thematic review

Thematic review series: The Immune System and Atherogenesis

Immune function in atherogenesis

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Abstract In this overview to a new thematic series on the immune system and atherogenesis, I provide a very brief summary of current conceptions of atherogenesis, of the innate and adaptive immune systems, and of the participation of the latter in atherogenesis, with particular emphasis on studies of the involvement of the immune system in atherosclerosis reported in the last 2 years. This is followed by a short outline of the eight reviews that will make up this thematic series. The overview is concluded with some caveats that should be considered in the analysis of atherosclerosis in experimental animals.—Getz, G. S. **The immune system in atherogenesis. *J. Lipid Res.* 2005.** 46: **1–10.**

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This issue inaugurates a new Thematic Series that highlights the role of immune function in atherosclerosis. To quote from an earlier editorial inaugurating another Thematic Series: "the complexities of lipid and lipoprotein metabolism, and the differing responses of vascular wall cells (including immune cells) to the interplay of lipoproteins and to the vast array of circulating cellular and blood elements, are enormous" (1). This viewpoint can be applied also to the interaction of the components of the immune system with lipid and lipoprotein metabolism in the context of the evolution of the atherosclerotic plaque.

A modern approach to the molecular pathogenesis of atherosclerosis has been well reviewed (2). The role of innate and adaptive immunity in its pathogenesis has been comprehensively dealt with in two recent reviews (3, 4). Readers are encouraged to consult each of these reviews alongside of this overview.

Because the following series deals in depth with important aspects of immune function in relation to atherosclerosis, in this overview I will briefly summarize the state of the subject, mostly as reflected in these aforementioned three reviews. Here, the focus will be on the lesion outcome, without dealing with the detailed cellular mechanisms. I will then update the information based upon studies published in the last 2–3 years. This will provide the backdrop for an introduction to this Thematic Series. The overview will conclude with a set of additional comments that might be helpful in the consideration of immune system participation in the modulation of atherosclerosis.

ATHEROSCLEROSIS AS CHRONIC INFLAMMATION

It is now widely recognized that atherosclerosis is a specific example of a chronic inflammatory response mainly to dyslipidemia and other risk factors. This notion was articulated in an excellent article by Russell Ross (5). In keeping with this formulation, the atherosclerotic plaque has as its major components macrophages, cells of the adaptive immune system, smooth muscle cells, and matrix components. As with most chronic inflammatory reactions, the cells of the immune system have the potential to significantly influence the outcome of the inflammation. The atherosclerotic plaque is notable for its focal nature, being mostly encountered in regions of the macrovasculature subject to disturbed flow hemodynamics.

In both human and experimental atherosclerosis, hypercholesterolemia is the major exciting factor for the development of vascular lesions. The increased cholesterol is carried either by LDL or VLDL remnants. It is now thought that increased plasma levels of LDL result in enhanced oxidation or perhaps other modifications of LDL within the vascular wall, representing a major initiating agent for the formation of the atherosclerotic response. Lipoproteins retained in the vessel wall by matrix compo-

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Abbreviations: apoE, apolipoprotein E; CD40L, CD40 ligand; CRP, C-reactive protein; dn, dominant negative; IL, interleukin; MHC, major histocompatibility complex; NF-κB, nuclear factor κB; NK, natural killer; NK-T, natural killer T; OxLDL, oxidized low density lipoprotein; RAG, recombination-activating gene; SAA, serum amyloid A; TCR, T-cell receptor; TGF, transforming growth factor; Th cell, T-helper cell; TNF, tumor necrosis factor.

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nents, the most prominent of which are the proteoglycans, are probably especially susceptible to oxidation. Oxidation of retained lipoproteins may be a function of the production of reactive oxygen species generated by the cells of inflammatory infiltrates or by enzymes such as lipoxygenases produced by infiltrating macrophages. Macrophages import oxidized LDL (OxLDL) into the endosomal system via a variety of scavenger-type receptors. The cholesterol so imported ultimately ends up in the cytoplasm, where it is esterified, generating cholesteryl ester droplets and forming foam cells that are the hallmark of early and growing atherosclerotic plaques. The accumulation of such foam cells constitutes the bulk of the early vascular lesion, designated by some as a fatty xanthoma (6). Oxidized phospholipid moieties of oxidized lipoproteins signal to many of the cells in the evolving plaque, especially to the endothelium overlying the accumulating OxLDL and to foam cells. Among other responses, this signaling increases the expression of adhesion molecules that facilitate the homing of monocytes and lymphocytes to this localized activated endothelium. The foam cells and activated endothelium may also produce proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, IFN-γ, and tumor necrosis factor-α (TNF-α) (7), which promote the further development of the inflammatory response. Also, the elaboration of chemotactic factors such as MCP-1 attracts the further influx of monocytes. The macrophage foam cell is a very versatile multifunctional cell, capable of elaborating reactive oxygen species, prostaglandins, nitric oxide, and growth factors. Foam cell homeostasis is the result of new recruitment and lipid loading on the one hand and efflux of lipid on the other. The latter is promoted by apolipoprotein E (apoE), apoA-I, HDL, and the ATP binding cassette proteins ABCA1, ABCG1, and ABCG4.

