Brief Reviews

Immune Mechanisms in Atherosclerosis

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Abstract—Atherosclerosis is an inflammatory disease. Its lesions are filled with immune cells that can orchestrate and effect inflammatory responses. In fact, the first lesions of atherosclerosis consist of macrophages and T cells. Unstable plaques are particularly rich in activated immune cells, suggesting that they may initiate plaque activation. We have seen a rapid increase in the understanding of the mechanisms that govern the recruitment, differentiation, and activation of immune cells in atherosclerosis. Experimental research has identified several candidate antigens, and there are encouraging data suggesting that immune modulation as well as immunization can reduce the progression of the disease. This review provides an overview of our current understanding of the role of immune mechanisms in atherosclerosis. (Arterioscler Thromb Vasc Biol. 2001;21:1876-1890.)

Key Words: atherosclerosis | pathophysiology | cell biology | cytokines

The atherosclerotic lesion contains large numbers of immune cells, particularly macrophages and T cells. Furthermore, the disease is associated with systemic immune responses and signs of inflammation. Histopathological and clinical investigations point to inflammatory/immune activation of plaques as a cause of acute coronary syndromes, and seroepidemiological studies have suggested links between atherosclerosis and microbial infections. During recent years, experiments in gene-targeted mice have provided mechanistic evidence in support of the hypothesis that immune mechanisms are involved in atherosclerosis. This review will provide an update on immune mechanisms in atherosclerosis, with particular focus on mechanistic studies.

Immune Cells in the Lesions of Atherosclerosis

Adaptive (ie, antigen-specific) immune reactions are initiated when a macrophage (or a dendritic cell of the macrophage lineage) displays a surface complex consisting of an antigenic peptide bound to a major histocompatibility complex (MHC) protein to a neighbor T cell. This can elicit T-cell activation, cytokine secretion, cytotoxicity, antibody production, and many other components of an immune reaction. There is now good evidence that atherosclerosis is associated with immune reactions and that antigen presentation occurs in atherosclerotic lesions. The cellular composition of an atheroma is illustrated in Figure 1. It has been known for many years that monocyte-derived macrophages are present in large numbers in atherosclerotic lesions.1–7 The discovery of the scavenger receptor pathway for uptake of modified lipoproteins8–10 provided an explanation for the finding that most foam cells bear macrophage differentiation markers.

The other main immune cell of atherosclerotic lesions, the T cell, remained undetected for a long time. It was observed that many vascular smooth muscle cells (SMCs) in human lesions express human leukocyte antigen (HLA)-DR, a cell surface protein that is induced by the T-cell cytokine interferon-γ (IFN-γ).11 By using T-cell–specific CD3 antibodies, it could be established that T cells are abundant in human plaques.12 A large proportion of them are in an activated state13–16; ie, they express adhesion molecules and other surface molecules, secrete cytokines, and may proliferate. The activation of T cells and macrophages leads to a cascade of cytokines that induce an inflammatory state. With the cDNA cloning and production of recombinant cytokines, it became possible to study their effects in the context of vascular biology. Such studies revealed that vascular endothelial cells and SMCs are important targets for inflammatory cytokines and that they are capable of producing significant amounts of cytokines themselves on stimulation.17,18

Studies during the last decade have identified other types of immune cells in atherosclerotic plaques. Among them, dendritic cells are “professional” antigen-presenting cells that may be particularly important in the initiation of immune responses to plaque antigens.19 Mast cells are immune effector cells with a capacity to modify lipoproteins and digest matrix components; they could be important in plaque activation and rupture.20 All these different cells may be involved in the initiation, progression, and/or development of complications to atherosclerosis. At present, there is evidence for pathogenic roles of monocyte-derived macrophages and T cells.

Recruitment of Immune Cells to the Vessel Wall

Leukocyte Adhesion to the Endothelium

During the initiation of atherosclerosis, mononuclear leukocytes, ie, monocytes and T cells, are recruited to the vessel wall across an intact endothelium. This requires activation of
Hypercholesterolemia leads to recruitment of immune cells to the vessel wall. LDL accumulates in the arterial intima. Lipid products of LDL oxidation induce VCAM-1 in endothelial cells. Monocytes and T cells adhere through their VLA-4 receptors. Their subsequent migration through the endothelial layer is stimulated by chemokines such as MCP-1 and also by complement activation products (Cpt), which can be generated by oxidized cholesterol aggregates. See text for explanation of other abbreviations.

TABLE 1. Animal Studies Demonstrating Important Roles for Leukocyte Adhesion and Migration in Atherosclerosis

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Function</th>
<th>Experiment</th>
<th>Atherosclerosis Model</th>
<th>Result</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-, E-selectin</td>
<td>Rolling (PMN, MC, Ly)</td>
<td>KO</td>
<td>ApoE-KO</td>
<td>↓ Fatty streaks</td>
<td>34</td>
</tr>
<tr>
<td>P-selectin</td>
<td>Rolling (PMN, MC, Ly)</td>
<td>KO</td>
<td>ApoE-KO</td>
<td>↓ Atheroma</td>
<td>35</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Firm adhesion (MC, Ly)</td>
<td>Ab</td>
<td>Rabbit</td>
<td>↓ Postinjury lesion</td>
<td>213</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Chemoattractant (MC, Ly)</td>
<td>KO</td>
<td>LDLR-KO, apoE-KO, apoB-Tg</td>
<td>↓ Atheroma</td>
<td>44, 46</td>
</tr>
<tr>
<td>CCR-2</td>
<td>MCP-1 rec (MC, Ly)</td>
<td>KO</td>
<td>LDLR, apoE-KO</td>
<td>↓ Atheroma</td>
<td>45</td>
</tr>
</tbody>
</table>

CCR-2 indicates C-C chemokine receptor-2; PMN, polymorphonuclear leukocytes; MC, monocytes; Ly, lymphocytes; rec, receptor; and Tg, transgenic. See text for explanation of other abbreviations.
velop fewer fatty streaks when bred into an atherosclerosis-prone mouse strain, the apoE-knockout (KO) mouse\textsuperscript{34,35} (Table 1).

**Chemotactic Transmigration**

In the classic inflammatory response, adhesion is followed by transmigration of the leukocytes through the endothelial layer and into the intima. This is governed by chemotactic factors produced in the subendothelial layer. Several studies of hypercholesterolemic rabbits and human samples show that the complement cascade is activated subendothelially during hypercholesterolemia.\textsuperscript{36,37} This leads to release of small, proteolytic peptide fragments of complement proteins. Such fragments include C5a, which is strongly chemotactic for monocytes and may be important for the recruitment of these cells to the intima.

