This Review is part of a thematic series on **Inflammatory Mechanisms in Atherosclerosis**, which includes the following articles:
- Anti-Inflammatory Mechanisms in the Vascular Wall
- Clinical Imaging of the High-Risk or Vulnerable Atherosclerotic Plaque
- Novel Clinical Markers of Vascular Wall Inflammation
- CD40 Signaling and Plaque Instability

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**Innate and Adaptive Immunity in the Pathogenesis of Atherosclerosis**

Göran K. Hansson, Peter Libby, Uwe Schönbeck, Zhong-Qun Yan

Abstract — This review considers critically the evidence for the involvement of mediators of innate and acquired immunity in various stages of atherosclerosis. Rapidly mobilized arms of innate immunity, including phagocytic leukocytes, complement, and proinflammatory cytokines, contribute to atherogenesis. In addition, adaptive immunity, with its T cells, antibodies, and immunoregulatory cytokines, powerfully modulates disease activity and progression. Atherogenesis involves cross talk between and shared pathways involved in adaptive and innate immunity. Immune processes can influence the balance between cell proliferation and death, between synthetic and degradative processes, and between pro- and antithrombotic processes. Various established and emerging risk factors for atherosclerosis modulate aspects of immune responses, including lipoproteins and their modified products, vasoactive peptides, and infectious agents. As we fill in the molecular details, new potential targets for therapies will doubtless emerge. (Circ Res. 2002;91:281-291.)

Key Words: lymphocytes ■ macrophages ■ antibody ■ inflammation ■ cytokines

Chronic inflammation characterizes atheroma, lesions that contain abundant immune cells, particularly macrophages and T cells. Identification of this immune/inflammatory infiltrate led us and others to postulate the involvement of immune mechanisms in atherogenesis. This notion received support from the association of ischemic heart disease with elevated antibody titers to several autoantigens and microbial antigens and from the observation that elevated circulating levels of immune cytokines accompany acute coronary syndromes. During recent years, experiments in genetically altered mice have lent further support to the hypothesis that immune mechanisms contribute to atheroma formation. Cells and molecules that mediate both adaptive immunity, which depends on antigen-specific immunologic memory, and innate immunity, characteristically antigen- and memory-independent, localize in atherosclerotic lesions.

Innate Immunity: Fast but Blunt

The first line of immune defense is based on detection of pathogen-associated molecular patterns (PAMPs) that evoke a toxic and inflammatory response (Figure). Pattern recognition receptors for PAMPs and antimicrobial peptides produced on PAMP ligation constitute the bulwark of host defenses in invertebrates. The germline genome encodes the mediators of innate immunity, obviating the need for a complicated somatic differentiation process. Constitutive ex-
Adaptive immunity recognizes specific molecular structures and depends on the generation of large numbers of antigen receptors, ie, T-cell receptors (TCRs) and immunoglobulins, by somatic rearrangement processes in blast cells. Once T cells recognize foreign antigens presented to them, they initiate adaptive immune responses against precisely these antigens. These responses include direct attack of antigen-bearing cells by cytotoxic T lymphocytes, stimulation of B cells to produce antibodies against the antigens, and induction of inflammation, with enhanced innate responses, in the area where the antigen is present. All these responses cooperate during host defenses to eliminate the foreign particle or microorganism. When expressed inappropriately, they can give rise to autoimmune diseases or allograft rejection.

Initial activation of “naïve” T cells requires strong activating stimuli best provided by the dendritic cell (DC), a specialized macrophage cell.20,21 DCs express on their surface major histocompatibility complex (MHC) class II molecules at high density as well as a set of costimulatory factors needed to instigate the adaptive immune response, ie, the activation of naïve T cells. Once successful activation has occurred, the remaining memory T cells have a lower activation threshold. Subsequent rounds of stimulation therefore require lower amounts of antigen. Regular macrophages, not just DCs, can accomplish this less stringent function and reactivation can therefore occur in nonlymphoid tissues such as the vessel wall.

Most of our antibodies are encoded by genes that have undergone somatic rearrangement. Activation of a specific B cell by antigen causes hypermutations in its immunoglobulin genes. Together with an evolutionary pressure caused by the antigen, this leads to an affinity maturation that generates antibodies of increasing specificity.