The progression of the lesion from the fatty xanthoma to a more complex lesion is characterized by the migration of smooth muscle cells from the media into the subendothelial intima and their subsequent proliferation. This is mediated, in part, by the growth factors secreted from macrophage foam cells. These smooth muscle cells may themselves become foam cells, but more importantly they are responsible for the synthesis of matrix proteins and proteoglycans. The foam cells may ultimately die either by necrosis or apoptosis, liberating their contained lipid and producing a necrotic core with extracellular lipid. The death of foam cells is probably related to the dysregulation of intracellular lipid metabolism and the formation of cytotoxic oxidized sterols and other lipids. Late atherosclerotic plaques may also undergo cartilaginous dysplasia with calcium deposition. The increase in the size of the evolving atherosclerotic plaque arises from the continued recruitment of monocytes and lymphocytes, the continued migration and proliferation of smooth muscle cells, the evolution of a necrotic core, and matrix protein synthesis. Activation of the macrophages in the lesions, particularly on the lesion shoulders, may lead to the release of proteases, with disruption of the plaque surface giving rise to the unstable plaque lesion becoming the nidus for thrombosis and consequent clinical complications.

BRIEF SUMMARY OF THE IMMUNE SYSTEM

The immune system has been recognized as an important component of atherosclerotic inflammation. The immune system can be thought of as two subsystems, the innate immune system and the adaptive immune system. The innate immune system is critical to the initial inflammatory response. Several cellular and mediator responses are involved. The acute cellular response in the context of atherosclerosis is centered on the monocyte-macrophage, whose participation has been summarized above. Other cells may also play some role. This includes natural killer (NK) cells, dendritic cells, mast cells, and B1 cells. NK cells, as the name implies, are able to kill tumor cells, virally infected cells, or antibody-coated cells. But they also secrete cytokines, the most prominent of which is IFN-γ. NK cells when activated are particularly efficient producers of IFN-γ, which activates macrophages (8). NK cell activity is influenced by other cytokines, such as IL-2, IL-15, and IL-12. Dendritic cells play a key role in antigen presentation, expressing high levels of scavenger receptors and class II major histocompatibility complexes (MHCs), which present antigens to cells of the adaptive immune system. They also express costimulatory molecules, B7-1 and B7-2. Mast cells on activation release histamine, leukotrienes, platelet-activating factor, proteases, and cytokines. In a sense, all of the cells included in the initial atherosclerotic response can be regarded as part of the innate immune response, because endothelial cells and smooth muscle cells can be induced, for example by IFN-γ, to express class I and class II MHC proteins. The MHCs on these cells are capable of presenting antigens, although not nearly with the efficiency of the more traditional antigen-presenting cells, macrophages, and dendritic cells. B1 cells bridge the innate and adaptive immune systems. They are responsible for the production of IgM antibodies, many of which react with oxidized phospholipids. These antibodies have been shown to block the uptake of OxLDL by macrophage scavenger receptors. The activation of the inflammatory response induces the synthesis and release of IL-1, TNF-α, and IL-6, which are responsible for inducing in the liver the transcription of the acute-phase plasma proteins, including C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen, and ferritin, as well as proteins of the complement system. An outline of the innate immune system is shown in **Fig. 1**.

The adaptive immune system (**Fig. 2**) reacts to endogenous neoantigens (e.g., apoptotic cells or OxLDL) or exogenous antigens, resulting in the activation of T-cells and B-cells. Some of these neoantigens are also targets of the innate immune system. The adaptive immune system may influence atherosclerosis in one of three ways: *1*) by cell-cell interaction (e.g., between antigen-presenting cells, macrophages, B-cells, or dendritic cells) and T-cells; *2*) by the secretion of a variety of cytokines from activated T-cells, which mediate an activation of macrophages and other cells of the atherosclerotic plaque; or *3*) by the production of antibodies by B-cells in a T-cell-dependent or -independent manner. Some of these antibodies have the ability