Several chemotactic cytokines called chemokines are produced by endothelial cells, SMCs, and intimal macrophages during lesion formation. The best-characterized of these chemokines is monocyte chemotactic protein-1 (MCP-1), which can be induced by complement activation or cytokines\textsuperscript{38,39} and promotes recruitment of monocytes and T cells.\textsuperscript{40–42} MCP-1 is expressed in significant amounts in all stages of atherosclerosis.\textsuperscript{40,43} When atherosclerosis-prone mice such as apoE-KO or LDL receptor (LDLR) KO mice were bred with mice deficient in MCP-1 or its receptors, CCR2, lesion formation was reduced drastically\textsuperscript{44–46} (Table 1). This demonstrates that chemokine-dependent migration of mononuclear cells into the intima is an important phenomenon in atherogenesis (Figure 2).

**Cellular Immunity of Atherosclerotic Lesions**

**Macrophages: Scavenging, Secretory, and Antigen-Presenting Cells**

Macrophages are major players in inflammation and innate (ie, antigen-independent) immune responses. These actions largely depend on their capacity to produce free oxygen radicals, proteases, complement factors, and cytokines. Importantly, the macrophage may also initiate adaptive immune responses by presenting foreign antigens to T cells. All these activities may be important in atherogenesis.

The differentiation from monocyte to macrophage is governed by macrophage colony stimulating factor (M-CSF), a cytokine that is produced not only by macrophages but also by vascular and stromal cells. Lack of M-CSF prevents macrophage differentiation, the consequences of which can be observed in many different organs. A striking phenotype of the M-CSF–deficient \textit{op/op} mouse is the lack of osteoclasts, which prevents bone remodeling and leads to the disease osteopetrosis. The \textit{op/op} phenotype is also characterized by a lack of macrophages in tissues. When \textit{op/op} mice were mated with apoE-KO mice, the offspring developed very little atherosclerosis despite high cholesterol levels in the blood.\textsuperscript{47} This demonstrates that macrophage differentiation is necessary for atherosclerosis.

Macrophage uptake of modified lipoproteins by way of scavenger receptors is tightly regulated by cytokines (Table 2 and Figure 1). IFN-γ, tumor necrosis factor-α (TNF-α), and IL-6 downregulate scavenger receptor-A (SR-A).\textsuperscript{48–50} IL-4 upregulates CD36,\textsuperscript{51} and the lectinlike, oxidized LDL receptor (LOX-1) is controlled by TNF-α and transforming growth factor-β (TGF-β).\textsuperscript{52} That cytokines regulate scavenger receptors probably reflects important roles for these receptors in the innate host defense. Additional support for this notion is the finding that SR-A–deficient mice are highly susceptible to infections by intracellular bacteria such as \textit{Listeria monocytogenes}.\textsuperscript{53} However, SR-A can also internalize antigens, which are routed for presentation to T cells.\textsuperscript{54,55} By binding foreign antigens and initiating their transfer to antigen-processing as well as degradative compartments, scavenger receptors may be important links between innate and adaptive immunity.

Components of oxLDL may not only be degraded or processed for antigen presentation but also may activate the macrophage itself. This was initially demonstrated in studies of peripheral human monocytes and macrophage cell lines\textsuperscript{56,57} and later confirmed in other cell culture systems. This effect may be mediated by proinflammatory lipids acting on specific receptors. OxLDL contains platelet-activating factor (PAF)–like lipids, which are strongly proinflammatory and activate macrophages as well as endothelial cells.\textsuperscript{58–61} In addition, oxLDL components might bind to signaling surface receptors such as TLRs or nuclear receptors such as lipid X receptors (LXR). Both of these receptor families are important in the regulation of macrophage function: TLRs mediate macrophage activation in response to bacterial toxins,\textsuperscript{62} whereas LXRs regulate the ATP binding cassette-I (ABCA1) transporter and other aspects of cholesterol metabolism in the macrophage.\textsuperscript{63}

**T-Cell Types and Their Functions**

**Important Role for Th1 Cells and Their Cytokines**

Most of the T cells in atherosclerotic lesions are CD3\textsuperscript{+} CD4\textsuperscript{+} T-cell receptor (TCR) \textalphaβ\textsuperscript{+} cells\textsuperscript{12,15} (Table 3). This implies that they recognize protein antigens presented to them by macrophages after uptake and processing through the endosomal pathway (Figure 3). Such cells represent approximately two thirds of all CD3\textsuperscript{+} T cells in advanced human lesions and most of the T cells in lesions of apoE-KO mice. Many of the cells exhibit surface markers indicative of a population of memory cells in a state of chronic activation.\textsuperscript{15} They are largely of the T-helper (Th1) subtype, which secretes IFN-γ, IL-2, and TNF-α and -β, which cause macrophage activation, vascular activation, and inflammation.\textsuperscript{64} At least 2 important stimuli for Th1 differentiation are present in the atherosclerotic plaque. The cytokine IL-12, which is produced by many lesion cells, is an important stimulus for Th1 differentiation.\textsuperscript{65} Osteopontin, also called early T-lymphocyte activation protein-1 (Eta-1), is needed for Th1 responses and promotes IL-12 expression and granuloma formation.\textsuperscript{66} It is expressed by macrophages, endothelial cells, and SMCs in plaques\textsuperscript{67,68} and may be important for local immunity as well as for mineralization.

IFN-γ is an important immune-activating cytokine that can prime macrophages for activation and induce inflammatory responses such as those observed in delayed-type hypersensitivity and granulomatous lesions (Table 2). It has been detected in human plaques, both on the mRNA and protein levels.\textsuperscript{13,64} Cell culture studies have shown it to be a powerful growth inhibitor for endothelial cells and SMCs.\textsuperscript{69,70} In
addition, IFN-γ induces expression of secretory phospholipase A_2, which can lead to production of inflammatory lipid mediators such as eicosanoids, lysophosphatidylcholine, and PAF.71

In vivo studies in rats have shown that the proliferative response of SMCs after arterial injury is inhibited by IFN-γ,72 which also inhibits smooth muscle contractility and collagen synthesis.70,73 It was therefore proposed that IFN-γ-producing T cells could play an important role in plaque destabilization by reducing the fibrous cap.74,75 Histopathological findings of activated T cells at sites of rupture in culprit lesions support this notion.76

Further support for a proatherogenic role came from studies of compound KO mice lacking the IFN-γ receptor as well as apoE. These mice had an ~60% reduction in atherosclerosis, supporting the notion that IFN-γ is a strongly proatherogenic cytokine77 (Table 4). The lack of IFN-γ signaling affected the lipoprotein profile (reduced apoA-IV) as well as the vessel wall (increased collagen), implying that both systemic and local effects could be important for the antiatherogenic action. However, IFN-γ was recently shown to aggravate transplant vasculopathy by its local action on the vessel wall.78 Even in severe combined-immunodeficient (SCID) mice, IFN-γ induced vascular pathology in xenografts, indicating that its direct vascular actions are sufficient to promote disease. Finally, recent experiments have shown that administration of this cytokine accelerates atherosclerosis in apoE-KO mice.79 All these data imply that IFN-γ is a proatherogenic cytokine.