Certain B cells called B1 cells do not undergo affinity maturation. Instead, they produce germline-encoded immunoglobulins that are usually low-avidity antibodies.22 Some of these “natural antibodies” recognize microbial components such as phosphorylcholine of pneumococci. Interestingly, this particular natural antibody also recognizes oxidized phospholipids of low-density lipoprotein (LDL).23 Sophisticated control mechanisms reduce the risk for inappropriate activation of the immune system. However, such activation can still occur, due to dysregulation or molecular mimicry. In the former case, a lower general

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### TABLE 1. Some Ligands for Pattern Recognition Receptors

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Scavenger Receptor (SR)</th>
<th>Toll-Like Receptor (TLR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS</td>
<td>SR-A</td>
<td>TLR2, TLR4</td>
</tr>
<tr>
<td>Lipoteichoic acid</td>
<td>SR-A, MARCO, SR-EC</td>
<td>?</td>
</tr>
<tr>
<td>Acetyl-LDL</td>
<td>SR-A, MARCO, SR-EC</td>
<td>TLR2, TLR4</td>
</tr>
<tr>
<td>Oxidized LDL</td>
<td>SR-A, CD36, SR-PSOX, LOX-1</td>
<td>?</td>
</tr>
<tr>
<td>HSP60</td>
<td>?</td>
<td>TLR2, TLR4</td>
</tr>
<tr>
<td>Bacterial CpG DNA</td>
<td>?</td>
<td>TLR9</td>
</tr>
</tbody>
</table>
Threshold for activation can lead to systemic autoimmune disease such as systemic lupus erythematosus. In the case of antigenic mimicry, endogenous molecules form that resemble foreign antigens. This situation can lead to organ-specific autoimmune in the tissues containing such autoantigens. Finally, production of endogenous molecules that can bind to pattern recognition receptors on macrophages may lead to inappropriate activation of innate immunity and pathological inflammation. All these situations cause human diseases, and at least the two latter types seem to contribute to atherogenesis.

**Innate and Adaptive Immune Mechanisms Operate Together and in Sequence During Host Defense**

In the course of evolution, the acquisition of adaptive immunity enabled a characteristic 2-tiered temporal sequence in immune responses. Rapidly deployed innate components furnish a first line of defense and beckon components of adaptive immunity, which mobilize more slowly. Adaptive immunity often harnesses effector pathways such as the complement cascade and adhesion molecules, which evolved as part of innate immunity. Consequently, shared mediators unite innate and adaptive immunity, the two limbs operating in concert as components of an integrated immune system. Table 2 summarizes some key features of innate and adaptive immunity.

**Vasculature as Part of the Immune System**

As integral components of the immune system, blood vessels are important in the surveillance of “self.” They play key roles in lymphocyte circulation and act as portals between tissue and blood compartments.

Endothelial cells (ECs) express TLRs, whose ligation induces expression of leukocyte adhesion molecules, inducible NO synthase 2 (NOS2), endothelin, interleukin-1, and other inflammatory molecules. These cells also express the scavenger receptors CD36 and LOX-1, and can internalize ligands such as modified LDL particles. Strategically located at the interface of blood and tissues, ECs play a pivotal role in the inflammatory response. Their activation causes leukocyte recruitment, increased permeability, edema, and other characteristic features of inflammation. Furthermore, ECs can activate adaptive immunity by presenting foreign antigens to specific T cells. Although they present antigen less efficiently than DCs or macrophages, the unique interfacial location of the endothelium renders ECs particularly important in recall responses to blood-borne antigens.

The blood vessels also occupy a central role in innate immune responses. Consider the extreme example of septic shock as a host response to a microbial invader. LPS from the microbe’s cell wall combine with soluble or cell-associated CD14 to ligate TLR4 on ECs, perivascular macrophages, and possibly vascular smooth muscle cells (SMCs). TLR signaling induces NF-κB activation and expression of genes such as NOS2. Production of abundant NO ensues, causing smooth muscle relaxation, vasodilation, and hypotension. NF-κB activation of tissue factor gene expression produces a hypercoagulable state. The combination of circulatory failure and disseminated intravascular coagulation often causes multiple organ failure and death. Other microbial components such as fimbriae on *Escherichia coli* can cause similar reactions, also probably through the TLR-NOS2 pathway. TNF-α and other cytokines released from activated macrophages act as amplifiers during septic shock in a positive feedback loop, probably via NF-κB activation, intensifying and disseminating the inflammatory response.

