondialdehyde, 4-hydroxynonenal and other molecular species generated through lipid peroxidation can form adducts on lysyl residues of ApoB100. Proteins with such modified lysyl residues can be immunogenic, as are modified phospholipid species. Antibodies to such phospholipids inhibit the binding of oxLDL to macrophages and have shown atheroprotective effects in animal experiments^{10–12}. These antibodies recognize not only oxidatively modified phospholipids in oxLDL and apoptotic cell membranes but also phosphocholine in the cell wall of *Staphylococcus aureus* (pneumococcus)¹⁰. The finding of immunological cross-reactions between oxLDL and the pneumococcal cell wall raises the question of whether molecular mimicry between pathogens and LDL could lead to atheroprotective immune activity.

The activation of endothelial cells by components of oxLDL, and possibly also by the turbulent blood flow at arterial branching points, lead to the expression of adhesion molecules such as E-selectin and VCAM-1 on the endothelial surface of the artery. This acts in synergy with chemokines such as CCL2, CCL5, CXCL10 and CX3CL1 to attract monocytes, dendritic cells (DCs) and T cells into the intima¹³ (Fig. 2). Monocytes in the intima are stimulated by macrophage colony-stimulating factor produced by activated endothelial cells to differentiate into macrophages; this process is necessary for development of atherosclerosis¹⁴. In the intima, macrophages upregulate their scavenger receptors that can then take up oxLDL. The ensuing cholesterol accumulation eventually turns these macrophages into the foam cells that are characteristic of the atherosclerotic lesion. DCs that patrol arteries may take up LDL components for subsequent antigen presentation in regional lymph nodes (Fig. 2). In the normal artery wall, resident DCs are thought to promote tolerization to antigen by silencing T cells; however, danger signals generated during atherogenesis may activate DCs, leading to a switch from tolerance to the activation of adaptive immunity^{15,16}.

T cells are recruited in parallel with macrophages, by similar mechanisms involving adhesion molecules and chemokines⁴ (Fig. 2). They are not as abundant, with a macrophage/T cell ratio of between approximately 4:1 and 10:1 in human lesions. However, T cells are activated in lesions, produce proatherogenic mediators and contribute to lesion growth and disease aggravation^{4,17}. Finally, B cells and mast cells are present only occasionally in lesions but are abundant on the abluminal, adventitial side of the atherosclerotic artery^{18,19}. Indeed, tertiary lymphoid structures are often associated with regions of advanced atherosclerosis (Fig. 1). All these observations indicate

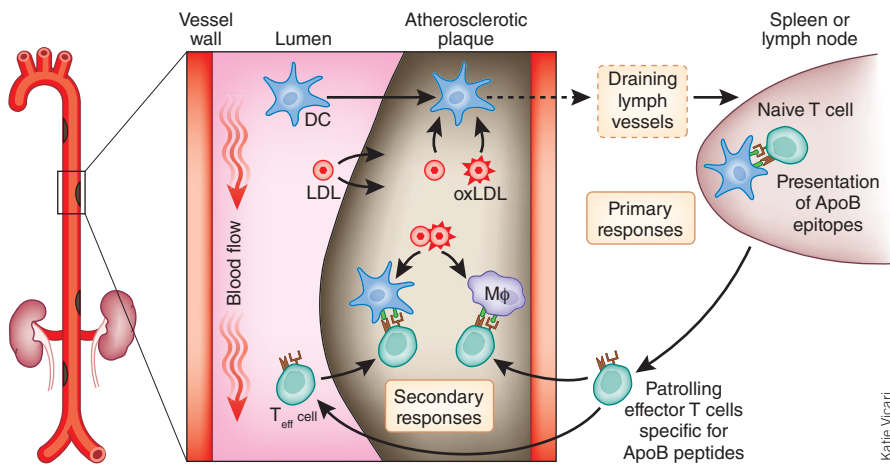


Figure 2 T cell activation in the vessel wall. The aorta at left has several atherosclerotic plaques (dark ovals). DCs emigrate from the blood to arteries, take up antigens such as ApoB100 of LDL, and migrate to draining lymph nodes, where they can present antigens to naive T cells. After activation, these cells develop into effector T (T_{eff}) cells that enter the bloodstream. When effector T cells are recruited into atherosclerotic plaques, they are reactivated by antigen presented by local macrophages (M ϕ) and DCs.

that adaptive as well as innate immune mechanisms have important roles in atherosclerosis.