Prolinflammatory Cytokines: Mediators of Innate and Th1 Responses

TNF-α and IL-1 are also present in human lesions.16,80,81 Similarly to IFN-γ, they affect smooth muscle proliferation, but their effects are more complex and indirect. IL-1 not only stimulates SMC replication by inducing an autocrine platelet-derived growth factor loop but also inhibits proliferation by inducing the growth-inhibitory autacoid, prostaglandin E_1.82,83 The net effect on SMC growth therefore depends on the precise conditions in experimental systems and remains

**TABLE 2. Some Immune Cytokine-Regulated Genes of Importance in Atherosclerosis**

<table>
<thead>
<tr>
<th>Gene Product</th>
<th>Cytokine</th>
<th>Cytokine Producers</th>
<th>Target Cells</th>
<th>Effect</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCAM-1</td>
<td>IL-1, TNF-α, IL-4</td>
<td>MΦ, Th2, SMC, EC</td>
<td>EC, SMC</td>
<td>Adhesion</td>
<td>214</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>IL-1, TNF-α, IFN-γ</td>
<td>MΦ, Th1, SMC, EC</td>
<td>EC</td>
<td>Adhesion</td>
<td>215</td>
</tr>
<tr>
<td>CD36</td>
<td>IL-4</td>
<td>Th2</td>
<td>MΦ</td>
<td>OxLDL uptake</td>
<td>216</td>
</tr>
<tr>
<td>SR-A</td>
<td>IFN-γ, TNF-α, IL-6</td>
<td>Th1, MΦ, SMC</td>
<td>MΦ</td>
<td>OxLDL uptake</td>
<td>48–50</td>
</tr>
<tr>
<td>NOS2</td>
<td>IFN-γ, TNF-α, IL-1</td>
<td>Th1, MΦ, SMC, EC</td>
<td>MΦ, SMC, EC</td>
<td>↑ NO</td>
<td>217, 218</td>
</tr>
<tr>
<td>NOS2</td>
<td>IL-4</td>
<td>Th2</td>
<td>MΦ, SMC, EC</td>
<td>↓ NO</td>
<td>101</td>
</tr>
<tr>
<td>COX2</td>
<td>IFN-γ, TNF-α, IL-1</td>
<td>MΦ, Th1, SMC, EC</td>
<td>MΦ, SMC, EC</td>
<td>↑ Eicosanoids</td>
<td>219</td>
</tr>
<tr>
<td>COX2</td>
<td>IL-4</td>
<td>Th2</td>
<td>MΦ, SMC, EC</td>
<td>↓ Eicosanoids</td>
<td>103</td>
</tr>
<tr>
<td>15–LO</td>
<td>IL-4, IL-13</td>
<td>Th2</td>
<td>MΦ, SMC</td>
<td>Oxidation, leukotrienes</td>
<td>220, 221</td>
</tr>
<tr>
<td>IL-1, TNF-α</td>
<td>IFN-γ</td>
<td>Th1</td>
<td>MΦ</td>
<td>Activ</td>
<td>222</td>
</tr>
<tr>
<td>IL-6</td>
<td>IL-1</td>
<td>MΦ, EC, SMC</td>
<td>SMC</td>
<td>B-cell activ, PTX prod</td>
<td>92</td>
</tr>
<tr>
<td>MMPs</td>
<td>TNF-α, IL-1, CD40L*</td>
<td>MΦ</td>
<td>MΦ, SMC, EC</td>
<td>Proteolysis</td>
<td>84, 201, 223, 224</td>
</tr>
<tr>
<td>MMPs</td>
<td>IFN-γ</td>
<td>Th1</td>
<td>MΦ, SMC, EC</td>
<td>↓ Proteolysis</td>
<td>84, 201</td>
</tr>
<tr>
<td>tPA, uPA</td>
<td>TNF-α, IL-1</td>
<td>MΦ, EC, SMC</td>
<td>EC</td>
<td>↑ Fibrinolysis</td>
<td>225</td>
</tr>
<tr>
<td>uPA</td>
<td>IFN-γ</td>
<td>Th1</td>
<td>EC</td>
<td>↓ Fibrinolysis</td>
<td>226</td>
</tr>
<tr>
<td>PAl-1, -2</td>
<td>IFN-γ, TNF-α, IL-1</td>
<td>MΦ, Th1</td>
<td>EC</td>
<td>Antifibrinolysis</td>
<td>227</td>
</tr>
<tr>
<td>PPAR-γ-dep genes</td>
<td>IL-4</td>
<td>Th2</td>
<td>MΦ</td>
<td>↑ CD36 expression</td>
<td>228</td>
</tr>
<tr>
<td>ApoA-IV</td>
<td>IFN-γ</td>
<td>Th1</td>
<td>Hepatocytes</td>
<td>↓ HDL</td>
<td>77</td>
</tr>
<tr>
<td>ABCA1</td>
<td>IFN-γ</td>
<td>Th1</td>
<td>MΦ, foam cells</td>
<td>Cholesterol efflux</td>
<td>229</td>
</tr>
<tr>
<td>sPLA2</td>
<td>IFN-γ</td>
<td>Th1</td>
<td>SMC</td>
<td>Eicosanoids, PAF, lysoPC</td>
<td>71</td>
</tr>
<tr>
<td>LPL</td>
<td>IFN-γ, TNF-α, IL-1</td>
<td>MΦ, Th1</td>
<td>MΦ</td>
<td>↓ Lipolysis</td>
<td>86, 87</td>
</tr>
</tbody>
</table>

NOS indicates nitric oxide synthase; COX, cyclooxygenase; LO, lipoxygenase; MMPs, matrix metalloproteinases; tPA, tissue plasminogen activator; uPA, urokinase-type plasminogen activator; PAl, plasminogen activator inhibitor; PPAR, peroxisome proliferator–activated receptor; dep, dependent; LPL, lipoprotein lipase; MΦ, macrophage; EC, endothelial cell; activ, activation; PTX, pentraxin; prod, products; and lysoPC, lysophosphatidylcholine. See text for explanation of other abbreviations.