**Mediators of Innate and Adaptive Immunity**

The molecular mediators of innate immunity include protein molecules, collectively denoted as cytokines. In addition, small molecules, such as vasoactive peptides, histamine, and eicosanoids, may function as effectors of innate immunity and have been associated with atherosclerosis. Although T and B lymphocytes, the detector cells of adaptive immune responses, differ entirely from those of innate immunity, the effector pathways overlap to a great extent. Thus, T cell activation leads to secretion of the cytokine interferon-γ (IFN-γ), which primes macrophages, lowering their threshold for TLR-dependent activation. In addition, T cells can produce TNF-α, a proinflammatory cytokine with NF-κB activating capacity. Moreover, the activated T cells express CD40 ligand (CD40L or CD154), which ligates its receptor, CD40, on macrophages; B cells; and many other cells including DCs, ECs, and SMCs. By involving inflammatory cells in the effector phase, T cells with the T helper-1 (Th1) phenotype tend to promote and amplify the same kind of inflammatory responses also induced when innate immune cells recognize PAMPs through their pattern-recognition receptors.

### Table 2. Key Features of Innate and Adaptive Immunity

<table>
<thead>
<tr>
<th></th>
<th>Innate</th>
<th>Adaptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance in evolution</td>
<td>Primitive organisms</td>
<td>Vertebrates</td>
</tr>
<tr>
<td>Induction time</td>
<td>Fast (hours to days)</td>
<td>Slow (days to decades)</td>
</tr>
<tr>
<td>Recognizes</td>
<td>Common “pathogen-associated microbial patterns” (PAMPs)</td>
<td>Unique epitopes on each pathogen/antigen</td>
</tr>
<tr>
<td>Cellular components</td>
<td>Macrophages; NK cells; mast cells</td>
<td>T and B cells</td>
</tr>
<tr>
<td>Generation of specificity</td>
<td>Encoded in germline</td>
<td>Somatic rearrangement</td>
</tr>
<tr>
<td>Effector mechanisms</td>
<td>Complement (alternative pathway); cytokines; chemokines; cell-mediated cytotoxicity</td>
<td>Antibodies; cytotoxic T cells (CTL); classical complement activation; antibody-dependent cell-mediated cytotoxicity; cytokines, chemokines</td>
</tr>
<tr>
<td>Characteristic transcription factors</td>
<td>NF-κB (+ JNK/AP1)</td>
<td>Jak/STAT, NF-κB, etc</td>
</tr>
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"Hansson et al Immunity in Atherosclerosis 283"

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Initiation of Atherosclerosis

Much of our knowledge regarding the details of cellular and molecular signaling during atherogenesis relies on observations in animal models. In view of the excessive lipid levels required for disease induction and the compressed time scale of disease development, we must frame the conclusions derived from such experimental preparations in tentative terms. Nonetheless, correlation with observations made on human material tends to corroborate the major conclusions derived from experimental models of atherosclerosis.

The animal studies, as well as cell culture experiments and studies of human tissue samples, support the view that the initiation of atherosclerosis often represents a response of the innate immune system to the accumulation and modification of lipoproteins in the arterial intima. Extracellular accumulation of lipids occurs very early in response to increased plasma lipoprotein levels in animals. Proteoglycan and protein-bound lipoprotein particles, perhaps in microenvironments shielded from plasma antioxidants, can undergo modification. Such modifications include oxidation of the lipid or protein moieties as well as nonenzymatic glycation of lipoproteins. Furthermore, the products of hypochlorous acid-induced modification of lipoproteins localize in atheroma. Of note, macrophages within atheroma can contain myeloperoxidase, the enzyme that produces hypochlorous acid.

Several lines of evidence suggest that microbial products may promote plaque growth and/or activation. Bacterial products such as LPS and heat shock proteins (HSPs) may act on vascular cells, DNA and proteins of certain microorganisms can be detected in lesions, and seroepidemiological data show correlations between antibody titers to microbes and disease. The recent finding of TLR expression in atherosclerotic plaques offers a possible mechanism by which microbial products may activate plaque cells.

Excellent in vitro evidence supports an important effector role for variously modified lipoproteins and their constituents in triggering the production of the mediators of innate immunity. In addition, nonlipid mediators implicated in vascular disease may also elicit cytokine gene expression. For example, angiotensin II, previously regarded primarily as a vasoconstrictor molecule, can induce the elaboration of cytokines from atheroma-associated cells.