A major role for innate immunity in atherosclerosis

The defense of the normal artery depends on innate immune responses mounted by endothelial cells and, after an inflammatory challenge, by macrophages and other cells of the immune response that are recruited to the artery wall. Such innate immune responses also have a major role in the initiation of atherosclerosis²⁰. They involve internalizing as well as signaling pattern-recognition receptors (PRRs; Fig. 3).

Scavenger receptors that internalize modified LDL particles are multifunctional PRRs that clear the local environment of cell debris, internalize microbes and assist in adhesion and antigen presentation²¹. Scavenger receptors that recognize oxidation-specific epitopes of oxLDL include SRA-1 and SRA-2, MARCO, CD36, SR-B1, LOX-1 and PSOX²¹. Although these receptors undoubtedly serve a major role as mediators of intracellular cholesterol accumulation, their importance in atherosclerosis remains unclear, and gene-knockout studies of hypercholesterolemic mice have provided contradictory results²¹. This may reflect a role for scavenger receptors in the pathway leading to cholesterol efflux from tissues. Intracellular cholesterol that accumulates after scavenger receptor-mediated uptake of oxLDL might be eliminated more easily than are accumulations of extracellular cholesterol in the forming lesion. In the former case, ABC-type cassette transporters can mobilize cholesterol to high-density lipoproteins for export through the liver and bile system²², whereas the extracellular cholesterol pool becomes a hydrophobic barrier that resists elimination. Interestingly, these cholesterol transporters modulate the differentiation of hematopoietic stem cells and thus control the number of circulating monocytes, which is associated with the extent of atherosclerosis²³.

The endothelium of normal and atherosclerotic arteries expresses a broad repertoire of signaling PRRs, including TLR1, TLR2, TLR3, TLR4, TLR5, TLR7 and TLR9 (refs. 24,25). Monocyte-derived macrophages recruited to forming lesions also express a broad range of TLRs as well as other signaling PRRs^{24,25}. Knockout studies of hypercholesterolemic mice have demonstrated a major proatherosclerotic

role for MyD88, a key adaptor protein in the signaling cascades of most TLRs^{26,27}. Targeted deletion of the gene encoding TLR4 also results in less atherosclerosis, albeit to a smaller extent. Of note, MyD88 also participates in the signal-transduction pathway downstream of the receptors for interleukin 1 (IL-1) and IL-18, two proatherosclerotic cytokines^{28,29}. Therefore, part of the diminished disease observed in MyD88-deficient mice probably also reflects the loss of signaling by IL-1 β and IL-18.

Oxidized LDL, and components thereof, can ligate particular TLRs (Fig. 3). Thus, oxLDL and also carboxyethylpyrrol, a phospholipid species generated during oxidation, have been reported to ligate TLR2 and induce vascular responses^{30,31}, whereas minimally modified LDL, an LDL preparation that has undergone brief or low-intensity oxidative attack, binds TLR4 (ref. 32). Further studies will be needed to clarify the role of these ligand-receptor interactions, particularly as

TLRs are PRRs and plasma lipoproteins can serve as transport vehicles for true TLR ligands such as endotoxins. Interestingly, TLR2 expression by vascular rather than blood-borne cells may be particularly proatherosclerotic³³.

In addition to the surface-bound TLRs, signaling PRRs are also present intracellularly. Some of these intracellular PRRs assemble into inflammasomes, which are molecular platforms that can trigger the secretion of IL-18 and IL-1 β ³⁴. The NLRP3 (also known as NALP3) inflammasome has been reported to be activated by cholesterol crystals present in macrophages^{35,36} (Fig. 3). Mice deficient in NLRP3 or IL-1 β expression in macrophages develop not only less inflammation but also smaller atherosclerotic lesions under hypercholesterolemic conditions.