*The cell surface molecule CD40L (CD154) has effects similar to those of a soluble cytokine.

**TABLE 3. T-Cell Types Potentially Involved in Atherosclerosis**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Antigen Rec</th>
<th>Epitope</th>
<th>Restriction Element</th>
<th>Present in Lesions</th>
<th>Effect Shown in Exp Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4</td>
<td>TCRαβ</td>
<td>Peptide</td>
<td>MHC class II</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>CD8</td>
<td>TCRαβ</td>
<td>Peptide</td>
<td>MHC class I</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>γδT</td>
<td>TCRγδ</td>
<td>Lipid</td>
<td>CD1</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

rec indicates receptor; exp, experimental. See text for explanation of other abbreviations.
controversial in the in vivo situation. TNF-α and IL-1 are powerful inducers of local inflammation in blood vessels and elsewhere (Table 2). For instance, they stimulate further activation of macrophages, induce secretion of matrix metalloproteinase-9,84 and promote expression of leukocyte adhesion molecules (see above). IL-1 is also an important costimulatory factor for T-cell activation and TNF-α, a proapoptotic cytokine.

The metabolic effects of IL-1 and TNF-α are even more striking than those of IFN-γ (Table 2). They are powerful inhibitors of lipoprotein lipase, which leads to increased systemic levels of VLDL and hypertriglyceridemia.85,86 In addition, they exert important effects on glucose and energy metabolism. High-dose stimulation with TNF-α therefore results in cachexia. It is possible that much lower TNF-α levels lead to metabolic changes of the kind observed in the metabolic syndrome, such as hypertriglyceridemia, redistribution of adipose tissue, and reduced insulin sensitivity.87–89 Interestingly, this syndrome is considered an important risk factor for atherosclerotic cardiovascular disease.

The proinflammatory cytokine pathway is a cascade that involves activation by TNF-α and IL-1 to IL-6 expression90 and also to secretion of pentraxins such as C-reactive protein (CRP).91 This cascade is amplified in each step. For instance, IFN-γ produced by Th1 cells stimulates macrophages to secrete IL-1. The latter cytokine stimulates SMCs to make copious amounts of IL-6,92 a mediator that is present in atherosclerotic lesions93,94 and that may also be induced by the terminal complement complex C5b-94 (Table 2). CRP and other pentraxins may not only be markers of inflammation but also exert direct effects on leukocyte recruitment and apoptosis in the vessel wall.95,96

Patients with unstable coronary syndromes have elevated levels of IL-6, CRP, and pentraxin-3,97–99 which may be due to increased inflammatory activity in their culprit lesions. Importantly, even a modestly elevated CRP level is a risk factor for coronary heart disease in healthy, middle-aged men.100 This suggests that the modest inflammatory activity of “silent” plaques results in a cascade leading to CRP production and also accelerates disease progression.

**Table 4. Effects of Immune Deficiency and Immunomodulation on Atherosclerosis**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Immune Defect</th>
<th>Immune Phenotype</th>
<th>Atherosclerosis Model</th>
<th>Effect on Disease</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound KO</td>
<td>RAG-1</td>
<td>SCID</td>
<td>ApoE</td>
<td>↓ Athero</td>
<td>194</td>
</tr>
<tr>
<td>Compound KO</td>
<td>RAG-2</td>
<td>SCID</td>
<td>ApoE + fatty diet</td>
<td>No effect</td>
<td>195</td>
</tr>
<tr>
<td>Compound KO</td>
<td>SCID/SCID</td>
<td>SCID</td>
<td>ApoE</td>
<td>↓ Athero</td>
<td>197</td>
</tr>
<tr>
<td>Compound KO</td>
<td>IFN-γ rec</td>
<td>Defective Th1</td>
<td>ApoE</td>
<td>↓ Athero</td>
<td>77</td>
</tr>
<tr>
<td>IV Ig inj</td>
<td></td>
<td>Immunosuppression</td>
<td>ApoE</td>
<td>↓ Athero</td>
<td>209</td>
</tr>
<tr>
<td>Anti-CD40 inj</td>
<td></td>
<td>Immunosuppression</td>
<td>LDLR</td>
<td>↓ Athero</td>
<td>204</td>
</tr>
<tr>
<td>Compound KO</td>
<td>CD40L</td>
<td>Reduced immune activation</td>
<td>ApoE</td>
<td>↓ Athero</td>
<td>205</td>
</tr>
<tr>
<td>IFN-γ inj</td>
<td></td>
<td></td>
<td>ApoE</td>
<td>↑ Athero</td>
<td>79</td>
</tr>
<tr>
<td>KO on B6 background</td>
<td>TNF-α rec</td>
<td>Defective inflammation</td>
<td>Fat feeding</td>
<td>Fatty streaks</td>
<td>230</td>
</tr>
<tr>
<td>KO on B6 background</td>
<td>IL-10</td>
<td>Increased proinflammatory cytokines, ↑ Th1</td>
<td>Fat feeding</td>
<td>Fatty streaks</td>
<td>106, 107</td>
</tr>
</tbody>
</table>

IV Ig inj indicates intravenous immunoglobulin injection; rec, receptor; and athero, atherosclerosis. See text for explanation of other abbreviations.
broader set of targets than Th1 cells alone, and its effects may not be equivalent to those of Th2 activity.

A third T-helper cell called Th3 was recently described. It produces TGF-β on activation. This cytokine stimulates collagen synthesis and is fibrogenic. Furthermore, it is strongly anti-inflammatory, as illustrated by the fact that TGF-β-KO mice die of fulminant inflammation by the age of 6 weeks. TGF-β and its receptor are present in the atherosclerotic lesion, where it may act to stimulate collagen synthesis and cap formation and to dampen inflammation. Several different cell types, including macrophages, SMCs, and Th3 cells, can express TGF-β, which might be important for plaque stabilization. The SMC growth-promoting action of T cells that release heparin-binding growth factors might add to this effect.

In conclusion, although Th1 cytokines are abundant in lesions, it seems less likely that atherosclerosis will be considered a strict Th1 disease. It might even be speculated that different phases of this chronic disease are promoted by different effector pathways. Clearly, the development and regulation of Th pathways in atherosclerosis require further studies.

