

Traditional risk factors like hypercholesterolemia are important for atherogenesis, but it is now apparent that the immune system also plays an important role. Uncovering the mechanisms by which specific components of the immune system impact atherogenesis will not only provide new insights into the pathogenesis of lesion formation, but could also lead to novel therapeutic approaches that involve immune modulation.

Innate and acquired immunity in atherogenesis

Atherosclerosis is a chronic disease that begins in fetal life, slowly progresses during childhood and adolescence, and then accelerates in fits and spurts in adult life to result in plaque erosion or rupture, effecting morbid or fatal clinical events. Autopsy studies have confirmed the presence of advanced and obstructive lesions in young and clinically robust individuals, and intracoronary ultrasound studies have demonstrated the remarkable prevalence of coronary atherosclerosis in 37% of 'healthy' heart donors 20–29 years of age, 60% of those 30–39 years of age and 85% of those over 50 years of age¹. Therefore, it is not unexpected that the initial manifestation of cardiovascular disease (CVD) in 50% of patients is either sudden death or myocardial infarction. These data emphasize the need to intervene early in the 'natural history' of atherosclerosis to prevent the development of clinical disease and death.

Hypercholesterolemia seems to be an absolute prerequisite for lesion initiation and progression. If all adults had lifelong ideal plasma cholesterol (below 150 mg/dl), there would probably be few if any cases of symptomatic disease. However, there is diversity in expression of disease even in cases of extreme hypercholesterolemia: a 3-year-old male with homozygous familial hypercholesterolemia developed myocardial infarction and died, whereas a 33-year-old female with the same mutation in the gene encoding the low-density-lipoprotein receptor (LDLR) and the same plasma cholesterol concentration (800 mg/dl) died from a non-coronary cause².

What accounts for these differences in the rate of lesion formation and clinical presentation? For the most part, this is unknown; furthermore, because atherosclerosis is a complex disease whose etiology is multifactorial, it is likely that different factors will dominate in different individuals³. There are many 'traditional' risk factors discovered by classical epidemiology that define much of the risk, such as hypercholesterolemia, smoking, male gender, hypertension, diabetes and age. However, newly defined 'non-traditional' risk factors are emerging as being equally important. Among these, it is now apparent that the immune system, when considered in the broadest context, is one of the dominant factors, aside from cholesterol, modulating atherogenesis^{4–6}. Here we will review the rationale for immune involvement—the evidence that both adaptive and innate immune mechanisms come into play in response to antigens in the atherosclerotic lesion and that these responses can modulate lesion development. Many of the immune responses involved in atherogenesis most likely evolved as responses to other pathogens that are more fundamentally important for the survival of the host, and provide examples of antigens

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that show molecular mimicry between epitopes of atherosclerosis-associated antigens and other endogenous and exogenous pathogens. Understanding the mechanisms by which the immune system affects atherogenesis not only will provide new insights into the pathogenesis of lesion formation but also

could lead to new therapeutic approaches that involve immune modulation.

Why is the immune system involved?

Many genes are involved in immune function, indicative of the vital survival advantage of such a complex system. Atherosclerosis is manifested well beyond the reproductive period and thus could not have exerted evolutionary pressure for any selected immune response. However, it is now recognized that there are responses to inflammatory components of the atherogenic process that are shared with similar disease processes in infectious as well as other acute and chronic diseases. It is likely that those other diseases have 'selected' certain immune responses that in turn affect atherogenesis for better or worse. The fundamental appreciation that inflammation is an important and possibly even obligatory component of lesion initiation and progression, and also participates in the plaque rupture that mediates thrombotic complications and clinical events, has fundamentally changed the view of the pathogenesis of atherosclerosis^{7,8}. Immune activation must be viewed in the context of responses to inflammatory components of atherogenesis.

Inflammation is a process in which blood leukocytes leave the vascular space and enter a tissue site in response to a perceived pathogen. In atherosclerotic lesions, the nature of the inciting pathogen is not entirely clear. There are probably several different candidate pathogens or pathogenic processes that can elicit localized inflammatory responses in the artery; one prime set of candidates is minimally oxidized LDL (called minimally modified LDL, or mmLDL) and late forms of oxidized LDL (oxLDL). Once trapped in the artery wall by binding to extracellular proteoglycans, a key event in atherogenesis^{9,10}, LDL is oxidized by mechanisms not yet understood¹¹ and/or undergoes other types of modifications, such as non-enzymatic glycation, enzymatic degradation, aggregation or combinations of these, all of which result in alterations of 'self'. The consequent generation of a wide spectrum of oxidation-specific (or other modification-specific) neo-epitopes renders the modified LDL immunogenic, and leads to both a cellular and a humoral response⁶. In addition, the oxidation of LDL generates oxidized lipids that are toxic, pro-inflammatory and ultimately pro-atherogenic¹². For example, oxidized cholesterol moieties can promote apoptosis and cell death¹³. Oxidized phospholipids can in-

duce artery wall cells to secrete chemotactic molecules, such as monocyte chemoattractant protein 1 (MCP-1); activate endothelial cells to express adhesion molecules for monocytes and T cells; and induce expression of growth factors, such as monocyte colony-stimulating factor, that facilitate the phenotypic transformation of monocytes into macrophages and stimulate the proliferation of smooth muscle cells^{14,15}. Macrophages, a central mediator of cellular innate (and adaptive) immunity, are essential in lesion initiation and progression^{3,4}. Once activated, they initiate the oxidation of LDL and rapidly take up oxLDL through specific scavenger receptors, leading to foam-cell formation¹⁶. This is a key event in disease progression, as mice deficient in scavenger receptor A and CD36 have significantly reduced atherosclerosis^{17,18}. Activated macrophages also secrete a variety of pro-inflammatory products that affect lesion progression and plaque stability³.

Other potential candidate pathogens include infectious agents¹⁹, pathogenic molecules that incite one or more events of the inflammatory cascade (such as lysophospholipids or oxidized lipids), and metabolic events, which can mediate increased production of reactive oxygen species (such as hypercholesterolemia or hypertension, by means of angiotensin II)²⁰. Evidence indicates that the chief risk factors for atherogenesis, such as dyslipidemia and diabetes as well as the insulin resistance associated with obesity, also contribute to inflammatory conditions through complex mechanisms, including enhanced lipid peroxidation, glycoxidation and increased secretion of pro-inflammatory cytokines, among other effects. Evidence that inflammation is important is strongly supported by many studies showing that increased plasma concentrations of markers of inflammation, such as C-reactive protein (CRP), interleukin 6 (IL-6), serum amyloid A, IL-1 receptor (IL-1R) antagonist and soluble adhesion molecules, are independent predictors of coronary events⁸ (Fig. 1).

Evidence of immune system involvement in atherogenesis

Both adaptive and innate immune responses modulate the rate of lesion progression as well as the composition of the lesions (Table 1). Both apolipoprotein E-deficient (*ApoE*^{-/-}) and *Ldlr*^{-/-} mice have been individually crossed into a recombination activating gene (Rag)-deficient background (*Rag1*^{-/-} or *Rag2*^{-/-}), generating hypercholesterolemic mice lacking both T and B cells. When such mice are fed an atherogenic diet to produce very high plasma cholesterol (above 1,300 mg/dl) for a sufficiently long period of time (more than 16 weeks), they do not show any change in the extent of lesion formation as compared with that of *ApoE*^{-/-} mice with normal immune function^{21–23}. However, when these same immunodeficient mice are examined at earlier time points (4–8 weeks)