The effector arms of innate immunity include antimicrobial peptides, nitric oxide, eicosanoids and several other molecular species released in response to PRR ligation. Antimicrobial peptides are produced in atherosclerotic lesions and might not only mediate pathogen killing but also promote inflammation³⁷. Whether they contribute to atherosclerosis remains unclear. Several prostaglandins affect vascular function by regulating platelet aggregation and exerting proinflammatory activities^{38,39}. Leukotriene B₄ is also proinflammatory and increases atherosclerosis in mouse models^{40,41}. The leukotriene pathway is expressed in human atherosclerosis, and polymorphisms in genes involved in leukotriene biosynthesis are associated with atherosclerosis and greater risk for myocardial infarction^{42–45}.

Adaptive immunity enters the scene

Components of adaptive immunity are present in human lesions throughout the course of atherosclerosis, and several studies have indicated an important role for antigen-specific adaptive immune responses in the atherogenic process⁴⁶. Studies of mouse models of atherosclerosis, such as *ApoE*^{−/−} or *Ldlr*^{−/−} mice, in combination with mice deficient in both B cells and T cells, have demonstrated a substantial role for the adaptive arm of immunity in atherosclerosis. The progeny of *ApoE*^{−/−} mice crossed with lymphocyte-deficient mice lacking recombination-activating gene 1 or 2 or mice with severe combined immunodeficiency have much less atherosclerosis^{47,48}.

Although the results noted above have been confirmed by studies showing a pathogenic role for proinflammatory CD4⁺ T cells

(discussed below), other experiments have suggested that B cells have a protective role. Splenectomy aggravates atherosclerosis in *Apoe*^{-/-} mice, whereas transfer of splenic B cells from aged atherosclerotic *Apoe*^{-/-} mice has a protective effect on splenectomized recipients⁴⁹. Transfer of bone marrow from B cell-deficient μ MT mice into *Ldlr*^{-/-} mice has shown that B cells and/or antibodies are protective in both early and late atherosclerosis⁵⁰. In line with those results, bone marrow–chimeric *Ldlr*^{-/-} mice lacking IL-5, a cytokine that promotes the population expansion of B-1 cells, have lower concentrations of immunoglobulin M (IgM) antibodies to phosphocholine and, concomitantly, more atherosclerosis⁵¹. Reports demonstrating the atheroprotective effects of B cell-depleting antibody to CD20 (anti-CD20)⁵² and the proatherosclerotic effects of transferred B-2 cells, but not of B-1 cells⁵³, suggest that certain subsets of B cells exert contrasting effects on disease. Of note, plasma cells are not depleted by anti-CD20, and B220^{lo}IgM⁺ B cells and IgM production are also affected less than IgG-producing B cells are.

Antibodies to oxLDL in particular are atheroprotective. Many experimental studies of rabbits and mice in which oxLDL is used for immunization have shown a positive correlation between high titers of anti-oxLDL and the degree of protection against atherosclerosis^{54–56}. Accordingly, infusion of anti-LDL results in less atherosclerosis in hypercholesterolemic mice¹². As is often the case, the situation is more complex in humans, with various studies showing a positive or negative correlation or no correlation between anti-LDL titers and atherosclerosis or its manifestations^{57–60}. Interestingly, titers of IgM and IgG antibodies to oxLDL have been found to show differences in their associations with CAD, which suggests that their biological roles also differ⁶¹.

T lymphocytes: key participants in atherogenesis

T cells of the atherosclerotic plaque are of the memory-effector phenotype and are mostly positive for the $\alpha\beta$ T cell antigen receptor (TCR $\alpha\beta$) and CD4⁺, although many CD8⁺ T cells can also be found, as well as a small population of TCR $\gamma\delta$ ⁺ cells⁴. Clonal expansion of T cells has been demonstrated in lesions from humans and *Apoe*^{-/-} mice^{62,63}; this suggests that antigen-specific reactions take place in the lesion (Fig. 2). This idea is also supported by the finding that *Ldlr*^{-/-} mice in which CD40 ligation is interrupted have smaller lesions⁶⁴. Reconstitution of *Apoe*^{-/-} mice with severe combined immunodeficiency using CD4⁺ T cells from atherosclerotic *Apoe*^{-/-} mice accelerates atherosclerosis, with homing of T cells to the lesions⁴⁸. CD8⁺ T cells stimulated by injection of an agonist to the tumor necrosis factor–like surface protein CD137 or activated toward an artificial antigen expressed by smooth muscle cells increase atherosclerosis in *Apoe*^{-/-} mice^{65,66}. *Ldlr*^{-/-} mice deficient in the inhibitory molecules PD-L1 and PD-L2 have larger plaques with massive lesional infiltration of CD8⁺ T cells, which indicates that these cells might be controlled by PD-1 in atherosclerosis⁶⁷.