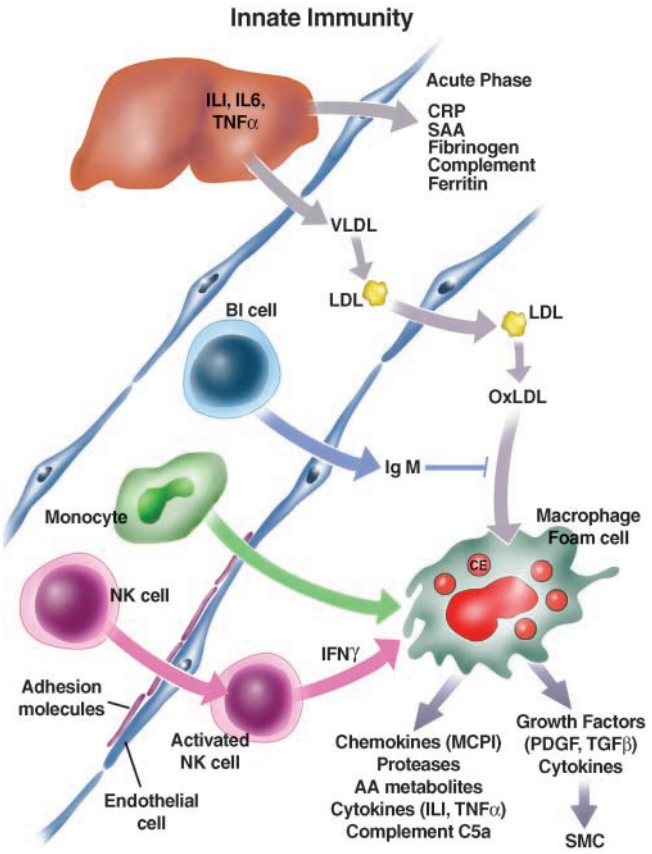

Fig. 1. Innate immunity. The liver produces the major actor in atherogenesis in the form of VLDL, which in transit through the plasma is converted to LDL. Some of the LDL enters the subendothelial space, where it may be oxidized, forming oxidized LDL (OxLDL). The liver in a proinflammatory state [i.e., increased cytokines interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α)] also produces many acute phase proteins, some of which are listed. Their direct participation in the process of atherogenesis is not yet clear. Components of the OxLDL elicit the activation of endothelial cells, resulting in the upregulation of adhesion molecules, which facilitate the entry of blood monocytes into the subendothelial space. Here, monocytes may be converted to macrophages expressing cell surface scavenger receptors, which mediate the import of OxLDL, which contains the cholesterol that is stored in the cell as cholesteryl ester (CE), forming the droplets characteristic of the foam cell of the evolving lesion. The monocyte macrophage is a multipotential cell, and some of its secreted products are listed. These products influence the evolution of the plaque, the autoactivation of the inflammatory reaction, and the migration of smooth muscle cells to the subendothelial space, where they contribute to lesion evolution. Natural killer (NK) cells may also enter the subendothelial space and produce many cytokines or chemokines, but mostly IFN-γ, which among other actions activates the monocyte macrophage. The B1 cell, which is not present in the lesion, bridges the innate and adaptive immune systems and may produce IgM antibodies that inhibit the uptake of OxLDL into the macrophage, slowing its conversion to a foam cell. CRP, C-reactive protein; MCP1, monocyte chemotactic protein 1; PDGF, platelet derived growth factors; SAA, serum amyloid A; SMC, smooth muscle cells; TGF, transforming growth factor.