**CD8 Cells and γδT Cells: Additional Players in the Plaque Orchestra?**

CD8+ T cells are found at varying proportions in human lesions (Table 3). Most of the cytotoxic activity associated with T cells is found in this subpopulation (Tc cells), which is activated by cells that express proteasomally processed peptide fragments of nascent proteins in the context of MHC class I. It was recently shown that expression of a “foreign” antigen on vascular SMCs can lead to cytotoxic attack by CD8+ T cells. In an apoE-deficient mouse, this results in significant aggravation of atherosclerosis. Thus, it is possible that CD8+ T cells cause some of the widespread apoptosis that is associated with atherosclerosis. However, apoptosis can also be induced by reactive oxygen and nitrogen species, which are generated by macrophages and SMCs on stimulation with proinflammatory cytokines. Therefore, activation of CD4+ as well as CD8+ T cells can lead to cell death in lesions. In addition, CD8+ T cells may respond to antigens not only by cytotoxic activity but also by secretion of cytokines in a manner parallel to that observed for Th1 and Th2 cells. Functional Tc1 and Tc2 subsets of CD8+ T cells seem to be important for the tissue response in mycobacterial infections, but it is not known whether they play a role in atherosclerosis.

A third type of T cell has a CD3+ 4-8 phenotype and expresses TCRs that are γδ rather than αβ dimers. Such γδT cells are important in mucosal immunity and in the recognition of complex lipids as antigens. TCRγδ binds such antigens, which are presented on CD1 rather than MHC proteins. The capacity of γδT cells to recognize lipids makes them potentially interesting in atherosclerosis. γδT cells and CD1-expressing cells have been found at varying proportions in arterial inflammatory lesions and atherosclerotic lesions, but their role remains unclear.

**Other Immune Cells May Also Participate in the Local Immune Response**

The 2 other types of lymphocytes, B cells and natural killer (NK) cells, are less frequent in atherosclerotic lesions than T cells. Relatively few B and NK cells are found in advanced human lesions, but prominent B-cell infiltrates may occur in the adventitia and the periadventitial connective tissue. They can develop into pathological perivascular lymphoid aggregates, a condition termed periaortitis. In lesions of hypercholesterolemic rabbits and mice, B cells are relatively abundant, and clones of immunoglobulin-producing cells can be found. In humans as well as animals, IgG accumulation is prominent in lesions. Some of these IgGs may be specific for antigens present in the tissue. Although a substantial proportion of the IgG may have entered the lesion by filtration, there is also evidence for local synthesis. Plasma cells, which may secrete large amounts of IgG, are present in lesions and are the likely source of this locally produced IgG.

A different kind of hematopoietic cell, the mast cell, has been identified in atherosclerotic lesions. Mast cells are less frequent than macrophages and T cells but may be important in plaque activation and acute coronary syndromes because they produce several proteases and accumulate at sites of plaque rupture. Factors released from mast cells may degrade the extracellular matrix and could also influence the functions of surrounding cells and modify locally deposited lipoproteins. Finally, the dendritic cell, which is a specialized member of the macrophage lineage, has been found in human and experimental atherosclerotic lesions.

This cell performs a key role in immune activation because it is specialized on antigen presentation and appears to be the only cell that can activate naive T cells. It has a high migratory capacity and may “patrol” tissues such as the artery wall in search of antigens, which are endocytosed and transported to regional lymph nodes, where presentation of the antigen to naive and memory T cells, and hence, induction of adaptive immunity, can take place.

**Immune Specificity in the Atherosclerotic Lesion**

**Restricted Heterogeneity Suggests Local Immune Activation**

The antigen specificity of T cells is encoded in the sequence of their rearranged TCR genes, which determine the conformation of the antigen-binding site in the CDR3 domain of the TCR protein. Because the rearrangement process is stochastic, each nascent T cell carries a unique TCR gene and protein. On activation, the stimulated T cell divides to give rise to a clone of cells with identical specificities. The presence in tissue of a population of T cells with an identical TCR is therefore indicative of clonal proliferation and hence, of expansion of these particular T cells due to antigenic stimulation. Such clonal expansions are found in early lesions of apoE-KO mice. This finding is suggestive of local clonal proliferation of T cells, which is compatible with activation by local antigens. Interestingly, similar TCRs containing the Vβ6 variable domain are frequently expressed by T cells that recognize oxidatively modified LDL, one of the candidate antigens in atherosclerosis (A. Nicoletti, G. Paulsson, and G.K. Hansson, unpublished observations, 2001).

In human lesions, the situation is more complex. A heterogeneous population of TCRs and therefore, of T cells is found in lesions, and the enormous allelic variation in
MHC molecules, ie, HLA proteins, between individuals makes it difficult to identify patterns that could reflect local expansions of HLA-restricted T cells. Although this may be partly due to technical difficulties, it seems unlikely that the immunological situation in atherosclerosis could be as simple as the one in type 1 diabetes, for example, where one specific HLA-DQ allele permits an autoimmune response that leads to β-cell destruction and disease. Instead, it is likely that several antigens and antigenic epitopes are involved and can be presented by several different HLA alleles. Nevertheless, autoantigens have also been identified in atherosclerosis.

**OxLDL Is a Candidate Autoantigen**

Antibodies to oxLDL can be detected in atherosclerotic patients and experimental animals and are present in atherosclerotic lesions. These studies identified oxLDL as a candidate antigen in atherosclerosis (Table 5). Further support for this notion came with the identification of cellular immune responses to oxLDL. T cells can be isolated from fresh human plaques, cloned, expanded in culture, and challenged with candidate antigens. By using this approach, oxLDL was shown to be a major autoantigen in the cellular immune response of atherosclerosis. One fourth of all CD4+ T cells cloned from human plaques recognized oxLDL in an HLA-DR-dependent manner. OxLDL-specific T cells are present in lymph nodes of apoE-KO mice, which have strong humoral as well as cellular immune responses to such modified lipoproteins. In humans, oxLDL induces activation of a subset of peripheral T cells, suggesting that it is a quantitatively important antigen.

The antibody specificity of IgG molecules in lesions can be determined by isolating them and permitting them to react with an antigen, eg, in ELISA or Western blot analyses. With this approach, it was demonstrated that IgGs of atherosclerotic lesions contain antibodies reactive with oxLDL. The antigens, ie, LDL carrying various kinds of oxidative modifications, could be demonstrated in the lesions by immunostaining and Western blot. Therefore, oxLDL is an autoantigen that is formed in the lesion, and it elicits a local cellular as well as a humoral immune response.