Role of helper T cell subsets

Atherosclerosis is driven by the T helper type 1 (T_H1) response. Interferon- γ , the signature T_H1 cytokine, is present in the human

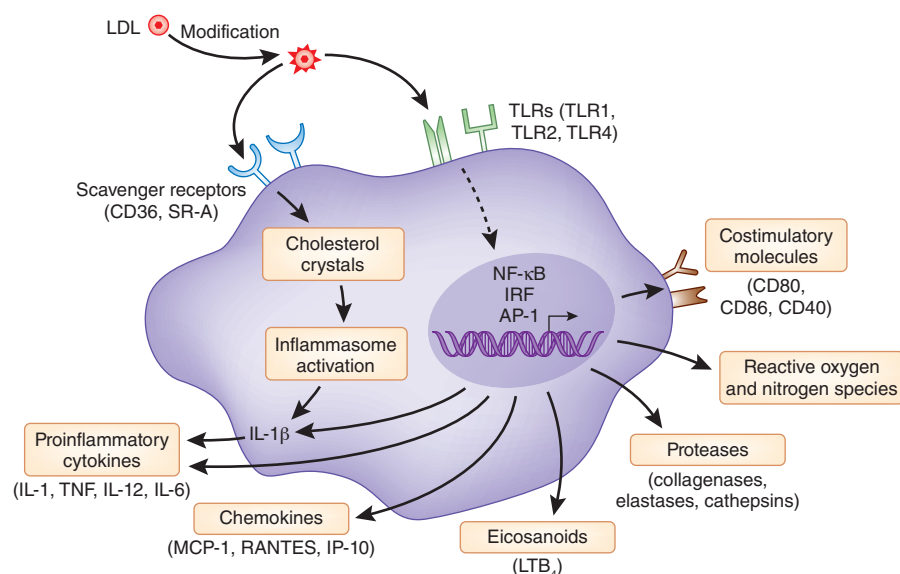
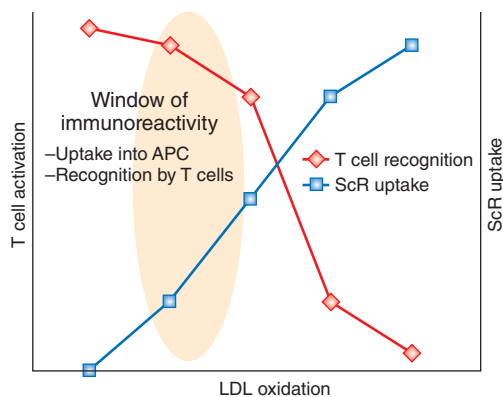


Figure 3 Activation of innate immune responses in the atheroma. Macrophages, DCs and endothelial cells display a large repertoire of PRRs. Uptake of modified LDL particles such as oxLDL through scavenger receptors leads to the intracellular accumulation of cholesterol that can activate the inflammasome, leading to IL-1 β secretion. Components of modified LDL can also ligate TLRs, triggering an intracellular signaling cascade that leads to the expression of a series of genes encoding proinflammatory molecules, including cytokines, chemokines, eicosanoids, proteinases, oxidases and costimulatory molecules. NF- κ B, IRF and AP-1 are transcription factors.