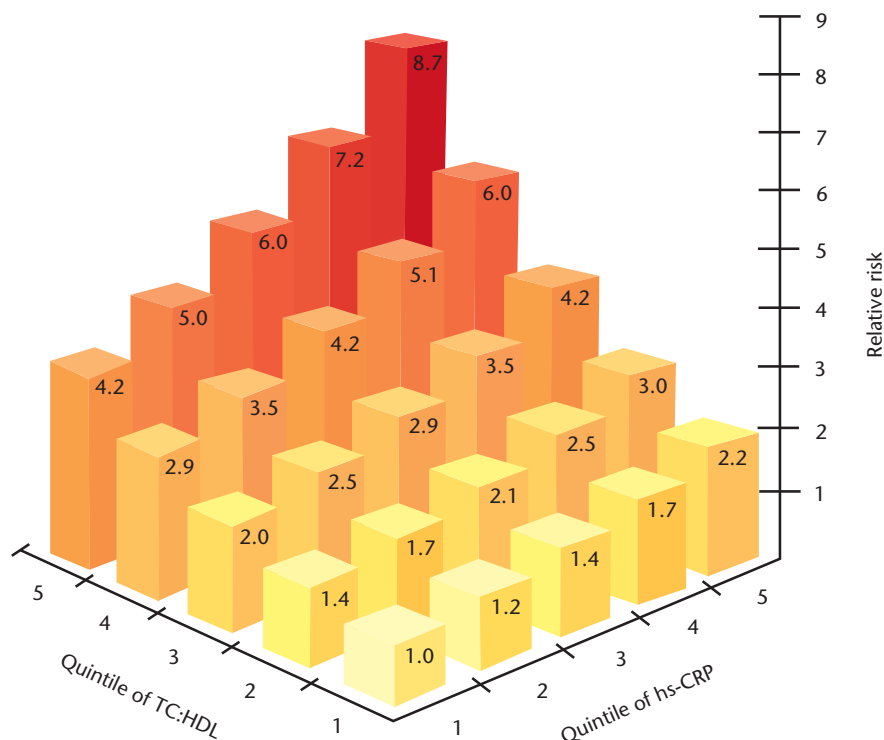


Fig. 1 Relationship of CRP to HDL cholesterol concentrations, demonstrating that CRP is an independent risk factor for CVD (from ref. 8). TC, total cholesterol; HDL, high density lipoprotein cholesterol; ha-CRR, high-sensitivity CRP.

or even at more extended periods of time but in the presence of much lower plasma cholesterol concentrations (about 600–800 mg/dl), the effect of immune deficiency results in a 40–80% decrease in the extent of lesions formed^{21,23,24}. In addition, crosses of *ApoE*^{-/-} mice with severe combined immunodeficiency mice, which also lack B and T cells, generated mice that also had 70% fewer lesions. However, when CD4⁺ T cells from atherosclerotic *ApoE*^{-/-} mice were transferred into these mice, they developed atherosclerosis similar to that of immunocompetent controls²⁵. These immune-mediated effects were site specific, as *ApoE*^{-/-} × *Rag2*^{-/-} mice showed a 60–80% reduction in lesions at the site where lesions first appear in mice, the aortic root, whereas there were no differences in lesions of the brachiocephalic trunk in male mice, although this was not the case in female mice²⁴.

Cumulatively, these studies indicate that T and B cells are not obligatory for lesion initiation or progression if there is sufficiently high atherogenic pressure generated by substantial hypercholesterolemia for a sufficient period of time. However, with less atherogenic pressure, these immune responses are influential in modulating the course of atherogenesis. These studies indicate a net pro-atherogenic effect of T- and B-cell function, but even these effects must be qualified by site and possibly even gender effects. Furthermore, there are many examples of specific protective mechanisms of immune activation as well, such as the demonstration that immunization of hypercholesterolemic animals with an immunodominant antigen of lesions, oxLDL, ameliorates the progression of lesion formation despite very high plasma cholesterol^{26–30}. Infusion of polyspecific immunoglobulin into *ApoE*^{-/-} mice is

Table 1. Immune modulation of atherogenic murine models

Defect or treatment	Immunological effect	Effect on atherosclerosis	Mouse model	Diet	Ref.
<i>Rag1^{-/-}Rag2^{-/-}</i>	T- and B-cell defect	↓	<i>Apoe^{-/-}</i>	Chow diet	21,24
<i>Rag1^{-/-}Rag2^{-/-}</i>	T- and B-cell defect	No effect	<i>Apoe^{-/-}</i>	High-fat diet	21,22
<i>Rag1^{-/-}</i>	T- and B-cell defect	↓ (early)	<i>Ldlr^{-/-}</i>	High-fat diet	23
Splenectomy		↑	<i>Apoe^{-/-}</i>	High-fat diet	72
plus B-cell transfer from old <i>Apoe^{-/-}</i>		Rescue	Normal/splenectomized <i>Apoe^{-/-}</i>	High-fat diet	72
plus T-cell transfer from old <i>Apoe^{-/-}</i>		Rescue	Splenectomized <i>Apoe^{-/-}</i>	High-fat diet	72
CD4 ⁺ cell transfer from old <i>Apoe^{-/-}</i>		↑	<i>Apoe^{-/-} × SCID</i>	Chow diet	25
Anti-CD40L	No CD40 signaling	↓	<i>Ldlr^{-/-}</i>	High-fat diet	56–58
CD40L ^{-/-}	No CD40 signaling	↓	<i>Apoe^{-/-}</i>	Chow diet	59
IFN- γ R ^{-/-}	No IFN- γ effects	↓	<i>Apoe^{-/-}</i>	High-fat diet	62
IFN- γ treatment	More IFN- γ effects	↑	<i>Apoe^{-/-}</i>	Chow diet	63
IL-10 transgenic	More IL-10 by T cells	↓	<i>Ldlr^{-/-}</i>	High-fat diet	66
IL-12 treatment	More IL-12 effects	↑	<i>Apoe^{-/-}</i>	High-fat diet	64
IL-18 treatment	More IFN- γ effects	↑	<i>Apoe^{-/-} / <i>Apoe^{-/-} × IFN-γ^{-/-}</i></i>	Chow diet	65
Anti-TGF- β 1,2,3	No TGF- β signaling	↑	<i>Apoe^{-/-}</i>	Chow diet	70,71
Polyspecific IgG	Immunosuppression	↓	<i>Apoe^{-/-}</i>	High-fat diet	31
Hsp65	Vaccination	↑	<i>Ldlr^{-/-}</i>	Chow diet	80
oxLDL	Vaccination	↓	<i>Ldlr^{-/-}</i>	High-fat diet	28
oxLDL	Vaccination	↓	<i>Apoe^{-/-}</i>	High-fat diet	29,30

SCID, severe combined immunodeficiency; Anti-CD40L, antibody against CD40L; IFN- γ R, IFN- γ receptor; Anti-TGF- β 1,2,3, antibody against TGF- β 1,2,3.

also protective³¹. The important lesson is that the impact of immune function is complex, as might be expected, and atherosclerosis can be both enhanced and inhibited by immune modulation. However, there is no evidence so far that immune mechanisms are primary causes of atherosclerosis.

Innate immunity and atherosclerosis

Innate immunity is characterized by a natural selection of germline-encoded receptors, which focuses on highly conserved motifs in pathogens. It provides the first line of defense for the host and is characterized by fast and blunt responses. It involves several cell types, most importantly macrophages (and dendritic cells), which express a limited repertoire (about 100) of highly conserved pattern-recognition receptors (PRRs), such as scavenger receptors and Toll-like receptors (TLRs). Such PRRs typically recognize a restricted pattern of ligands, called pathogen-associated molecular patterns (PAMPs). In addition to being ligands on pathogens, PAMPs include a diverse array of compounds, including lipopolysaccharides, teichoic acids, aldehyde-derivatized proteins, mannans, bacterial DNAs and denatured DNAs. After receptor ligation, cells either endocytose PAMP-expressing particles or are activated (for example, through nuclear factor- κ B), which elicits an inflammatory response^{32,33}.

The recruitment of monocytes is essential for lesion formation (Fig. 2), as hypercholesterolemic mice that are deficient in MCP-1 or in expression of CCR2, its cognate receptor on monocytes, have a greatly reduced incidence of atherosclerosis^{34,35}. Similarly, hypercholesterolemic *Op/Op* (*Csf1^{-/-}*) mice, which lack monocyte colony-stimulating factor and therefore lack differentiated macrophages in their tissues, show minimal atherosclerosis³⁶. During early events in atherosclerosis, activated endothelial cells express various adhesion molecules, resulting in leukocyte rolling and adhesion³⁷. This activation is triggered most prominently by products of oxLDL. For example, the oxidized phospholipid 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphorylcholine (POVPC) selectively induces connecting segment-1 (CS-1) fibronectin,

whereas other oxidized phospholipids show an induction of vascular cell adhesion molecule 1, which might explain the preferential recruitment of monocytes and T cells because of their interaction with the integrin very late antigen 4 (ref. 14). Cytokines such as tumor necrosis factor- α (TNF- α) and IL-1 are also involved. Oxidized LDL and locally secreted chemokines, such as MCP-1, effect the chemotactic migration of monocytes and T cells, but not neutrophils, into the subendothelial space³⁸. In turn, the expression of MCP-1 by arterial cells is strongly stimulated by oxidized phospholipids as well as cytokines and activated complement.