Cytokines elicited by such atherogenic stimuli augment the expression of the genes encoding various leukocyte adhesion molecules, increasing their expression on the surface of ECs in regions of nascent atheroma formation. Candidate molecules for endothelial-leukocyte adhesion in early atherogenesis validated by experiments in genetically altered mice include vascular cell adhesion molecule-1 (VCAM-1) and E-selectin. In addition, lipid components of modified lipoproteins can directly induce adhesion molecules. Lyso-phosphatidyl choline and other phospholipid species generated during lipid peroxidation act as proinflammatory stimuli, inducing VCAM-1 in ECs. Reactive oxygen species may also induce VCAM-1 due to their capacity to activate NF-κB.

Once adherent, the leukocytes migrate into the underlying intima in response to chemoattractant stimuli. The chemoattractant cytokines (chemokines) probably participate in this process. Interruption of signaling due to monocyte chemoattractant protein-1 (MCP-1), for example, will retard lesion formation in hypercholesterolemic mice. MCP-1 attracts mononuclear leukocytes bearing the chemokine receptor CCR-2. Different categories of chemokines may participate in recruitment of distinct leukocyte classes to the atheroma. For example, a trio of CXC chemokines (IP-10, Mig, and I-Tac) selectively attract T and B lymphocytes, which bear the CXC R3 receptor. Recent work has localized eotaxin in human atherosclerotic plaques. This CC chemokine, in addition to recruiting mononuclear phagocytes, may participate in mast cell accumulation within atheroma.

Once resident in the arterial intima, monocytes differentiate into macrophages, which accumulate intracellular lipid. This process depends on the expression of scavenger receptors, including SR-AI and II, CD36, MARCO, SR-PSOX, and CD68, also known as macrosin. As discussed above, these receptors recognize structural motifs shared by a wide variety of microbial macromolecules, as well as apoptotic cells and modified lipoproteins. Uptake through SR-A can lead to presentation of processed ligands to specific T cells, and this receptor therefore links innate and adaptive immunity.

Other potentially important features of the lipid-laden macrophage include proliferation and the elaboration of certain cytokines and growth factors. In addition to MCP-1, macrophage-colony stimulating factor (M-CSF) appears to play a key role in the activation of various macrophage functions implicated in atherogenesis. Studies of human and experimental atherosclerosis have documented overexpression of M-CSF within lesions. Moreover, mutant mice lacking the ability to produce M-CSF display delayed atheroma development in a gene dosage–dependent fashion.

Complement constitutes an additional family of effector proteins involved in innate immunity. In experimental atheroma, complement activation can actually precede the development of lesions. Products of the complement cascade including anaphylatoxin can attract leukocytes. In addition, the terminal membrane attack complex of complement can promote damage to cell membranes and eventual cell death. Sublethal injury may permit the release of growth factors, such as fibroblast growth factor, from cells. C-reactive protein, an acute phase reactant whose serum and lesional levels appear elevated during atherogenesis, may activate complement. However, the role of complement in atherosclerosis remains controversial.

Mobilization of Adaptive Immunity in the Atheroma

T cells participate in the formation of atherosclerotic lesions as early as monocytes. Indeed, several of the adhesion molecules and chemokines that promote monocyte recruitment also stimulate T cells to enter lesions (eg, the selectins, VCAM-1, ICAM-1, and MCP-1). In advanced human plaques, T cells constitute approximately 10% to 20% of the
cells. 74,85 By exhibiting cytotoxic activity and producing

mice, arterial lesions contain Th2 cytokines only in condi-

tions of extreme hypercholesterolemia. 82 Notably, Th1 and Th2 cytokines exhibit cross-regulation: IL-10 inhibits the Th1 pathway, whereas IL-12 reciprocally inhibits Th2 responses. The low level of Th2 activity in lesions may therefore result at least in part from local IL-12 secretion. Interleukin-10 may correspondingly dampen the Th1 response. Accordingly, IL-10–deficient mice have increased fatty streak development. 83,84 In addition to regulatory cytokines, the level of antigen expressed in the vicinity of the antigen-presenting cell and the T cell may also influence the Th1/Th2 bias. Therefore, the level of autoanti-
gens such as modified lipoproteins in the lesions and regional lymph nodes may act in concert with IL-10/IL-12 regulation to control the balance between the two Th effector pathways.