The immune response to oxLDL plays a pathogenetic role in atherosclerosis because lesion progression can be inhibited by immunization or induction of neonatal tolerance to oxLDL. It seems paradoxical that both tolerization and hyperimmunization can reduce the extent of disease; this may be due to the different effector pathways activated by these two kinds of treatment.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Type</th>
<th>Processing</th>
<th>Presentation</th>
<th>Recognition</th>
<th>Effect of Immun.</th>
</tr>
</thead>
<tbody>
<tr>
<td>OxLDL</td>
<td>Autoantigen</td>
<td>Endosomal</td>
<td>MHC-II</td>
<td>CD4⁺T(γδT?)</td>
<td>↓ atheroma</td>
</tr>
<tr>
<td>hsp60</td>
<td>Autoantigen</td>
<td>Endosomal, cytosolic</td>
<td>MHC-II</td>
<td>CD4</td>
<td>↑ atheroma</td>
</tr>
<tr>
<td>β2-Gp1b</td>
<td>Autoantigen</td>
<td>Endosomal</td>
<td>MHC-II</td>
<td>CD4</td>
<td>fatty streak</td>
</tr>
<tr>
<td>C pneum</td>
<td>Microbe</td>
<td>Endosomal</td>
<td>MHC-II</td>
<td>CD4</td>
<td>?</td>
</tr>
<tr>
<td>CMV</td>
<td>Virus</td>
<td>Cytosolic</td>
<td>MHC-I</td>
<td>CD8</td>
<td>?</td>
</tr>
</tbody>
</table>

* C pneum indicates Chlamydia pneumoniae, CMV, cytomegalovirus; and immun, immunization. See text for explanation of other abbreviations.

**Heat Shock Proteins Are Common Autoantigens in Inflammatory Disease**

Heat shock proteins have also been implicated in the pathogenesis of atherosclerosis (Table 5). Such proteins are involved in protein folding in the normal cell, are produced in large amounts by injured cells, are released on tissue injury, and serve as targets for autoimmune responses in many inflammatory diseases. Interestingly, heat shock proteins are also released by monocytes exposed to oxLDL. Antibodies to heat shock protein 60 (hsp60) and its prokaryotic homologue hsp65 are frequently detected in rheumatoid arthritis and other inflammatory conditions.

Xu and coworkers immunized rabbits with hsp65/60 and recorded induction of vascular inflammation, with endothelial activation and mononuclear cell adhesion. The developing lesions also contained T cells, and cell lines derived from such infiltrates exhibited anti-hsp60 reactivity. Anti-hsp60 antibodies occurred in peripheral blood, and immunization with hsp60 was found to increase fatty streak development in hypercholesterolemic rabbits and mice. In humans, antibodies to hsp 65/60 are elevated in hypertension and early atherosclerosis and may predict progression of atherosclerotic disease. Because heat shock proteins of humans and microbes are structurally and antigenically similar, it is possible that molecular mimicry between immune responses to microbial heat shock proteins and homologues expressed by vascular cells could account for the association between infections and atherosclerosis.

A third autoantigen, β2-glycoprotein Ib (β2-GPI), is present on platelets but may also be expressed by endothelial cells (Table 5). Autoantibodies to β2-GPI are produced in several inflammatory disorders, including atherosclerosis. The immune response to β2-GPI appears to be proatherogenic, because hyperimmunization with β2-GPI or transfer of β2-GPI-reactive T cells aggravates fatty streak formation in LDLR⁻/⁻ mice. The pathogenetic mechanism by which β2-GPI acts remains unclear, but it may be related to this protein’s capacity to bind phospholipids.

**A Role for ‘Phospholipid Antibodies’ in Atherosclerosis?**

Antibodies reacting with phospholipids such as cardiolipin are associated with recurrent thrombosis and accelerated atherosclerosis. Many of these antibodies recognize phospholipid-binding plasma proteins such as β2-GPI, and it could be speculated that β2-GPI affects atherosclerosis by targeting immune responses to membrane lipids. Interestingly, another set of “phospholipid antibodies” recognizes...
oxidized phospholipids in oxLDL\(^{160}\) and may represent "natural" antibodies that appear in early life and also bind phospholipids on apoptotic cells and certain bacteria.\(^{161}\) Perhaps pathogenic immune responses are due to "molecular mimicry" between oxLDL and modified lipids on microbes and apoptotic cells.

**Microbes as Antigens in Atherosclerosis**

In 1988, Saikku et al\(^{162}\) discovered that patients with cardiovascular diseases often have high-titer antibodies to *Chlamydia pneumoniae* (Table 5). This common pathogen can cause pneumonia, but it has also been associated with a variety of other chronic diseases. Further investigations revealed that *C pneumoniae* survives intracellularly in macrophages, can be detected in atherosclerotic lesions, and can be isolated and grown from such lesions.\(^{163}\)–\(^{165}\) This established that *C pneumoniae* is involved in atherosclerosis. However, the extent of *C pneumoniae* infiltrates in lesions remains controversial,\(^{166}\) as does the importance of *C pneumoniae* infection as an aggravating factor in experimental atherosclerosis.\(^{167}\)–\(^{170}\) It therefore remains to be clarified how important *C pneumoniae* is for disease development and whether the microorganism or the immune response against it can be harmful.

Viruses of the Herpesviridae family have also been implicated in atherosclerosis (Table 5). Both herpes simplex type 1 and cytomegalovirus have been detected in human lesions.\(^{171}\)–\(^{173}\) An avian analogue, Marek’s disease virus, aggravates cholesterol-induced atherosclerosis in chickens. Cytomegalovirus has been linked to transplant arteriosclerosis\(^{174}\) and may also be associated with "garden-variety" atherosclerosis and restenosis after angioplasty.\(^{176}\)–\(^{177}\) Several pathogenic mechanisms have been proposed.\(^{178}\) Cell culture studies have identified a role for this virus as a stimulus for smooth muscle migration and scavenger receptor expression, which may be important in vascular pathology.\(^{179}\) However, it appears that direct action of the virus, rather than immune responses against it, is playing the important pathogenic role.

**Systemic Immune Responses and Serodiagnosis**

Systemic humoral immune responses are characteristic for atherosclerotic disease in humans and experimental models. Antibodies to oxidatively modified LDL are commonly found in patients; as expected from the incidence of silent disease, they are frequent also in healthy individuals.\(^{133}\),\(^{180}\) Immunodominant epitopes in modified LDL include malondialdehyde-conjugated lysine residues.\(^{139}\),\(^{181}\) These modifications are generated by enzymatic or nonenzymatic attack on fatty acid residues, which leads to release of reactive aldehydes that can bind to the polypeptide chain of apoB-100. Other B-cell epitopes on oxLDL include oxidized phospholipids such as phosphorylcholine, which is also present on several microbes and apoptotic cells and is recognized by "natural" antibodies present in most individuals.\(^{161}\)

Anti-oxLDL antibody titers are correlated with the progression of advanced atherosclerosis in some studies,\(^{133}\),\(^{182}\) whereas others have been unable to find any such correlation. Interestingly, titers are correlated negatively with early cardiovascular diseases, including borderline hypertension and carotid intima/media thickening.\(^{183}\)–\(^{185}\) It appears that no simple, positive correlation occurs between antibodies and disease throughout its natural history. Instead, it may be that bursts of plaque activity boost immune responses, possibly by releasing antigenic material, and give rise to titer increases. Such episodic activity might be interspersed with periods of slow, silent growth of lesions and fading immune activity. It is also possible that antibodies can serve to eliminate modified lipoproteins from the circulation, thus reducing the load of atherogenic lipoproteins in arterial lesions. Antibodies may therefore be viewed both as markers of disease activity and as vehicles for clearance of antigen. The situation with regard to other antigens appears to be similar to that for modified LDL. Anti-hsp65/60 antibodies have been positively correlated with the progression of carotid atherosclerotic disease in an epidemiological study,\(^{151}\) but more data are needed before these assays find their place in clinical diagnostics.