Macrophages (and dendritic cells) are important in adaptive immunity in their capacity to ingest pathogens and present antigens to initiate adaptive immune responses. Macrophages are fundamental innate immune cells through their production of reactive oxygen species, proteases and cytokines as well as their scavenging functions mediated through PRRs. The scavenger receptors CD36 and SR-B1, as well as SR-A, are examples of such PRRs, which internalize oxLDL, leading to foam-cell formation, a rate-limiting step in atherogenesis^{17,18}. TLRs are expressed in atherosclerotic lesions³⁹ and may also participate in inflammatory signaling. Although TLR4 deficiency does not decrease atherosclerosis in cholesterol-fed *Apoe^{-/-}* mice⁴⁰, a TLR4 polymorphism that attenuates receptor signaling is associated with decreased atherosclerosis in humans⁴¹. The function of other TLRs has not yet been examined. CD14, the non-transmembrane receptor for lipopolysaccharide, initiates inflammatory responses through interactions with TLRs. A polymorphism in the CD14 promoter, resulting in a significantly higher density of CD14 on monocytes, has been identified as a risk factor for myocardial infarction⁴². These data indicate a convergence of innate immunity to pathogens and atherosclerosis. A variety of stimuli can activate macrophages to secrete an array of cytokines that tightly regulate scavenger receptor expression and inflammatory responses in general. Components of oxLDL, in turn, can also activate macrophages and affect gene expression, such as expression

of genes encoding peroxisome proliferator-activated receptor- γ , CD36 and ATP-binding cassette transporter A1, which profoundly influence macrophage inflammatory and atherogenic activity (discussed in the accompanying article by Li and Glass⁴³).

Soluble factors of innate defense, such as complement and CRP, which act together to rapidly eliminate microbes, are also involved in atherogenesis. Deposition of C3, C4 and terminal C5b–C9 complement complexes occur in atherosclerotic lesions⁴⁴. *Ldlr*^{-/-} mice deficient in C3 develop increased atherosclerosis, indicating an important function for the complement system⁴⁵. As discussed above, CRP reflects inflammation and is an independent risk factor for CVD. In the context of immunity, it functions as an acute-phase protein to rapidly clear pathogens⁴⁶. CRP may be involved in atherogenesis, however, as indicated by the observation that it is secreted by intimal macrophages, as discussed below⁴⁷.

Natural antibodies (generated in the absence of known antigen stimulation), mainly immunoglobulin M (IgM), mediate immediate responses against systemic bacterial and viral infections, preventing their dissemination. Many of these antibodies are produced by a specialized set of innate B lymphocytes, the B1 cells, which typically express a restricted set of germline-encoded antigen receptors with specificity for natural or self ligands and generate a link between the innate and adaptive immune responses. In atherosclerosis, IgM responses to oxLDL have been found in both animal models of atherosclerosis and in humans (discussed below). Immunoglobulins, including IgM, are deposited in atherosclerotic lesions, and some of these have been shown to have specificity for epitopes of oxLDL (ref. 48). CD1d-restricted natural killer T cells, equivalent to B1 cells as innate T lymphocytes, have not yet been reported to be involved in atherosclerosis.

Adaptive immunity and atherosclerosis

In contrast to innate immunity, adaptive immunity is more precise but slower. Specific molecular structures on antigens are recognized by antigen receptors, such as T-cell receptors (TCRs) and B-cell receptors (BCRs), which provide great specificity and affinity by somatic rearrangements in blast cells. In contrast to the limited number of PRRs, TCRs have been estimated to number about 10^{18} and BCRs about 10^{14} , providing an almost unlimited number of receptors.

Dendritic cells and macrophages can activate T cells. This process involves the presentation of antigen to the antigen-specific TCR and additional co-stimulatory signals, such as the interaction of CD40 ligand (CD40L) with CD40. Most antigens cannot stimulate B cells without assistance from CD4⁺ T cells, which recognize these peptide–major histocompatibility complex (MHC) complexes on B cells, become activated and provide signals (co-stimulatory molecules and cytokines) that promote somatic hypermutation and immunoglobulin class switching. Most CD4⁺ cells are cytokine-

secreting T-helper (Th) cells and express $\alpha\beta$ -TCR, which interacts with MHC class II molecules. A smaller portion expresses $\gamma\delta$ -TCR; most of these cells interact with the non-polymorphic non-classical MHC molecule CD1, which presents certain antigens (particularly lipids and glycolipids). Based on the cytokines they secrete, Th cells are traditionally divided into Th1 cells, which secrete interferon- γ (IFN- γ) and IL-2 and promote cell-mediated immunity, and Th2 cells, which secrete IL-4, IL-5, IL-10 and IL-13 and help B cells produce antibodies. These two subsets of Th cells also cross-regulate each others' activity. CD8⁺ T cells are mainly cytotoxic killer cells, although they too can secrete cytokines, such as TNF- α , lymphotoxin and IFN- γ .

Some T cell-independent antigens can activate B cells without the help of cognate T-cell function; these responses are dominated by IgM, because class switching is severely limited⁴⁹. Such thymus-independent antigens are typically represented by closely spaced repeated epitopes expressed at high density⁵⁰. Oxidized LDL, which expresses multiple copies of oxidation-specific epitopes on a single LDL particle, is probably an example of such an antigen.

In general, antibodies provide protection against exogenous pathogens and endogenous altered self molecules to maintain homeostasis by neutralization and clearance.

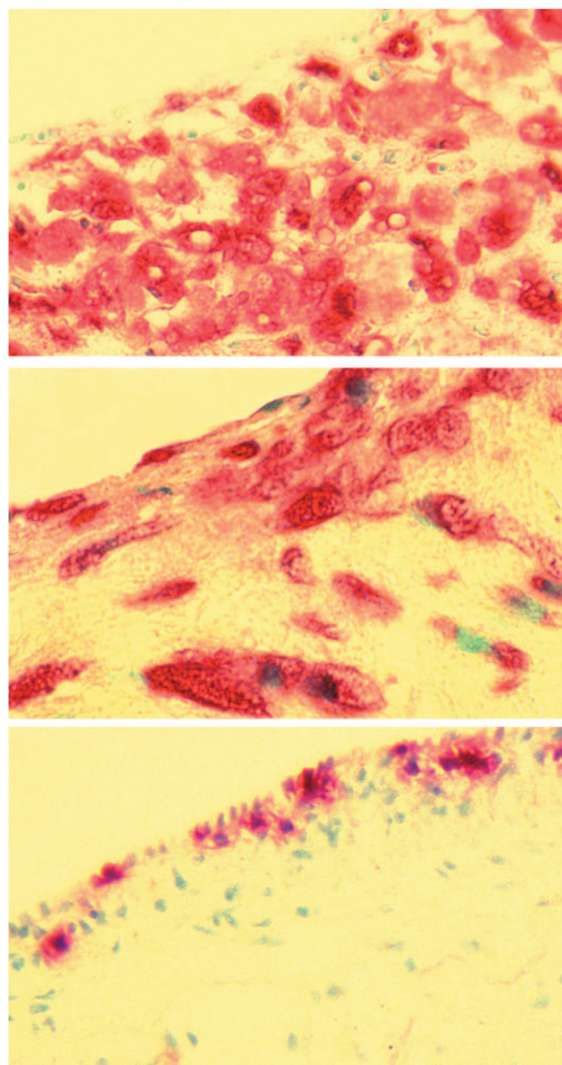


Fig. 2 Fatty streaks of human coronary arteries and aortae from animal models of atherosclerosis immunostained for macrophages (top), oxidation-specific epitopes (middle) or T cells (bottom). Oxidation-specific epitopes were visualized using a natural monoclonal antibody cloned from atherosclerotic *ApoE*^{-/-} mice that recognizes oxLDL and oxidized phospholipids and is structurally and functionally identical to T15-clonospesific antibodies against PC. (Courtesy of W. Palinski, Department of Medicine, University of California, San Diego.)