In addition to helper T cells, atherosclerotic plaques harbor moderate numbers of CD8+ T cells as well as occasional B cells. 74,85 By exhibiting cytotoxic activity and producing antibodies, respectively, both these cell types may wield an importance greater than indicated by their sheer numbers. 7

As explained, antigen presented by macrophages or ECs readily activate memory-effector T cells. Naïve T cells, however, require presentation by DCs. Interestingly, such cells have been detected in human as well as experimental atherosclerotic lesions. 86,87 DCs have a high migratory capacity and may “patrol” tissues such as the artery wall in search for antigens. Foreign materials encountered during such surveillance when endocytosed, transported to regional lymph nodes, and presented by DCs, could activate both naïve and memory T cells. 20,21

In addition to T effector cells and macrophages, athero-
sclerotic lesions contain another immune effector cell, the mast cell. 88 Although macrophages and T cells by far outnumber mast cells, they may nonetheless function importantly in plaque activation and acute coronary syndromes because they produce a host of proteases (including some not made by macrophages) and accumulate at sites of plaque rupture. 89–91 Factors released from mast cells may degrade the extracellular matrix and could also influence the functions of surrounding cells and modify locally deposited lipoproteins. 92

Specific Antigens Initiate Adaptive Immunity in Atherosclerosis

T cells owe their antigen specificity to the unique sequence of the antigen-binding site in the CDR3 domain of the receptor protein. 89 On activation, the stimulated T cell divides to give rise to a clone of cells with identical specificities. The presence in a tissue of a population of T cells with identical TCRs therefore indicates clonal proliferation, which is usually due to antigenic stimulation.

The early lesions of apoE-deficient mice show evidence for such clonal T cell expansion. 94 Human lesions, studied of necessity at later stages and by nature less uniform than their experimental counterparts, present a more complex situation. Advanced human atheroma contain a heterogeneous population of TCRs and therefore T cells. 95–98 Clonal expansions may occur in earlier phases of disease; however, the limited availability of early lesions makes it difficult to assess this possibility.

Autoimmune conditions are often linked to certain MHC alleles. In type I diabetes, for example, one specific HLA-DQ allele favors an autoimmune response that leads to β-cell destruction and disease. However, the ubiquity and multifac-
torial nature of human atherosclerosis make a simple rela-
tionship with a single MHC determinant unlikely. Again, the access to mouse models of human disease has made it possible to study immunogenetic aspects of atherosclerosis. Such studies have demonstrated a disease-promoting role for (MHC class II) I- Aβ restricted Th1 cells, contrasting to an antiatherosclerotic effect of I-Eα and I- Aβ in the development of fatty streaks in fat-fed C57BL/6 mice. 99

Evidence from studies of human disease supports the involvement of autoantigens in atherosclerosis. T cells can be isolated from fresh human plaques, cloned and expanded in culture, and challenged with candidate antigens. Such experiments identified oxidized LDL as a major autoantigen in the cellular immune response of atherosclerosis. 100 This finding, together with the detection of anti-oxLDL antibodies in atherosclerotic patients and experimental animals, 101 supports the concept that immune responses to oxLDL operate in atheroma. Lymph nodes and spleens of apoE-deficient mice can give rise to oxLDL-specific T and B cell lines that display strong humoral as well as cellular immune responses to such modified lipoproteins. 102–105

HSPs comprise further candidate antigens in atherosclerosis. These proteins produced in large amounts by injured cells act as chaperones to limit denaturation of other cellular proteins. HSPs serve as targets for autoimmune responses in many inflammatory diseases, including rheumatoid arthritis and Crohn’s disease. 106