plaque⁴ and has pathogenic effects, including less collagen fiber formation, higher expression of major histocompatibility complex class II, enhanced protease and chemokine secretion, upregulation of adhesion molecules, induction of proinflammatory cytokines, and enhanced activation of macrophages and endothelial cells⁴. Mice deficient in interferon- γ or its receptor have a lower lesion burden, and mice that receive interferon- γ have larger lesions than those of control mice^{68–71}. Injection of IL-12 also promotes the formation of early lesions⁷², whereas targeted deletion of the gene encoding IL-12 or vaccination against IL-12 inhibits early but not late lesion development^{73,74}. Furthermore, mice lacking IL-18, a T_H1-promoting cytokine, have smaller lesions²⁹, whereas mice treated with IL-18 have more atherosclerosis⁷⁵. Finally, targeted deletion of *Tbx21*, which encodes the major T_H1-differentiating transcription factor T-bet, leads to much less lesion development in *Ldlr*^{-/-} mice⁷⁶. Collectively these data demonstrate that T_H1 cells have a major role in the pathogenesis of atherosclerosis. IL-4, the signature cytokine of the T_H2 lineage, is not frequently observed in human plaques⁷⁷, and experimental studies examining the involvement of T_H2 cells are contradictory, with some showing proatherosclerotic effects^{73,78} and others showing protective effects⁷⁹ or no significant effect⁸⁰. IL-33, a powerful inducer of T_H2 responses, results in less atherosclerosis in *Apoe*^{-/-} mice⁸¹. On balance, then, the role of T_H2 immune responses in atherosclerosis remains unclear.

Contradictory data have also been presented for IL-17-producing helper T cells (T_H17 cells). Although IL-17 mRNA seems to be present at low abundance in atherosclerotic plaques, IL-17 protein has been detected in several cell types of human atherosclerotic tissue, including T cells, mast cells, B cells, neutrophils and smooth muscle cells^{82,83}. Studies of *Apoe*^{-/-} mice treated with antibodies or decoy receptors to IL-17, and of *Ldlr*^{-/-} mice reconstituted with IL-17 receptor-deficient bone marrow, suggest a proatherogenic role for this cytokine^{84–86}. In contrast to those studies, mice with a

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Figure 4 Inverse relationship between the uptake of antigen-presenting cells and T cell recognition of oxLDL. With increasing oxidation of LDL, clustered negative charges on its surface molecules are generated and become ligands for scavenger receptors (ScR), leading to uptake by antigen-presenting cells. T cells, in contrast, recognize peptide motifs of native but not oxidized forms of the LDL protein ApoB100. Optimal conditions for antigen uptake, presentation and T cell recognition may exist within a narrow range of LDL oxidation.

preponderance of T_H17 cells due to deficiency of SOCS3, a suppressor of signaling from IL-17 (and several other cytokines), show less disease development⁸⁷. Further studies will be needed to determine the role of T_H17 cells in atherosclerosis, but at present the possibility that these cells and their products have different roles in different phases of atherosclerosis cannot be ruled out.

Several studies have demonstrated a protective effect of various subsets of regulatory T cells (T_{reg} cells) in models of atherosclerosis. Foxp3⁺ cells have been found in the plaques of mice as well as humans, although in low numbers^{88,89}. The T_{reg} cell cytokine products TGF- β and IL-10 have profound atheroprotective effects in mouse models, but it should be kept in mind that these cytokines are also produced by several other cell types. Further evidence for the atheroprotective effect of T_{reg} cells has been provided by mice deficient in CD80-CD86 or CD28, which have fewer T_{reg} cells. Reconstitution of atherosclerotic mice with bone marrow deficient in CD80-CD86 or CD28 leads to more disease⁹⁰. Transfer of natural Foxp3⁺ T cells has also been shown to be protective against experimental atherosclerosis^{90,91}.

Peripheral T_{reg} cells can be induced by mucosal administration of antigen or anti-CD3. Nasal immunization of *ApoE*^{-/-} mice with an ApoB100 peptide fused to the B subunit of cholera toxin that binds to mucosal gangliosides leads to the induction of ApoB100-specific regulatory Tr1 cells that produce IL-10, as well as less atherosclerosis⁹². *ApoE*^{-/-} mice that receive oral anti-CD3 also have less atherosclerosis associated with the induction of CD4⁺CD25⁺ T_{reg} cells that express the latency-associated peptide of TGF- β ⁹³.