Antibodies can also induce other components of the immune system, such as complement pathways and effector functions of other immune cells (macrophages, B cells, mast cells and so on). The latter process involves binding to antibody class–corresponding Fc receptors, leading to either negative or positive regulation of immune cell responses, the efficient uptake of immune complexes for the degradation of antigens, and efficient antigen presentation. These adaptive responses cooperate in a highly regulated manner and arm the host for quick and specific responses in subsequent encounters with the antigen.

T cells are a prominent component of both early and late lesions (Fig. 2). Most T cells in lesions bear CD3 and CD4 markers and $\alpha\beta$ -TCR. These represent about two-thirds of all CD3⁺ cells in advanced human lesions and more than 90% of T cells in lesions of *ApoE*^{-/-} mice⁵. There is also evidence for the presence of dendritic cells, which could be part of a hypothesized Langerhans cell–like network in the vessel wall⁵¹. In addition, moderate numbers of CD8⁺ T cells are found. Small numbers of natural killer cells are also found in early lesions; however, cytolytic activity mediated by natural killer cells does not affect lesion progression⁵². In contrast, there are relatively few B cells, although they are found in the adventitia surrounding lesions^{53,54}.

All lesion cells express MHC class II molecules, indicative of IFN- γ -mediated activation. In turn, MHC class II molecules can interact with the TCRs of CD4⁺ Th cells⁵. Unexpectedly, the co-stimulatory factors CD40 and CD40L have been reported to be expressed widely in all cells in the lesion, not just in T cells and B cells⁵⁵. These interactions are important, as genetic disruption of CD154 (CD40L) in *ApoE*^{-/-} mice⁵⁶, as well as treatment of cholesterol-fed *Ldlr*^{-/-} mice with antibody against CD40L, reduced lesion formation by more than 60% (ref. 57). Furthermore, treatment with antibodies against CD40L inhibited the evolution of already established lesions and induced a stable plaque phenotype^{58,59}. IFN- γ induces CD40, and CD40 ligation induces matrix metalloproteinase expression by macrophages, which can cause plaque destabilization.

Th1 cells are dominant in atherosclerotic lesions, especially early lesions, as the cytokines IFN- γ , IL-2 and TNF- α are highly expressed, whereas only low amounts of the Th2 cytokines IL-4, IL-5 and IL-10 can be detected. In addition, IgG2a antibodies against oxLDL epitopes, typical of Th1 help, predominate in plasma during early stages of atherosclerosis in *Ldlr*^{-/-} and *ApoE*^{-/-} mice. IL-4 expression was detected in lesions of *ApoE*^{-/-} mice only at very advanced stages of disease in the presence of excessive hypercholesterolemia. Only at this later stage were IgG1 antibodies, typical of Th2 help, found more prominently⁵.

In the context of atherogenesis, the Th1 cytokine IFN- γ is pro-inflammatory and pro-atherogenic. In addition to activating macrophages, it inhibits smooth muscle cell proliferation and collagen synthesis, and thereby promotes plaque destabilization. IL-1 and TNF- α have similar functions in promoting inflammatory responses (IL-6 and CRP) and the induction of matrix metalloproteinase 9. IL-12 and IL-18 are also highly expressed in atherosclerotic lesions and can further augment IFN- γ secretion^{60,61}. Th1-mediated effects appear to be atherogenic: *ApoE*^{-/-} mice deficient in the IFN- γ receptor have significantly decreased lesions and increased collagen content⁶²; daily administration of IFN- γ promoted

atherosclerosis in *ApoE*^{-/-} mice⁶³; and daily administration of IL-12 or IL-18, both of which promote Th1 effects, also increased atherosclerosis in *ApoE*^{-/-} mice^{64,65}. The pro-atherogenic effects of IL-18 are mediated through IFN- γ (ref. 65). Thus, Th1 cells secrete IFN- γ , which activates macrophages and leads to their release of IL-12, which in turn augments IFN- γ secretion by T cells. IFN- γ also inhibits the production of the Th2 cytokines IL-4 and IL-10.

In contrast, Th2 responses seem to antagonize pro-atherogenic Th1 effects and thereby confer atheroprotection. IL-10 can potentially suppress IL-12 and IFN- γ secretion. *In vitro* studies have shown that recombinant IL-10 inhibited the oxLDL-induced production of IL-12 by human monocytes, indicating a protective function for IL-10. Indeed, cholesterol-fed *Ldlr*^{-/-} mice that overexpress IL-10 in T cells under control of the human IL-2 promoter had 50% smaller lesions⁶⁶ compared to *Ldlr*^{-/-} mice. These same mice also had decreased circulating IFN- γ -secreting CD4⁺ cells in peripheral blood and in the spleen, and showed an increased ratio of IgG1 to IgG2a antibodies against malondialdehyde-(MDA)–LDL. Furthermore, treatment of *ApoE*^{-/-} mice with pentoxifylline, which inhibits Th1 differentiation, decreases lesion formation⁶⁷. In addition, mildly hypercholesterolemic, genetically modified C57Bl/6 mice demonstrated a protective function for Th2-biased responses in fatty streak formation⁶⁸. The function of the Th2 cytokine IL-4 might be more complex, as other studies found this cytokine to be pro-atherogenic⁶⁹. In summary, Th2 responses seem to have a protective effect. T cells and macrophages engage in an interactive ‘dialog’⁶⁰, and the local dominance of one subset of Th cells could well influence the course of lesion progression and stability.

The anti-inflammatory cytokine transforming growth factor- β (TGF- β) is secreted by macrophages, smooth muscle cells and the subset of Th cells, Th3, that exerts regulatory functions. TGF- β could be involved in plaque stabilization, because, in contrast to IFN- γ , it stimulates collagen synthesis and is fibrogenic. Indeed, inhibition of TGF- β signaling by neutralizing antibodies led to larger lesion size with a less stable plaque phenotype^{70,71}.

Adding to the complexity of immune effects is the recent report that splenectomy of cholesterol-fed *ApoE*^{-/-} mice led to significantly increased atherosclerosis, indicating that the spleen has anti-atherogenic activity^{72,73}. Further experiments in this study established that transfer of either purified B cells or T cells from the spleens of atherosclerotic *ApoE*^{-/-} donors, which presumably were already ‘educated’ with respect to relevant atherosclerotic antigens, could rescue this effect. The mechanisms for these important findings remain to be elucidated. Similar effects may be found in humans, moreover, as long-term studies of soldiers who underwent splenectomy after trauma showed they had a twofold elevated incidence of coronary artery disease⁷⁴.

Antigens in atherosclerosis

There is ample evidence of immune activation in the atherosclerotic lesion. Of specific interest is the finding that atherosclerotic lesions of *ApoE*^{-/-} mice showed preferential expression of certain TCR-variable gene segments, suggesting the oligoclonal expansion of T cells and indicating that a limited number of antigens mediate specific proliferation⁷⁵. What are the antigens involved? There are many candidates,

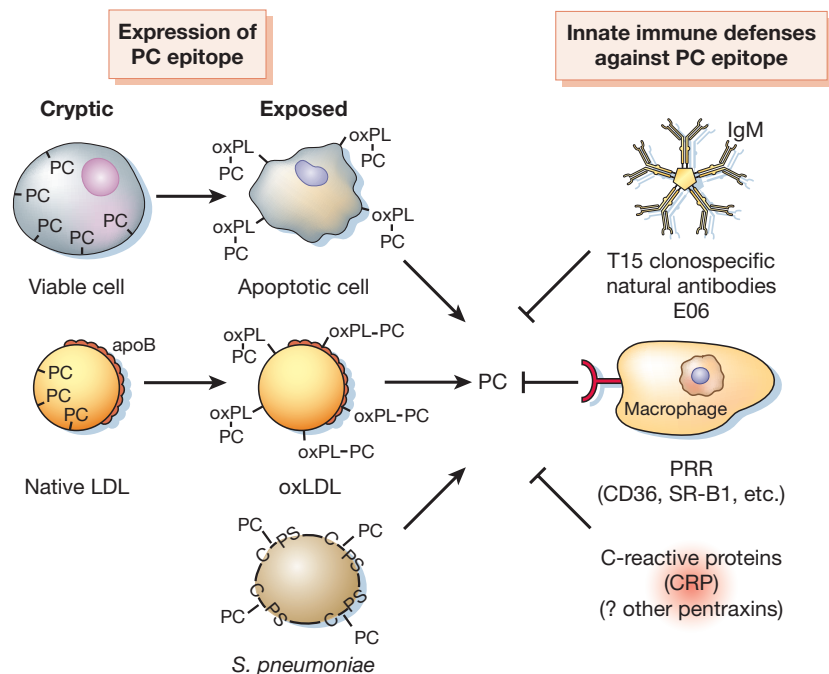


Fig. 3 Molecular mimicry between epitopes of oxLDL, apoptotic cells and the PC of the C-PS of pathogens. For native LDL and viable cells, the PC-containing phospholipids need to be oxidized (oxPL) to have the PC moiety exposed for recognition by innate immune defenses, represented by natural antibodies of the T15/E06 type, macrophage scavenger receptors, such as CD36 and SR-B1, and CRP.