Immunization with HSP65/60 induces vascular inflammation, with infiltrates of HSP60-reactive T cells, 107–109 Peripheral blood of atherosclerotic animals contains anti-HSP60 antibodies, and immunization with HSP60 can aggravate disease in rabbits and mice. 107,110 Interestingly, HSP60 can activate TLR4, in a CD14-dependent manner, similar to bacterial endotoxin. 111 Therefore, HSP60 release may not only induce specific antibodies and T cells but also directly activate innate immunity.
A third proposed autoantigen, β2-glycoprotein Ib (β2GpIb), is present on platelets and under some circumstances on ECs. In several inflammatory disorders, including atherosclerosis, lupus, and the antiphospholipid antibody syndrome, plasma contains autoantibodies to β2GpIb. The immune response to β2GpIb appears to promote atherosclerosis; however, the mechanism involved remains obscure. Some evidence has implicated microbial pathogens in atherogenesis, and bacteria may induce innate immunity, molecular mimicry, and autoimmunity as well as direct infection of tissues. Several studies suggest a role for Chlamydia pneumoniae in atherosclerosis. Interestingly, HSP60 of this microbe resembles human HSP60 and can elicit inflammatory responses. Another putative vascular pathogen, cytomegalovirus, encodes a chemokine receptor that renders infected SMCs susceptible to CC chemokine–induced migration. It remains uncertain whether immune reactions to microbes and/or molecular mimicry between microbes and autoantigens contribute to atherosclerosis.

**Immune Cytokines Regulate Vascular Cells**

Once activated by either antigen, in the case of adaptive immunity, or by PAMP, in the case of innate immunity, both pathways converge in their use of cytokines and other major effectors. Cytokines produced by immune cells exert profound effects on vascular ECs and SMCs. Thus, growth factors such as FGF-2 and PDGF elaborated by macrophages and heparin-binding growth factors released by T cells can promote the proliferation of SMCs. Similarly, FGF-1 can stimulate the growth of ECs. In contrast, proinflammatory cytokines tend to inhibit vascular cell proliferation and rather sensitize them to apoptosis. Immune cytokines also influence the development and maintenance of differentiated properties in the vasculature. In SMCs, the gene for the contractile protein, α-actin, is stimulated by TGF-β but inhibited by IFN-γ. Similarly, TGF-β strongly promotes the synthesis of interstitial collagens (types I and III) by human SMCs, whereas IFN-γ powerfully inhibits their synthesis of collagen as well as α-actin. ECs respond to proinflammatory cytokines and chemokines by expressing adhesion molecules (see earlier) but also by reducing the continuity of interendothelial junctions, leading to increased permeability across the endothelial barrier.

As lesions progress, they frequently accumulate calcium mineral, which is a tightly regulated process. Cytokines control the expression of osteopontin/Eta-1, a protein implicated in lesion calcification but also in Th1 immunity. Members of the TGF-β family of cytokines, including the bone morphogenetic proteins (BMPs), may also participate in this process. Mice lacking M-CSF exhibit exaggerated calcification of atherosclerotic lesions in response to hyperlipidemia. This support the view that intraluminal macrophages may function as osteoclasts during atherogenesis. The steady-state level of calcium in a lesion at any given time probably reflects the balance between mineralization and dissolution due to this osteoclastic activity of the M-CSF–activated macrophage.

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**TABLE 3. Regulation of Vascular Cell Functions by Cytokines**

<table>
<thead>
<tr>
<th>Target</th>
<th>Positive Stimuli</th>
<th>Inhibitory Stimuli</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMC proliferation</td>
<td>PDGF (TGF-β)</td>
<td>IFN-γ (TGF-β)</td>
</tr>
<tr>
<td>SMC matrix/collagen</td>
<td>TGF-β (IL-1, PDGF)</td>
<td>IFN-γ</td>
</tr>
<tr>
<td>SMC contractile proteins</td>
<td>TGF-β</td>
<td>IFN-γ</td>
</tr>
<tr>
<td>Ig-type adhesion molecules</td>
<td>IL-1, TNF-α, IFN-γ, CD40L</td>
<td>...</td>
</tr>
<tr>
<td>SMC NOS2</td>
<td>IL-1, TNF-α, IFN-γ</td>
<td>TGFB-1, IL-4</td>
</tr>
<tr>
<td>EC/SMC COX2</td>
<td>IL-1, TNF-α, IFN-γ, CD40L</td>
<td>...</td>
</tr>
<tr>
<td>EC E-selectin</td>
<td>IL-1, TNF-α, IFN-γ, CD40L</td>
<td>...</td>
</tr>
<tr>
<td>EC/SMC tissue factor</td>
<td>IL-1, TNF-α, CD40L</td>
<td>...</td>
</tr>
<tr>
<td>EC/SMC MMPs</td>
<td>IL-1, TNF-α, CD40L</td>
<td>IFN-γ</td>
</tr>
</tbody>
</table>

Ig indicates immunoglobulin superfamily; COX-2, cyclooxygenase 2; NOS, nitric oxide synthase; and MMP, matrix metalloproteinase.