**Evidence That Immunity Affects Atherosclerosis**

**Atherosclerosis in Patients With Immune Disorders**

It is by now well established that atherosclerosis is accompanied by adaptive immune responses and that the early phase of the disease is dominated by immune cells, particularly macrophages. These facts do not necessarily imply that atherosclerosis can be treated or prevented by interfering with immune mechanisms. To clarify whether that could be possible, it has been important to determine whether the extent of atherosclerosis is different (reduced or increased) in individuals with immune defects.

The situation in immune-deficient patients is complicated and difficult to interpret. Individuals with severe combined immune deficiencies have not survived long enough to develop cardiovascular disease, and most of the congenital immune deficiencies affect too few patients to make epidemiological investigations into cardiovascular disease meaningful. Individuals with selective, congenital defects in the humoral immune responses are relatively frequent, and the clinical syndrome is compatible with life into adult age. These patients do not exhibit any reduced heart disease, suggesting that humoral immunity does not aggravate atherosclerosis.\(^{186}\) HIV patients, who lack CD4+ T cells (and particularly Th1 activity), develop aggressive cardiovascular disease, particularly when modern highly active antiretroviral treatment has been used to prevent rapid development of AIDS.\(^{187}\)–\(^{189}\) However, the protease inhibitor treatment may itself cause metabolic syndromes, and it is difficult to determine whether the increased cardiovascular morbidity is due to the disease or its treatment.

Some of the most remarkable data in support of a link between immunity and atherosclerosis come from epidemiological studies of patients with autoimmune disorders. Patients with rheumatoid arthritis have a 2- to 5-fold increase in cardiovascular morbidity and mortality.\(^{190}\) and patients with systemic lupus erythematosus exhibit an even higher increase in cardiovascular disease.\(^{191}\),\(^{192}\) Although some of this morbidity is clearly due to small-vessel vasculitis associated with the underlying autoimmune disease, there is also evidence for atherosclerotic large-vessel disease in many cases. Interestingly, patients with systemic lupus erythematosus share several autoimmune phenomena with atherosclerotic patients.\(^{193}\) Further studies into these aspects are clearly needed.
Mechanistic Insights From Gene-Targeted Mouse Models

To clarify the role for adaptive immunity in atherosclerosis, it was necessary to generate immunodeficient animal models (Table 4). The apoE-KO mouse was crossed with the recombinase-activating gene-1−/− (RAG-1−/−) mouse, which lacks functional T and B cells due to a mutation in the rearrangement machinery. The offspring exhibited a 40% reduction of atherosclerosis, indicating that adaptive immunity accelerates disease. However, the impact of the immune defect was undetectable in cases of excessive hypercholesterolemia. This could be due to either an overwhelming effect of very high cholesterol levels or a qualitative difference in immune activity in this metabolic condition. The finding of a Th1→Th2 switch in severe hypercholesterolemia supports the latter conclusion. Interestingly, the atheroprotective effect of estrogens was recently found to be lacking in immunodeficient mice, suggesting that the atheroprotective effect of estrogen depends on estrogen action via immune mechanisms. Whether this involves switches in effector functions remains to be determined.

A more recently constructed apoE−/− × scid/scid mouse, which also lacks adaptive immunity, shows an even more striking phenotype, with a 70% reduction of disease in immunodeficient female apoE−/− × scid/scid mice compared with immunocompetent apoE−/− scid/+ littermates (Table 4). Neither the RAG nor the SCID immunodeficient mice exhibited any changes in serum cholesterol compared with single-KO apoE−/− mice; this points to important effects on the vascular wall rather than on systemic metabolism for the effect on atherosclerosis.

This conclusion was further supported when immune cell populations were transferred into the apoE−/− × scid/scid mice. Transfer of CD4+ T cells from immunocompetent apoE−/− mice to immunodeficient apoE−/− × scid/scid mice increased atherosclerosis dramatically in the recipient, from 27% to 79% of the level in fully immunocompetent apoE−/− mice. Transferred T cells migrated to lesions and induced expression of MHC class II genes, probably through IFN-γ secretion. These data show that immune activity increases atherosclerosis and identifies CD4+ T cells as the proatherogenic subset.

In another recent study, a T-cell–mediated response to a vascular autoantigen was found to aggravate atherosclerosis in apoE-KO mice. These mice were bred with a mouse strain that carries the β-galactosidase gene under the control of a smooth muscle–specific promoter activated by a tamoxifen-inducible Cre recombinase. When such mice were immunized by injections of dendritic cells presenting β-galactosidase, CD8+ T cells were activated to specifically recognize this antigen. Subsequent induction of β-galactosidase expression in SMCs by tamoxifen treatment led to a lytic attack of the SMCs by antigen-specific CD8+ T cells. This resulted in a dramatic increase in atherosclerosis.

Important Roles for Immunoregulatory Cell Surface Molecules

Immune activation depends on interactions between proteins displayed on the surfaces of adjacent cells as well as on soluble cytokines. Such proteins include adhesion molecules, such as ICAM-1, and the more narrowly expressed pairs of costimulatory molecules. The latter include the T-cell protein CD28, which binds 2 alternative counterreceptors, B7.1 (CD80) and B7.2 (CD86). CD28 ligation promotes T-cell activation, whereas ligation of B7 proteins to the alternative T-cell surface receptor, cytotoxic T-lymphocyte antigen-4 (CTLA-4, CD152), induces a negative signal for cell-mediated immune responses. Soluble CTLA-4 proteins have been used to inhibit immune reactions and can prevent chronic rejection of organ transplants. However, they do not appear to reduce experimental atherosclerosis.