including exogenous infectious pathogens such as bacteria and viruses, and endogenous proteins such as heat-shock proteins (Hsps) and β 2GP-1 (ref. 76), which could lead to true autoimmune responses, modified artery wall proteins and oxLDL, as discussed above. There has been much interest in infectious agents as pathogens in atherogenesis¹⁹, such as *Chlamydia pneumoniae*, as patients with CVD have high titers of antibodies against these agents. In addition, *C. pneumoniae*, herpes simplex and cytomegalovirus have been detected in human lesions. Infectious agents are not necessary for lesion development in *ApoE*^{-/-} mice⁴⁰, although this does not rule out the possibility of a modifying function of infection in disease progression. Infection at a site distant from the lesion, such as gingivitis, could also influence cellular activation in the lesion through systemic events mediated by cytokines or antibodies⁷⁷. Many Hsps of microbes (such as Hsp65) and humans (such as Hsp60) are highly conserved and show molecular mimicry. Consequently, antibodies against Hsp65 could target arterial cells, such as endothelial cells, which express Hsp60 in large amounts when exposed to stress⁷⁸. Indeed, antibodies against Hsp65 and Hsp60 correlated with the progression of carotid disease in one study in humans⁷⁹, and immunization of *Ldlr*^{-/-} mice with Hsp65 promoted early atherosclerosis⁸⁰, whereas mucosal administration of antigens led to tolerance and decreased atherogenesis⁸¹.

The most extensive data obtained so far support the idea that oxLDL is important as an antigen⁸². Oxidized LDL is present in the atherosclerotic lesions of all animal models and humans examined¹⁶ (Fig. 2). When polyunsaturated fatty acids of LDL phospholipids undergo peroxidation, a variety

of highly reactive breakdown products are formed, such as MDA, which in turn can form covalent adducts with the lysine residues of the protein of LDL, apoB. In addition, reactive oxidation products derived from phospholipids, such as POVPC, can also form covalent adducts with apoB, and these adducts retain the intact phosphorylcholine (PC) headgroup⁶. These 'neo-self' determinants have been called oxidation-specific epitopes and have been shown to trigger a substantial humoral response specific to the given modification. For the measurement of these immune responses, two models of oxLDL are widely used: MDA-LDL, which is generated by the derivatization of LDL with MDA, yielding mainly MDA-lysine epitopes, and CuSO₄-oxidized LDL (CuOx-LDL), which has many different oxidation-specific epitopes, although the oxidized PC-containing phospholipid seems to be an immunodominant epitope⁸²⁻⁸⁴.

Circulating IgG and IgM antibodies against both MDA-LDL and CuOx-LDL are present in the plasma of animals and humans and form immune complexes with oxLDL in atherosclerotic lesions^{48,85}. These antibodies closely correlate with atherosclerosis

progression and regression in murine models and correlate with measures of lipid peroxidation^{6,86,87}. In humans, many (but not all) studies have shown that plasma titers of antibodies against oxLDL epitopes, particular IgG, correlate with risk factors for CVD, and can even be used to predict the progression of carotid disease as well as myocardial infarction and death⁸². However, many variables affect such titers in humans, and the clinical utility of such measurements remains to be determined, particularly for the individual patient. Further evidence of the importance of oxLDL as an immunodominant antigen was obtained from the observation that 15% of CD4⁺ T cells cloned from human carotid atherosclerotic plaques specifically proliferated in response to oxLDL in a MHC class II-restricted way⁸⁸. Compelling evidence that the immune response to oxLDL is important in modulating lesion formation comes from studies demonstrating that immunization of hypercholesterolemic rabbits and mice with MDA-LDL or CuOx-LDL reduced the progression of lesion formation²⁶⁻³⁰.

Molecular mimicry between PC of oxLDL and pathogens

This review began with the thesis that many of the immune responses found in atherosclerosis were in fact directed at specific components of inflammatory responses in general. One example of this was discovered by detailed study of a specific immune response noted in *ApoE*^{-/-} mice. Because the titers of IgM autoantibodies against oxLDL are so high in these mice, it was possible to clone a panel of IgM monoclonal autoantibodies from their spleens that specifically bound to CuOx-LDL, but not native LDL, and specifically to

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oxidized phospholipids with the PC headgroup, such as POVPC (refs. 89,90). These monoclonal antibodies, such as the prototypic EO6, bind to intact oxLDL and block its binding and uptake by macrophages in a dose-dependent way, and specifically block the binding of oxLDL to the scavenger receptors CD36 and SR-B1 (refs. 90–92). In addition, POVPC covalently bound to bovine serum albumin could also block the uptake of oxLDL by these cells. These studies showed that oxidized phospholipids bearing the PC headgroup as ligands on oxLDL mediated uptake by macrophage scavenger receptors. A wide variety of PC-bearing oxidized phospholipids present in oxLDL have this property and are present in lesions^{93,94}. Furthermore, EO6 binds to apoptotic cells, which are known to be under oxidative stress and would be expected to express common oxidation-specific epitopes on their surface. EO6 blocks apoptotic cell uptake by macrophages, as does POVPC bound to bovine serum albumin⁹⁵. Thus, PC-oxidized phospholipids represent a previously unrecognized PAMP, which is bound by EO6 as well as by the PRRs CD36 and SR-B1, and possibly others. These data strongly indicate a common innate immune response to apoptotic cells and oxLDL.

Further evidence in support of this hypothesis came from studies in which the DNA sequences of the antigen-binding domains of these IgM antibodies were determined. All of the cloned IgM antibodies against oxLDL (including EO6) were shown to be 100% homologous in their entire VH–VL regions to an IgA natural antibody cloned more than 30 years ago⁴⁸. This antibody, made by the T15 clone, is directed against PC and confers optimal protection to mice against lethal infection with pathogens, such as *Streptococcus pneumoniae*⁹⁶. PC, the headgroup of many phospholipids, is a prominent component of the capsular polysaccharide (C-PS) of many bacteria. T15 (or EO6) bound equally well to C-PS and oxLDL, and each of these could compete for the binding to the other. T15 is a natural IgA antibody made by B1 cells that has the T15 idiotype, and even arises in mice grown in completely pathogen-free conditions. Thus, the actual positive selection agent for expansion of the B1 cell clone secreting T15/EO6 antibodies is postulated to be apoptotic cells or oxidized membranes or both; only later in life would pathogens contribute to further selection⁴⁸. This represents positive selection of a natural antibody by an endogenous ‘neo-self’ antigen; there is an enhanced expansion of B cells secreting T15/EO6 clonospesific antibodies in *ApoE*^{-/-} mice because of their atherogenic burden. Thus, PC-containing phospholipids, which are prominent components of mammalian membranes and LDL, are ‘cryptic’ epitopes in viable cells and native LDL. However, after oxidation, conformational changes occur that expose the PC epitope for recognition by EO6 (ref. 84) and by macrophage scavenger receptors. In contrast, the PC moiety of the pathogen C-PS is constitutively exposed for antibody recognition. Although this has not been tested, it is hypothesized that the pathogen PC also would be recognized by one or more of the same scavenger receptors (Fig. 3).