Importantly, vascular cells do not merely respond to cytokines but also can produce large amounts of these molecules. Both ECs and SMCs respond to stimulation with IL-1, TNF-α, or CD40 ligand by producing large amounts of IL-6 and also by augmented expression of IL-1 and CD40. They also express PTX-3, a member of the pentraxin gene family, which also includes CRP. PTX3 is found in atherosclerotic plaques and elevated serum levels of this cytokine is an early marker of myocardial infarction. In summary, vascular cells participate in and propagate the inflammatory response at sites of microbial challenge or pathological processes. Table 3 summarizes some important cytokine-vascular interactions.

**Importance of Immune Mechanisms in Atherosclerosis Deduced From Genetically Altered Mice**

Studies in genetically altered mice, particularly the hypercholesterolemic apolipoprotein E (apoE) and LDL-receptor (LDLR) knockout strains, show important roles for components of innate and adaptive immunity in atherosclerosis (Table 4). Reduced atherosclerosis in hyperlipidemic mice lacking scavenger receptor SR-A or CD36 implicates these pattern-recognition receptors in this disease. In contrast, TLR-4 deficiency does not change disease substantially in

**TABLE 4. Effects of Immune-Related Genes on Atherosclerosis in Knockout Mouse Models**

<table>
<thead>
<tr>
<th>Immune Defect</th>
<th>Disease Model</th>
<th>Effect on Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-CSF</td>
<td>apoE</td>
<td>↓</td>
</tr>
<tr>
<td>MCP-1, CCR2</td>
<td>apoE, LDLR</td>
<td>↓</td>
</tr>
<tr>
<td>P-, E-selectin</td>
<td>apoE</td>
<td>↓</td>
</tr>
<tr>
<td>SR-A</td>
<td>apoE</td>
<td>↓</td>
</tr>
<tr>
<td>CD36</td>
<td>apoE</td>
<td>↓</td>
</tr>
<tr>
<td>RAG</td>
<td>apoE</td>
<td>↓</td>
</tr>
<tr>
<td>SCID</td>
<td>apoE</td>
<td>↓</td>
</tr>
<tr>
<td>IFNgR</td>
<td>apoE</td>
<td>↓</td>
</tr>
<tr>
<td>CD40L</td>
<td>apoE</td>
<td>↓</td>
</tr>
<tr>
<td>IL-10</td>
<td>apoE</td>
<td>↑</td>
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</table>
apoE−/− mice. However, this result does not exclude participation of other TLRs in atherogenesis.

Several studies demonstrate a modulating role for adaptive immunity. The complete lack of adaptive immunity caused by the RAG or SCID mutations results in a 40% to 70% reduction of atherosclerosis in apoE−/− mice. Although these findings imply that atherosclerosis develops also in the absence of adaptive immunity, they point to an important modulating role of the arm of host defense. When conducted under conditions of extreme hypercholesterolemia, the modulatory effect of this immune defect is diminished. Importantly, reconstitution of SCID×apoE−/− mice with CD4+ T cells from immunocompetent apoE−/− mice accelerates disease almost to the level of the fully immunocompetent apoE−/− mouse. This result pinpoints CD4+ T cells as playing a proatherosclerotic role, a conclusion consistent with the substantial reduction of atherosclerosis observed in apoE−/− mice lacking interferon-γ signaling. CD40 ligation also plays an important proatherogenic role because interruption of CD40 signaling substantially reduces murine atheroma formation or evolution. Moreover, interruption of CD40/CD40L interaction yields lesions that express features of plaque stabilization, including diminished lipid and enhanced collagen content.

Immunization with oxidized LDL reduces atherosclerosis in hypercholesterolemic rabbits and mice and transfer of immunoglobulins also inhibits disease development. These observations point to atheroprotective immunity that may modulate the proatherogenic immune pathways. Support for this notion was recently obtained when protection against atherosclerosis was achieved by transferring B cells from atherosclerotic apoE KO mice to young apoE KO mice that had not yet developed disease. It is not yet known whether such atheroprotective immunity depends on circulating antibodies or on T-B cell interactions. However, the demonstration of transferable, atheroprotective immunity encourages further studies of immunization and immunomodulation as possible means for treatment of atherosclerosis.