Another costimulatory molecule, CD40, is expressed by B cells and dendritic cells and can be induced on many different cell types. It ligates the constitutively expressed T-cell protein, CD40 ligand (CD40L, or CD154). This interaction is necessary for T-B cell cooperation in the induction of antibody responses. CD40 is constitutively expressed by endothelial cells and can be induced in both vascular endothelial cells and SMCs by stimulation with proinflammatory cytokines and IFN-γ. Similarly, CD40L could be induced on these cells by cytokine stimulation. Ligation of CD40 leads to tissue factor expression by endothelial cells and can stimulate protease secretion from macrophages and SMCs.

Interestingly, CD40 and CD40L are present on vascular cells and macrophages of atherosclerotic lesions, and their activation may be involved in the progression of atherosclerotic disease. Recent animal experiments support this notion (Table 4). First, anti-CD40L antibody injections reduced fatty streak development in LDLR mice. Second, CD40L−/− apoE−/− compound-KO mice exhibited slower induction of lesions than did the CD40L+−/− apoE−/− controls. These data suggest that inhibition of the CD40 pathway might be a means of treating atherosclerosis. However, the mechanism of action for inhibitors such as anti-CD40L antibodies remains unclear and could involve not only immunomodulation but also direct effects on vascular cells.

Can We Prevent Atherosclerosis by Immunization?

Encouraging Experimental Data for the Immune Strategy

After having cited a wealth of studies that establish adaptive—and innate—immunity as proatherogenic, it may seem totally unrealistic to ask whether immunization, ie, deliberate activation of specific immunity, might protect against atherosclerosis. However, one should consider that several infections in which cellular immunity plays a pathogenic role can be prevented by vaccination. Experimental autoimmune diseases such as experimental autoimmune encephalomyelitis can be prevented by protective immunization. It is now evident that entirely different immune effector responses can be induced against the same antigen, with some important ones elicited by local antigen release in parenchymal tissue and others by subcutaneous or oral immunization.

As discussed, several different antigens have been implicated in the pathogenesis of atherosclerosis. Interestingly, immunization with one of them, oxLDL, can reduce disease in several animal models (Table 4), whereas immunization with hsp65/60 or β2-GP1 aggravates lesion development (Table 4). The reason for this apparent discrepancy is not known; however, there are important differences between the antigens. Hsp60 is an intracellular
molecule that can be expressed on the surface of stressed cells. OxLDL, in contrast, is an extracellular particle that is present in the interstitial space of the intima and may even circulate in the blood.\textsuperscript{208} It could be speculated that protective immunization against atherosclerosis works by inducing high-titer antibodies that increase the clearance of oxLDL by way of Fc and C3 receptors. In support of this, the protective effect of oxLDL immunization can be correlated with the titer of T-cell–dependent IgG antibodies,\textsuperscript{142} and transfer of polyclonal immunoglobulins, which contain anti-oxLDL antibodies, reduces atherosclerosis in apoE\textsuperscript{−\textemdash}/− mice.

The detrimental effect of immunization with hsp60 may depend on the fact that this protein can appear on the surface of endothelial cells and/or SMCs. This could lead to antibody binding that is followed by complement activation and lysis of the vascular cells. In addition, peptides derived from hsp60 synthesis might bind to MHC class I molecules, which would permit attack by cytolytic CD8\textsuperscript{+} T cells. The potential importance of the latter pathway was recently demonstrated in mice in which an autoimmune response to a transgene expressed on SMCs led to a CD8\textsuperscript{+} T-cell attack on the vessel wall and aggravation of atherosclerosis.\textsuperscript{112} It is possible that endogenous antigens such as hsp60 could elicit a similar attack once a cellular immune response has developed toward the protein.

**Therapeutic Potential of Vaccination and Immunomodulation**

Can we use our current knowledge about immune activity in atherosclerosis to prevent or treat the disease? Although no such therapy is used clinically today, there is reason for optimism. The success in protective immunization with oxLDL as an immunogen in experimental models suggests that this could also be a fruitful approach in humans. However, oxLDL is a heterogeneous particle that may be unsuitable for parental administration in patients. It may also be extremely difficult to standardize to the extent that is required for vaccines. For these reasons, it will be necessary to identify the most important B- and T-cell epitopes on oxLDL and to test whether either of them can be used as an immunogen with the same success as the intact particle. If this turns out to be the case, clinical trials should be encouraged.

Immunomodulation is also attractive as a possible therapeutic principle. However, broadly acting immunosuppressants such as corticosteroids and cyclosporins have undesirable, direct vascular effects that make them unsuitable for this purpose. Instead, we should try to develop more specific immunomodulators that act on the key mechanisms in the pathogenesis of atherosclerosis. Potential targets are proinflammatory lipids such as PAF and lysophosphatidylcholine, lipoprotein-derived antigens, and signal transduction pathways leading to inflammatory innate and adaptive immune responses.

Interestingly, certain drugs targeting lipid and carbohydrate metabolism may also act as immunomodulators. Statins act as repressors of MHC class II–mediated T-cell activation\textsuperscript{210} and can improve the outcome of cardiac transplantation.\textsuperscript{211} Glitazones, which are agonists for peroxisome-proliferator activating receptor-\γ, can also inhibit T-cell activation and cytokine secretion.\textsuperscript{212} It is tempting to speculate that some of the beneficial effects of these types of compounds are due to their immunomodulatory effects rather than their action on cholesterol or glucose metabolism.

Because the proinflammatory/Th1 pathway appears to play a key role, interference with signal transduction molecules, receptors, or cytokines involved in this cascade could be interesting as a potential therapeutic approach. TNF-α antagonists are already in clinical use for rheumatoid arthritis and have been tested clinically for heart failure. It will be important to determine whether they also act against atherosclerosis. Similarly, IFN antagonists and inhibitors of the NF-κB and Janus kinase/signal transducer and activator of transcription (Jak/STAT) pathways could turn out to be useful. Experimental studies have already shown beneficial effects of blockers of the costimulatory molecules CD40/CD40L; inhibition of this or similar costimulatory proteins might also be effective against atherosclerosis (see above).

Finally, it would be theoretically attractive to apply an immunomodulatory treatment that promotes anti-inflammatory immunity. This might be accomplished by enhancing the Th2 or TGF-β/Th3 effector mechanisms, for example, or by the more general inhibition of cell-mediated immunity that can be achieved by treatment with large doses of polyclonal immunoglobulins.\textsuperscript{209} The latter treatment, if useful clinically, would have to be reserved for selected cases of rapidly accelerating atherosclerosis. However, it may point to a useful principle for immunomodulation that could be used to develop more defined pharmaceuticals.

To summarize, several interesting drugs and immunogens have shown effects against atherosclerosis in experimental systems, and an additional set of molecules is attractive from a theoretical point of view. We should have some interesting years ahead of us.

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