CRP is a member of the phylogenetically ancient and highly conserved pentraxin family of proteins that is thought to be important in primary, innate host defenses. It shows a rapid and substantial increase in plasma in response to inflammatory events such as tissue injury and infection. It is known to bind to PC of infectious pathogens such as *S.*

pneumoniae and to mediate their clearance⁴⁶. CRP binds to apoptotic cells as well, although the identity of the ligand involved has not been determined⁹⁷. CRP also binds to oxLDL and exclusively to oxidized PC-containing phospholipids, and not to native LDL (ref. 98). In addition, the binding of CRP to apoptotic cells is also mediated by the PC moiety of oxidized phospholipids. Although CRP does not bind to native LDL, it does bind to LDL whose native structure was altered by plating on a microtiter well or by aggregation, indicating that PC exposure for CRP could occur by non-oxidative events as well⁹⁸ (Fig. 3).

These observations indicate that CRP, the natural antibody EO6 and certain scavenger receptors of macrophages are all PRRs, with the PC moiety as the PAMP to which they bind. Thus, PC exposure generates a potent pathogen, mediating highly conserved and concerted innate responses. With infectious pathogens, these responses are undoubtedly protective, which is the basis for their conservation. In atherogenesis, however, the responses could have complex effects, and further study will be needed to sort out the potential benefits or ‘penalties’. An increased titer of EO6 antibodies would be expected to be protective, as these antibodies potentially block macrophage uptake of oxLDL. Although scavenger receptor-mediated uptake of oxLDL is itself apparently atherogenic, it could be protective if the concentration of oxLDL generated were minimized by low concentrations of LDL or inhibition of oxidative events. With CRP, the situation is equally complicated: CRP could bind to the PC moiety of oxLDL and block uptake by macrophages. However, CRP could also enhance the uptake of opsonized oxLDL through Fcγ receptors. In addition, ligand-bound CRP can activate complement pathways in a complex way, and can activate macrophages to release pro-atherogenic cytokines such as IFN-γ (ref. 46). Because CRP is known to be present in atherosclerotic lesions, and to co-localize with oxidized⁹⁸ and otherwise modified LDLs, these observations indicate that CRP could be involved in complex ways in modulating atherogenesis. Undoubtedly, this complexity characterizes all immune interactions in the course of atherosclerosis.

Summary

There is now much evidence that both innate and adaptive immune mechanisms are involved in atherogenesis, as might be anticipated for a disease that is a chronic inflammatory process. Immune mechanisms have both protective and adverse effects in animal models. Elucidation of the pathways involved could lead to insights into pathogenic events that could explain in part the diversity in the expression of this disease in individuals apparently equal in regard to risk factors, such as plasma LDL. In turn, new therapeutic options could be developed, such as immunization with oxidation-specific epitopes of oxLDL, or interference with Th1-mediated pathways that lead to secretion of IFN-γ. Study of the action of the immune system in atherogenesis is in its infancy, and much remains to be learned. Most of the work so far has been done on experimental animals, mainly mice, and the relevance of these observations to human disease remains to be determined. However, lesion formation in humans occurs with much less atherogenic pressure and thus proceeds at a more leisurely pace than in hypercholesterolemic mice. Therefore, there may be more opportunity

for immune mechanisms to function. That inflammatory markers are a powerful predictor of CVD in humans, autoantibody titers against epitopes of oxLDL and Hsp are increased in CVD patients, and antigen-specific T cells and immunoglobulins are present in lesions indicates that immune mechanisms are relevant to humans as well. The challenge will be to translate what has been learned already, and what will be learned through future experimental studies, to human populations.