**Immune Mediators Regulate the Thrombotic Complications of Atherosclerosis**

The dreaded clinical manifestations of atherosclerosis generally involve thrombosis. Chronic stable angina or claudication of the lower extremities, while limiting effort, themselves do not threaten life. In contrast, acute myocardial infarction and stroke comprise by far the leading cause of mortality in developed nations. The mediators of innate immunity can regulate aspects of the thrombotic complications of atheroma. Tissue factor, the principal protagonist of thrombosis in atheroma. The expression of tissue factor in human macrophages, ECs, and SMCs depends on triggers to, and effectors of, immunity. LPS, TNF-α, IL-1, interferon-γ, and PTX-3 all induce tissue factor gene expression in human ECs. CD40 ligation can also induce tissue factor gene expression in human monocyte/macrophages. In atheroma, macrophages, SMCs, and ECs, can all express CD40 Ligand (CD154). In addition, activated platelets express functional CD154.

The accumulation of thrombus depends not only on its formation due to tissue factor–induced activation of the coagulation cascade, but also on clot breakdown due to thrombolysis. The principal atheroma-associated fibrinolytic mediators, tissue type, and urokinase plasminogen activators vary depending on the milieu of mediators of innate immunity. Soluble cytokines such as interleukin-1 and TNF-α can alter the activity of plasminogen activators, as well as thrombomodulin, an antagonist of coagulation. Cytokines can also augment the expression of inhibitors of the endogenous plasminogen activators. In this manner, mediators of innate immunity can modulate the delicate balance between clot formation and dissolution.

A physical disruption of the atherosclerotic plaque precipitates thrombus formation in a majority of cases. Rupture of the plaque’s fibrous cap allows contact of the blood coagulation proteins with the tissue factor procoagulant found within the intima. It is likely that a dynamic balance between collagen synthesis and degradation determines the fragility of the plaque’s fibrous cap, and hence the tendency to rupture and cause thrombosis. Interferon-γ inhibits collagen synthesis and may therefore limit the immune response to weakening of the fibrous cap. Matrix metalloproteases, cathepsins, and mast cell proteases can impair the integrity of the fibrous cap by degrading its collagen cap. Proinflammatory cytokines can regulate the release of these matrix-degrading proteases.

Because the SMC produces most of the interstitial collagen that lends strength to the plaque’s fibrous cap, paucity of these cells may render a plaque weak and vulnerable to rupture. Indeed, death, including death by apoptosis, of SMCs in the advanced atheroma might impair the ability of this cell type to repair and maintain the extracellular matrix that regulates the integrity of the plaque’s all-important fibrous cap, not only inhibits smooth muscle proliferation, but may also, together with TNF-α and IL-1, promote apoptosis of SMCs. Loss of SMCs, in part governed by mediators of immunity, can influence the susceptibility of a plaque to rupture. The findings of activated macrophages, T cells, and mast cells and of proteolytic enzymes at sites of plaque rupture in human coronary atheroma support the link between immunity and thrombosis.

**Conclusion**

This review has highlighted the involvement of mediators of innate immunity in all stages of atherosclerosis, from the earliest stage of lesion initiation to the ultimate clinical complication, thrombosis. Although phagocytic leukocytes often instigate innate immunity and their proinflammatory cytokine products contribute to disease as well as defense, adaptive immunity, with its T cells and immune regulatory cytokines, powerfully modulates disease activity and progression. Atherogenesis undoubtedly involves cross talk between pathways principally involved in adaptive and innate immunity. The complexity of the signaling pathways involved in atherogenesis appears daunting at first glance. Few human diseases have a longer “incubation period” than atherosclerosis. This disease can progress virtually unnoticed over
many decades and present clinically later in life or even evade trespassing the clinical horizon at all.

Despite the plethora of mediators and pathways that prevail during this prolonged period of lesion evolution, a unifying principle can simplify the fundamental concepts. The dynamism of plaque biology emerges as a major simplifying concept. Balances between positive and negative signals, between synthetic and degradative processes, between life and death, regulated by the alphabet soup of mediators ultimately determine the tempo of lesion evolution, complication, and clinical manifestations. By evoking elements of host defense reaction, atherosclerosis shares much with other inflammatory and/or fibrotic diseases. Future work will no doubt add to the list of mediators involved in regulating these processes. As we fill in the molecular details, new potential targets for therapies will doubtless emerge.

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