Antigens of atherosclerosis

The clonal expansion of T cells and their clustering in close proximity to DCs and macrophages point to a local immune response in the plaque (Fig. 2). Autoantigens as well as microbial molecules have been linked to this. Both bacterial and viral pathogens have been detected in plaques and may conceivably trigger a local immune response. However, modest (if any) effects on atherosclerosis have been detected in hypercholesterolemic mice treated with bacterial pathogens such as *Chlamydomydia pneumoniae*, and no beneficial effects have been registered in clinical trials using antibiotics to prevent a second myocardial infarction in patients³. Cytomegalovirus and certain bacteria of the oral

flora remain candidate vascular pathogens and could be linked to the disease-associated immune response⁹⁴.

The case for the involvement of autoantigens in the promotion of atherosclerosis is stronger than that for exogenous antigens, although the possibility that the former may be triggered by molecular mimicry cannot be excluded. Two antigens have emerged as being potentially important in this: heat-shock protein 60 (hsp60) and LDL. For both, experiments with hypercholesterolemic mice and rabbits have shown substantial effects on the promotion of disease development, and seroepidemiological studies have also supported the proposal that they have a role in human cardiovascular disease⁹⁵. The antigen hsp60 is extremely well conserved phylogenetically; therefore, antigenic similarities exist between prokaryotic and human hsp60 that could permit cross-reactivity. Normally intracellular, hsp60 is released after necrosis in many tissues. Several studies have shown that adaptive immune responses to hsp60 affect atherosclerosis⁹⁶, with more fatty streak formation after parenteral immunization against this antigen⁹⁷ and atheroprotective immunity after oral tolerization to this protein^{98,99}. The antigen hsp60 has been linked to several inflammatory conditions, including arthritis; therefore, anti-hsp60 reactions are not specific for atherosclerosis. Both adaptive and innate immune responses have been reported to be triggered by hsp60; however, such findings are controversial. An intracellular chaperone, hsp60 is prone to bind other macromolecules, including lipopolysaccharide, and studies suggest that its reported ability to activate TLR4 is in fact due to contamination by lipopolysaccharide¹⁰⁰.

LDL elicits both cellular and humoral immune responses during the course of atherosclerosis. It is a complex particle that contains several B cell and T cell epitopes. When it accumulates in vascular tissue, it undergoes a series of oxidative and enzymatic modifications that generate additional, potentially immunogenic structures¹⁰¹. Indeed, circulating antibodies in patients and experimental animals recognize oxidation-induced epitopes on LDL particles. Although some of these antibodies represent T cell-dependent IgG responses, others are natural antibodies, usually of the IgM class, that recognize phosphocholine present not only on oxLDL but also in the cell wall of *Streptococcus pneumoniae*¹⁰.

T cell clones reactive to LDL preparations have been isolated from human plaques¹⁰², and antibodies to LDL are abundant in patients with atherosclerosis. Adoptive transfer of LDL-reactive T cells accelerates atherosclerosis in hypercholesterolemic mice¹⁰³, whereas immunization against oxidized LDL particles results in less atherosclerosis^{55,56}. Interestingly, parenteral immunization with native LDL⁵⁶ or peptides derived from its ApoB100 protein¹⁰⁴, as well as mucosal immunization to native LDL peptides, also produce atheroprotective effects^{11,92}.

Antigen-presenting macrophages and DCs readily take up oxLDL. Scavenger receptors on these cells internalize oxLDL and other antigens not only for degradation²¹ but also for antigen processing and presentation to T cells¹⁰⁵. DCs loaded with oxLDL and injected into *ApoE*^{-/-} mice induce a T cell response to components of LDL; this response is associated with more atherosclerosis¹⁰⁶. In contrast, 'tolerogenic' DCs that had been treated with IL-10 while being loaded with ApoB100 inhibit disease¹⁰⁷. Therefore, the DC phenotype, cytokines present in the local milieu, concentration of antigen and possibly other factors together determine the type of immune response—proatherosclerotic or atheroprotective—elicited by LDL preparations.