- Tuzcu, E.M. *et al.* High prevalence of coronary atherosclerosis in asymptomatic teenagers and young adults: Evidence from intravascular ultrasound. *Circulation* **103**, 2705–2710 (2001).
- Leitersdorf, E., Tobin, E.J., Davignon, J. & Hobbs, H.H. Common low-density lipoprotein receptor mutations in the French Canadian population. *J. Clin. Invest.* **85**, 1014–1023 (1990).
- Glass, C.K. & Witztum, J.L. Atherosclerosis: The road ahead. *Cell* **104**, 503–516 (2001).
- Hansson, G.K., Libby, P., Schonbeck, U. & Yan, Z.Q. Innate and adaptive immunity in the pathogenesis of atherosclerosis. *Circ. Res.* **91**, 281–291 (2002).
- Hansson, G.K. Immune mechanisms in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **21**, 1876–1890 (2001).
- Hörkkö, S. *et al.* Immunological responses to oxidized LDL. *Free Radic. Biol. Med.* **28**, 1771–1779 (2000).
- Ross, R. Atherosclerosis—an inflammatory disease. *N. Engl. J. Med.* **340**, 115–126 (1999).
- Libby, P., Ridker, P.M. & Maseri, A. Inflammation and atherosclerosis. *Circulation* **105**, 1135–1143 (2002).
- Williams, K.J. & Tabas, I. The response-to-retention hypothesis of atherogenesis reinforced. *Curr. Opin. Lipidol.* **9**, 471–474 (1998).
- Skalen, K. *et al.* Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. *Nature* **417**, 750–754 (2002).
- Gaut, J.P. & Heinecke, J.W. Mechanisms for oxidizing low-density lipoprotein. Insights from patterns of oxidation products in the artery wall and from mouse models of atherosclerosis. *Trends Cardiovasc. Med.* **11**, 103–112 (2001).
- Pratico, D. Lipid peroxidation in mouse models of atherosclerosis. *Trends Cardiovasc. Med.* **11**, 112–116 (2001).
- Colles, S.M., Maxson, J.M., Carlson, S.G. & Chisolm, G.M. Oxidized LDL-induced injury and apoptosis in atherosclerosis. Potential roles for oxysterols. *Trends Cardiovasc. Med.* **11**, 131–138 (2001).
- Berliner, J.A., Subbanagounder, G., Leitinger, N., Watson, A.D. & Vora, D. Evidence for a role of phospholipid oxidation products in atherogenesis. *Trends Cardiovasc. Med.* **11**, 142–147 (2001).
- Marathe G.K., Prescott, S.M., Zimmerman G.A. & McIntyre T.M. Oxidized LDL contains inflammatory PAF-like phospholipids. *Trends Cardiovasc. Med.* **11**, 139–142 (2001).
- Witztum, J.L. & Steinberg, D. The oxidative modification hypothesis of atherosclerosis: Does it hold for humans? *Trends Cardiovasc. Med.* **11**, 93–102 (2001).
- Suzuki, H. *et al.* A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection. *Nature* **386**, 292–296 (1997).
- Febbraio, M. *et al.* Targeted disruption of the class B scavenger receptor CD36 protects against atherosclerotic lesion development in mice. *J. Clin. Invest.* **105**, 1049–1056 (2000).
- Libby, P., Egan, D. & Skarlatos, S. Roles of infectious agents in atherosclerosis and restenosis: An assessment of the evidence and need for future research. *Circulation* **96**, 4095–4103 (1997).
- Landmesser, U. & Harrison, D.G. Oxidant stress as a marker for cardiovascular events—Ox marks the spot. *Circulation* **104**, 2638–2640 (2001).
- Dansky, H.M., Charlton, S.A., Harper, M.M. & Smith, J.D. T and B lymphocytes play a minor role in atherosclerotic plaque formation in the apolipoprotein E-deficient mouse. *Proc. Natl. Acad. Sci. USA* **94**, 4642–4646 (1997).
- Daugherty, A. *et al.* The effects of total lymphocyte deficiency on the extent of atherosclerosis in apolipoprotein E^{-/-} mice. *J. Clin. Invest.* **100**, 1575–1580 (1997).
- Song, L., Leung, C. & Schindler, C. Lymphocytes are important in early atherosclerosis. *J. Clin. Invest.* **108**, 251–259 (2001).
- Reardon, C.A. *et al.* Effect of immune deficiency on lipoproteins and atherosclerosis in male apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **21**, 1011–1016 (2001).
- Zhou, X., Nicoletti, A., Elhage, R. & Hansson, G.K. Transfer of CD4⁺ T cells aggravates atherosclerosis in immunodeficient apolipoprotein E knockout mice. *Circulation* **102**, 2919–2922 (2000).
- Palinski, W., Miller, E. & Witztum, J.L. Immunization of low density lipoprotein (LDL) receptor-deficient rabbits with homologous malondialdehyde-modified LDL reduces atherogenesis. *Proc. Natl. Acad. Sci. USA* **92**, 821–825 (1995).
- Ameli, S. *et al.* Effect of immunization with homologous LDL and oxidized LDL on early atherosclerosis in hypercholesterolemic rabbits. *Arterioscler. Thromb. Vasc. Biol.* **16**, 1074–1079 (1996).
- Freigang, S., Hörkkö, S., Miller, E., Witztum, J.L. & Palinski, W. Immunization of LDL receptor-deficient mice with homologous malondialdehyde-modified and native LDL reduces progression of atherosclerosis by mechanisms other than induction of high titers of antibodies to oxidative neopeptides. *Arterioscler. Thromb. Vasc. Biol.* **18**, 1972–1982 (1998).
- George, J. *et al.* Hyperimmunization of apo-E-deficient mice with homologous malondialdehyde low-density lipoprotein suppresses early atherogenesis. *Atherosclerosis* **138**, 147–152 (1998).
- Zhou, X., Caligiuri, G., Hamsten, A., Lefvert, A.K. & Hansson, G.K. LDL immunization induces T-cell-dependent antibody formation and protection against atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **21**, 108–114 (2001).
- Nicoletti, A., Kaveri, S., Caligiuri, G., Bariaety, J. & Hansson, G.K. Immunoglobulin treatment reduces atherosclerosis in apo E knockout mice. *J. Clin. Invest.* **102**, 910–918 (1998).
- Medzhitov, R. & Janeway, C.A., Jr. Decoding the patterns of self and nonself by the innate immune system. *Science* **296**, 298–300 (2002).
- Medzhitov, R. Toll-like receptors and innate immunity. *Nature Rev. Immunol.* **1**, 135–145 (2001).
- Gosling, J. *et al.* MCP-1 deficiency reduces susceptibility to atherosclerosis in mice that overexpress human apolipoprotein B. *J. Clin. Invest.* **103**, 773–778 (1999).
- Boring, L., Gosling, J., Cleary, M. & Charo, I.F. Decreased lesion formation in CCR2^{-/-} mice reveals a role for chemokines in the initiation of atherosclerosis. *Nature* **394**, 894–897 (1998).
- Smith, J.D. *et al.* Decreased atherosclerosis in mice deficient in both macrophage colony-stimulating factor (op) and apolipoprotein E. *Proc. Natl. Acad. Sci. USA* **92**, 8264–8268 (1995).
- Cybulsky, M.I. & Gimbrone, M.A., Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science* **251**, 788–791 (1991).
- Witztum, J.L. & Berliner, J.A. Oxidized phospholipids and isoprostanes in atherosclerosis. *Curr. Opin. Lipidol.* **9**, 441–448 (1998).
- Edfeldt, K., Swedenborg, J., Hansson, G.K. & Yan Z.Q. Expression of toll-like receptors in human atherosclerotic lesions: A possible pathway for plaque activation. *Circulation* **105**, 1158–1161 (2002).
- Wright, S.D. *et al.* Infectious agents are not necessary for murine atherogenesis. *J. Exp. Med.* **191**, 1437–1442 (2000).
- Kiechl, S. *et al.* Toll-like receptor 4 polymorphisms and atherogenesis. *N. Engl. J. Med.* **347**, 185–192 (2002).
- Hubacek, J.A. *et al.* C(-260)→T polymorphism in the promoter of the CD14 monocyte receptor gene as a risk factor for myocardial infarction. *Circulation* **99**, 3218–3220 (1999).
- Li, A.C. & Glass, C.K. The macrophage foam cell as a target for therapeutic intervention. *Nat. Med.* **8**, 1235–1242 (2002).
- Vlaicu, R., Niculescu, F., Rus, H.G. & Cristea, A. Immunohistochemical localization of the terminal C5b-9 complement complex in human aortic fibrous plaque. *Atherosclerosis* **57**, 163–177 (1985).
- Buono, C. *et al.* Influence of C3 deficiency on atherosclerosis. *Circulation* **105**, 3025–3031 (2002).
- Volanakis, J.E. Human C-reactive protein: Expression, structure, and function. *Mol. Immunol.* **38**, 189–197 (2001).
- Yasojima, K., Schwab, C., McGeer, E.G. & McGeer, P.L. Generation of C-reactive protein and complement components in atherosclerotic plaques. *Am. J. Pathol.* **158**, 1039–1051 (2001).
- Shaw, P.X. *et al.* Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity. *J. Clin. Invest.* **105**, 1731–1740 (2000).
- Vos, Q., Lees, A., Wu, Z.Q., Snapper, C.M. & Mond, J.J. B-cell activation by T-cell-independent type 2 antigens as an integral part of the humoral immune response to pathogenic microorganisms. *Immunol. Rev.* **176**, 154–170 (2000).
- Ochsenbein, A.F. *et al.* Correlation of T cell independence of antibody responses with antigen dose reaching secondary lymphoid organs: Implications for splenectomized patients and vaccine design. *J. Immunol.* **164**, 6296–6302 (2000).
- Millonig, G., Schwentner, C., Mueller, P., Mayerl, C. & Wick, G. The vascular-associated lymphoid tissue: A new site of local immunity. *Curr. Opin. Lipidol.* **12**, 547–553 (2001).
- Schiller, N.K., Boisvert, W.A. & Curtiss, L.K. Lesion formation in LDL receptor-deficient mice with perforin and *Lys^{269G}* mutations. *Arterioscler. Thromb. Vasc. Biol.* **22**, 1341–1346 (2002).
- Sohma, Y. *et al.* Accumulation of plasma cells in atherosclerotic lesions of Watanabe heritable hyperlipidemic rabbits. *Proc. Natl. Acad. Sci. USA* **92**, 4937–4941 (1995).
- Zhou, X. & Hansson, G.K. Detection of B cells and proinflammatory cytokines in atherosclerotic plaques of hypercholesterolaemic apolipoprotein E knockout mice. *Scand. J. Immunol.* **50**, 25–30 (1999).
- Mach, F. *et al.* Functional CD40 ligand is expressed on human vascular endothelial cells, smooth muscle cells, and macrophages: Implications for CD40-CD40 ligand signaling in atherosclerosis. *Proc. Natl. Acad. Sci. USA* **94**, 1931–1936 (1997).
- Lutgens, E. *et al.* Requirement for CD154 in the progression of atherosclerosis. *Nature Med.* **5**, 1313–1316 (1999).
- Mach, F., Schönbeck, U., Sukhova, G.K., Atkinson, E. & Libby, P. Reduction of atherosclerosis in mice by inhibition of CD40 signalling. *Nature* **394**, 200–203 (1998).
- Schönbeck, U., Sukhova, G.K., Shimizu, K., Mach, F. & Libby, P. Inhibition of CD40 signaling limits evolution of established atherosclerosis in mice. *Proc. Natl. Acad. Sci. USA* **97**, 7458–7463 (2000).
- Lutgens, E. *et al.* Both early and delayed anti-CD40L antibody treatment in-