Tolerance and reactivity to LDL

LDL is a major circulating plasma component with a concentration of approximately 2–3 mM; therefore, immunological tolerance to this

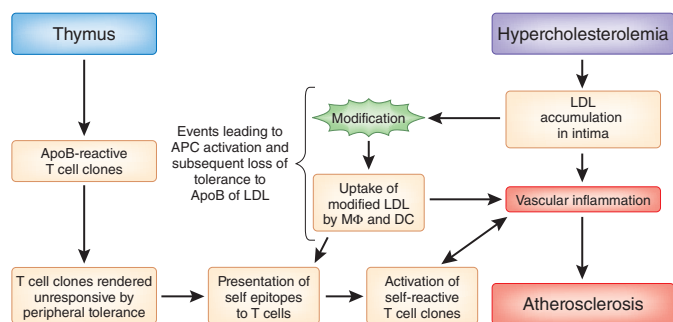


Figure 5 Mechanisms of LDL tolerance and autoreactivity: a hypothesis. ApoB100-reactive T cell clones that escape thymic education are probably kept in check by peripheral tolerance mechanisms. When LDL accumulates in the vessel wall, it undergoes modifications that elicit an inflammatory response and also permits uptake by antigen-presenting cells and antigen presentation of ApoB100 epitopes. This leads to the activation of ApoB100-reactive T cells, which contribute to the atherogenic process.

particle is necessary for survival. LDL-reactive T cells were thought to be eliminated by negative selection, leading to central tolerance. Oxidation of LDL was thought to generate neoantigens, and all T cell clones reactive to these would thus not be removed during thymic education. Data have now challenged that hypothesis by showing that peripheral T cells in atherosclerotic mice recognize peptide motifs of native LDL particles and ApoB100, the protein moiety of LDL¹⁰⁸. Surprisingly, oxidation extinguishes rather than promotes LDL-dependent T cell activation¹⁰⁸ (Fig. 4). Immunization against a TCR involved in the recognition of ApoB100 not only induces blocking antibodies that diminish T cell responses to this antigen but also diminishes the extent of disease¹⁰⁸. This indicates that cellular immunity toward native LDL protein might have a pathogenetic role in atherosclerosis. The existence of peripheral T cells that recognize native LDL suggests that central tolerance to this autoantigen is far from complete. Accordingly, potentially pathogenic T cells able to recognize LDL epitopes might be present in the adult organism but are probably kept in check by peripheral tolerance mechanisms (Fig. 5).

As discussed above, LDL oxidation generates a range of modifications with various physicochemical properties. Whereas heavily oxidized LDL particles show little similarity to native ones, more subtle oxidative events initially cause limited changes to LDL and the particles maintain most of the features of native particles, including antigenicity. Such minimal modifications are difficult to detect by biochemical methods; it is also difficult to completely prevent minimal oxidation when LDL is prepared from human blood. For all these reasons, the understanding of LDL immunochemistry is still limited and further studies will be needed to clarify the role of oxidation for autoimmune responses to LDL.

Metabolic regulation of immunity and inflammation

Inflammatory responses generated through the adaptive arm as well as the innate arm of immunity are modulated by signals that are generated in cellular and systemic metabolism and are targeted by several commonly used drugs. By binding to promoter elements of key genes of the immune response, nuclear receptors such as the glucocorticoid receptor, estrogen receptors, vitamin D receptor, retinoic acid receptors, lipid X receptors and proteasome proliferator-activated receptors regulate a broad spectrum of immune effector responses. For example, estrogen receptors inhibit activation of the transcription factor NF- κ B, whereas retinoic acid receptors modulate T cell proliferation and T_{reg} cell development. Proteasome proliferator-activated receptor- γ inhibits T cell activation by interacting with the transcription factor NFAT and also the transcription of genes encoding IL-1 β , CCL2, IL-12 and other proinflammatory effector molecules. These events probably affect atherogenesis; several excellent reviews have provided details on these processes^{109,110}.

An additional level of regulation depends on products of the cholesterol biosynthesis pathway. Farnesyl and geranyl-geranyl intermediates generated downstream of mevalonic acid bind to a set of enzymes and cotranscription factors, thus regulating their activity. Such events, usually called 'isoprenylation', control the activity of endothelial nitric oxide synthase, the major histocompatibility complex class II transactivator, and the small GTPase RhoA¹¹¹. By lowering the cholesterol content of cellular membranes, statins may also affect receptor clustering in lipid rafts. This is thought to be important for signaling through the TCR as well as hematopoietic growth factors^{112,113}. Consequently, statins dampen the activity of several autoimmune conditions, including experimental autoimmune encephalomyelitis and rheumatoid arthritis^{114,115}.

Atherosclerosis emphasizes the role of inflammation

A difficult case for genetic epidemiology

Atherosclerotic cardiovascular disease is among the most thoroughly investigated disease groups from an epidemiological point of view. Although classical epidemiology has established that high concentrations of plasma cholesterol, high blood pressure, cigarette smoking and diabetes are independent risk factors for CAD and other manifestations of atherosclerosis, genetic epidemiology has until now provided limited additional information. Familial hypercholesterolemia is one of the more common monogenic disorders, with an allele frequency of about 1:300–1:500, but it is too rare to show up in most genome-wide association studies. A set of genetic risk factors have been identified in small and medium-sized studies of single-nucleotide polymorphisms, including genes encoding costimulatory factors (such as OX40L), the major histocompatibility complex class II transactivator and components involved in the biosynthesis pathway of proinflammatory leukotrienes^{45,116–118}. However, such genes have not shown up in large genome-wide association studies. Genes in the HLA-DR locus are associated with plasma lipid concentrations¹¹⁹, but they have not risen to the top of the skyline in Manhattan plots of genome-wide association studies focusing on CAD. It is unclear whether this reflects a limited importance or other reasons. Of note, CAD is approximately 10–20 times more common than rheumatoid arthritis and is nearly 100 times more prevalent than multiple sclerosis. Therefore, it is unlikely that a single HLA allele would carry disease susceptibility.

Atherosclerosis emphasizes the role of inflammation

Case-control studies have shown that patients with several chronic inflammatory diseases have a significantly greater risk of coronary artery disease. Patients with rheumatoid arthritis have a twofold higher incidence of CAD, those with systemic lupus erythematosus have an even higher risk, and patients with psoriasis also develop more CAD¹²⁰. Ongoing studies suggest that CAD starts to manifest a few years after the debut of rheumatoid arthritis but is not prevalent before its start¹²¹. Therefore, it seems more likely that the inflammatory status of rheumatoid arthritis promotes the vascular inflammation of atherosclerosis rather than that rheumatoid arthritis and CAD share risk genes. Follow-up studies suggest that when administered early in the course of rheumatoid arthritis, blockade of tumor necrosis factor results in a lower risk of CAD¹²². In contrast, blockade of tumor necrosis factor does not have a beneficial effect in heart

failure, an end-stage condition that can be caused not only by CAD but also by cardiomyopathy and several other diseases¹²³. It will be important to continue to expand such studies to assess the effect of anti-inflammatory therapy on CAD¹²⁵.

Conclusions

Clinical and histopathological studies of patient groups have identified inflammatory mechanisms as being pathogenetically important in atherosclerosis. They have shown that components of innate immunity as well as adaptive immunity are involved in the disease process and that biomarkers of inflammation carry a predictive value for CAD. Components of plasma lipoproteins that accumulate in atherosclerotic arteries can trigger PRRs of innate immunity and serve as autoantigens for cellular and humoral immune reactions. Many experimental studies support the idea of a major role for such immune mechanisms in atherosclerosis and have identified several potential targets for therapy.

In humans, inflammation is an independent risk factor for manifestations of atherosclerosis, but the gene-environment interactions and pathogenetic mechanisms involved remain unclear. However, studies showing more cardiovascular morbidity in patients with chronic inflammatory diseases point to a disease-promoting role for systemic inflammation in atherosclerosis. Further studies will be needed to evaluate the use of immune-directed therapies in atherosclerotic cardiovascular disease.

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The authors declare competing financial interests: details accompany the full-text HTML version of the paper at <http://www.nature.com/natureimmunology/>.

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