- duces a stable plaque phenotype. *Proc. Natl. Acad. Sci. USA* **97**, 7464–7469 (2000).
60. Uyemura, K. *et al.* Cross-regulatory roles of interleukin (IL)-12 and IL-10 in atherosclerosis. *J. Clin. Invest.* **97**, 2130–2138 (1996).
 61. Gerdes, N. *et al.* Expression of interleukin (IL)-18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells, and macrophages: Implications for atherogenesis. *J. Exp. Med.* **195**, 245–257 (2002).
 62. Gupta, S. *et al.* IFN- γ potentiates atherosclerosis in ApoE knock-out mice. *J. Clin. Invest.* **99**, 2752–2761 (1997).
 63. Whitman, S.C., Ravisankar, P., Elam, H. & Daugherty, A. Exogenous interferon- γ enhances atherosclerosis in apolipoprotein E^{-/-} mice. *Am. J. Pathol.* **157**, 1819–1824 (2000).
 64. Lee, T.S., Yen, H.C., Pan, C.C. & Chau, L.Y. The role of interleukin 12 in the development of atherosclerosis in ApoE-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **19**, 734–742 (1999).
 65. Whitman, S.C., Ravisankar, P. & Daugherty, A. Interleukin-18 enhances atherosclerosis in apolipoprotein E^{-/-} mice through release of interferon- γ . *Circ. Res.* **90**, E34–E38 (2002).
 66. Pinderski, L.J. *et al.* Overexpression of interleukin-10 by activated T lymphocytes inhibits atherosclerosis in LDL receptor-deficient mice by altering lymphocyte and macrophage phenotypes. *Circ. Res.* **90**, 1064–1071 (2002).
 67. Laurat, E. *et al.* *In vivo* downregulation of T helper cell 1 immune responses reduces atherosclerosis in apolipoprotein E-knockout mice. *Circulation* **104**, 197–202 (2001).
 68. Huber, S.A., Sakkinen, P., David, C., Newell, M.K. & Tracy, R.P. T helper-cell phenotype regulates atherosclerosis in mice under conditions of mild hypercholesterolemia. *Circulation* **103**, 2610–2616 (2001).
 69. King, V.L., Szilvassy S.J. & Daugherty, A. Interleukin-4 deficiency decreases atherosclerotic lesion formation in a site-specific manner in female LDL receptor^{-/-} mice. *Arterioscler. Thromb. Vasc. Biol.* **22**, 456–461 (2002).
 70. Mallat, Z. *et al.* Inhibition of transforming growth factor- β signaling accelerates atherosclerosis and induces an unstable plaque phenotype in mice. *Circ. Res.* **89**, 930–934 (2001).
 71. Lutgens, E. *et al.* Transforming growth factor- β mediates balance between inflammation and fibrosis during plaque progression. *Arterioscler. Thromb. Vasc. Biol.* **22**, 975–982 (2002).
 72. Caligiuri, G., Nicoletti, A., Poirier, B. & Hansson, G.K. Protective immunity against atherosclerosis carried by B cells of hypercholesterolemic mice. *J. Clin. Invest.* **109**, 745–753 (2002).
 73. Witztum, J.L. Splenic immunity and atherosclerosis: A glimpse into a novel paradigm? *J. Clin. Invest.* **109**, 721–724 (2002).
 74. Robinette, C.D. & Fraumeni, J.F., Jr. Splenectomy and subsequent mortality in veterans of the 1939–45 war. *Lancet* **2**, 127–129 (1977).
 75. Paulsson, G., Zhou, X., Tornquist, E. & Hansson, G.K. Oligoclonal T cell expansions in atherosclerotic lesions of apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **20**, 10–17 (2000).
 76. George, J. *et al.* Adoptive transfer of β_2 -glycoprotein I-reactive lymphocytes enhances early atherosclerosis in LDL receptor-deficient mice. *Circulation* **102**, 1822–1827 (2000).
 77. Beck, J.D., Pankow, J., Tyroler, H.A. & Offenbacher, S. Dental infections and atherosclerosis. *Am. Heart J.* **138**, S528–S533 (1999).
 78. Wick, G., Perschinka, H. & Millonig, G. Atherosclerosis as an autoimmune disease: An update. *Trends Immunol.* **22**, 665–669 (2001).
 79. Mayr, M., Kiechl, S., Willeit, J., Wick, G. & Xu, Q. Infections, immunity, and atherosclerosis: Associations of antibodies to *Chlamydia pneumoniae*, *Helicobacter pylori*, and cytomegalovirus with immune reactions to heat-shock protein 60 and carotid or femoral atherosclerosis. *Circulation* **102**, 833–839 (2000).
 80. Afek, A. *et al.* Immunization of low-density lipoprotein receptor deficient (LDLRD) mice with heat shock protein 65 (HSP-65) promotes early atherosclerosis. *J. Autoimmun.* **14**, 115–121 (2000).
 81. Maron, R. *et al.* Mucosal administration of heat shock protein-65 decreases atherosclerosis and inflammation in aortic arch of low-density lipoprotein receptor-deficient mice. *Circulation* **106**, 1708–1715 (2002).
 82. Palinski, W. & Witztum, J.L. Immune responses to oxidative neoepitopes on LDL and phospholipids modulate the development of atherosclerosis. *J. Intern. Med.* **247**, 371–380 (2000).
 83. Gilotte, K.L., Hörrkö, S., Witztum, J.L. & Steinberg, D. Oxidized phospholipids, linked to apolipoprotein B of oxidized LDL, are ligands for macrophage scavenger receptors. *J. Lipid Res.* **41**, 824–833 (2000).
 84. Friedman, P., Hörrkö, S., Steinberg, D., Witztum, J.L. & Dennis, E.A. Correlation of antiphospholipid antibody recognition with the structure of synthetic oxidized phospholipids. Importance of Schiff base formation and aldol concentration. *J. Biol. Chem.* **277**, 7010–7020 (2002).
 85. Ylä-Herttua, S. *et al.* Rabbit and human atherosclerotic lesions contain IgG that recognizes epitopes of oxidized LDL. *Arterioscler. Thromb.* **14**, 32–40 (1994).
 86. Cyrus, T. *et al.* Absence of 12/15-lipoxygenase expression decreases lipid peroxidation and atherogenesis in apolipoprotein e-deficient mice. *Circulation* **103**, 2277–2282 (2001).
 87. Tsimikas, S., Palinski, W. & Witztum, J.L. Circulating autoantibodies to oxidized LDL correlate with arterial accumulation and depletion of oxidized LDL in LDL receptor-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **21**, 95–100 (2001).
 88. Stemme, S. *et al.* T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. *Proc. Natl. Acad. Sci. USA* **92**, 3893–3897 (1995).
 89. Palinski, W. *et al.* Cloning of monoclonal autoantibodies to epitopes of oxidized lipoproteins from apolipoprotein E-deficient mice. Demonstration of epitopes of oxidized low density lipoprotein in human plasma. *J. Clin. Invest.* **98**, 800–814 (1996).
 90. Hörrkö, S. *et al.* Monoclonal autoantibodies specific for oxidized phospholipids or oxidized phospholipid-protein adducts inhibit macrophage uptake of oxidized low-density lipoproteins. *J. Clin. Invest.* **103**, 117–128 (1999).
 91. Gilotte-Taylor, K., Boullier, A., Witztum, J.L., Steinberg, D. & Quehenberger, O. Scavenger receptor class B type I as a receptor for oxidized low density lipoprotein. *J. Lipid Res.* **42**, 1474–1482 (2001).
 92. Boullier, A. *et al.* The binding of oxidized low density lipoprotein to mouse CD36 is mediated in part by oxidized phospholipids that are associated with both the lipid and protein moieties of the lipoprotein. *J. Biol. Chem.* **275**, 9163–9169 (2000).
 93. Podrez, E.A. *et al.* Identification of a novel family of oxidized phospholipids that serve as ligands for the macrophage scavenger receptor CD36. *J. Biol. Chem.* **277**, 38503–38516 (2002).
 94. Podrez, E.A. *et al.* A novel family of atherogenic oxidized phospholipids promotes macrophage foam cell formation via the scavenger receptor CD36 and is enriched in atherosclerotic lesions. *J. Biol. Chem.* **277**, 38517–38523 (2002).
 95. Chang, M.K. *et al.* Monoclonal antibodies against oxidized low-density lipoprotein bind to apoptotic cells and inhibit their phagocytosis by elicited macrophages: Evidence that oxidation-specific epitopes mediate macrophage recognition. *Proc. Natl. Acad. Sci. USA* **96**, 6353–6358 (1999).
 96. Briles, D.E., Forman, C., Hudak, S. & Clafflin, J.L. Anti-phosphorylcholine antibodies of the T15 idotype are optimally protective against *Streptococcus pneumoniae*. *J. Exp. Med.* **156**, 1177–1185 (1982).
 97. Gershov, D., Kim, S., Brot, N. & Elkon, K.B. C-Reactive protein binds to apoptotic cells, protects the cells from assembly of the terminal complement components, and sustains an antiinflammatory innate immune response: Implications for systemic autoimmunity. *J. Exp. Med.* **192**, 1353–1364 (2000).
 98. Chang, M.K., Binder, C.J., Torzewski, M. & Witztum, J.L. C-reactive protein binds to both oxidized LDL and apoptotic cells through recognition of a common ligand: Phosphorylcholine of oxidized phospholipids. *Proc. Natl. Acad. Sci. USA* **99**, 13043–13048 (2002).

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