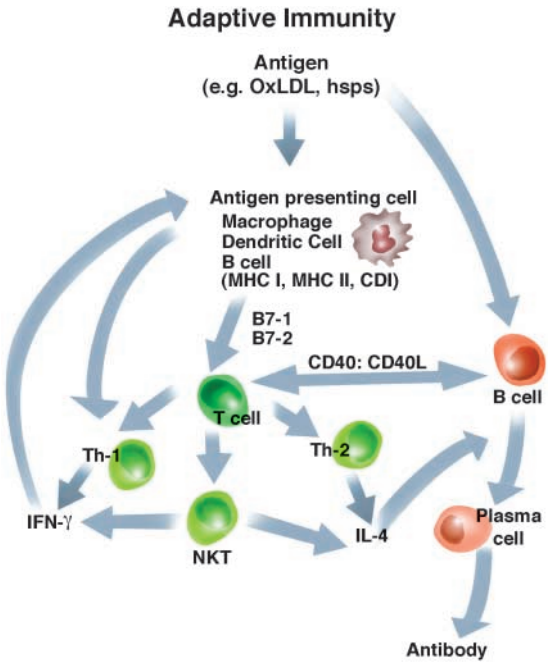

Fig. 2. Adaptive immunity. This network is activated by exposure to either neoantigens such as OxLDL and heat shock proteins (hsps) or exogenous antigens such as chlamydial antigens. These antigens are processed and presented by antigen-presenting cells (dendritic cells, macrophages, or B-cells) in the context of major histocompatibility complex (MHC) class I or II or CD1 for dendritic cells and macrophages and the B-cell receptor in the case of B-cells. The antigen-presenting cells present antigen to CD4 T-cells bearing cognate T-cell receptors, resulting in activation of the T-cells but only in the presence of the second signal produced by costimulatory molecules (e.g., B7-1, B7-2) interacting with CD28. B-cells activated by antigens may also interact with T-cells via the CD40-CD40 ligand (CD40L) pair of costimulatory molecules predominantly involved in this interaction but also in other cell-cell interactions. The activated and selected T-cells may differentiate into T-helper cell 1 (Th1) or Th2 subsets, producing predominantly IFN-γ and IL-4, respectively. IFN-γ may positively feed back on the activity of the antigen-presenting cells, whereas IL-4 facilitates the differentiation of B-cells into antibody-producing cells. The third subset of CD4+ cells are the natural killer T (NK-T) cells, which most often see lipid antigen in the setting of CD1 on dendritic cells or macrophages. NK-T cells, unlike most of the other CD4+ T-cells, may produce both IFN-γ and IL-4. The B-cells produce antibodies of the IgM or IgG types that interact with the antigens of modified LDL or other relevant antigens. This figure is modified by permission from the figure in Hansson et al. (4).

to block the import of modified lipoproteins via macrophage scavenger receptors.

Antigenic molecules are processed within the endosomal system of antigen-presenting cells and are presented on their surface in the context of MHC class I or II molecules. Such antigenic epitopes interact with cognate T-cell receptors (TCRs) on the surface of T-cells, which respond either by anergy or activation. The decision between these responses is largely determined by the availability of costimulatory molecules (e.g., B7-1 or B7-2) on antigen-presenting cells that interact with CD28 on T-cells. The pool of individual TCRs is enormous, arising during T-cell development as a result of gene rearrangements involving many genes, including the obligatory recombination-activating genes (RAG 1 and 2). The bulk of the T-cells express the TCR consisting of a heterodimer of α and β chains. A small proportion of T-cells express a TCR consisting of γ

and δ subunits. The latter T-cells recognize antigens without the necessity that they be presented on MHC molecules. Although these cells are capable of generating a large variety of antigen receptors, most of them recognize a limited number of antigens (9). Some of these cells respond to heat shock proteins (10).

T-cells may be divided into CD4+ and CD8+ subclasses. The majority of T-cells in atherosclerotic plaques are CD4+ cells, although smaller numbers of CD8+ cells have been detected. CD4+ cells recognize antigen loaded on MHC class II molecules of the antigen-presenting cell, whereas CD8+ cells recognize antigens presented in the context of MHC class I molecules. Among the CD4+ cells are several subgroups, three of which have been investigated in murine atherosclerosis. These subgroups are distinguished by the complement of cytokines they produce. Th1 cells mainly secrete proinflammatory cytokines such as IFN-γ, which activates macrophages and facilitates the production of antibodies of the IgG2a class by B-cells. T-helper 1 (Th1) cells do not secrete IL-4 or IL-5. Th2 cells, on the other hand, secrete IL-4 and IL-5 but not IFN-γ. These cells provide help for the synthesis of other antibody classes. The Th2 cytokines may be anti-inflammatory. There is cross-regulation among these two subsets of T-cells, so that each tends to inhibit the other. IFN-γ inhibits Th2 cells and IL-4 inhibits Th1 cell cytokine secretion. Also, IL-10 inhibits the Th1 pathway, whereas IL-12 reduces the Th2 responses (4). A third special subset of T-cells are the natural killer T cells (NK-T cells), which bear some of the same markers as NK cells, but unlike the latter they express rearranged cell surface TCRs of limited diversity. They mainly recognize lipid antigen in the context of the MHC-like compound CD1. There are other T-cell subsets that have been less studied in atherosclerosis (e.g., T-regulatory cells, Th3 cells, etc.).

B-cells are the other major group of circulating lymphocytes. They recognize antigen via the B-cell antigen receptor, in which cell surface IgM plays a central role. Like T-cells, B-cells, through these receptors, express unique specificity derived from the rearrangement of immunoglobulin genes, a process that is also RAG dependent. Mature B-cells, designated as plasma cells, secrete specific antibodies. The production of antibodies to protein antigen requires T-cell help. The interaction between T-cells and B-cells is facilitated by CD40 ligand (CD40L) expressed on T-cells and CD40 on B-cells.

IMMUNE MODULATION OF ATHEROSCLEROSIS

This topic is studied in both human atherosclerosis and experimental models of atherosclerosis, with the mouse now holding pride of place because of the capacity to manipulate almost at will its genetic program. Indeed, different strains of mice exhibit quite different susceptibilities to atherosclerosis (11), indicating the importance of genetic modifiers in the evolution of the atherosclerotic lesion. The murine lesion bears many similarities to the human lesion, but it is worth noting that there are some differences both in the distribution of lesions (e.g., aortic root dominance in the mouse) and in the quality of the lesions.

Several lines of evidence have been adduced to implicate the immune system in the process of atherosclerosis. First, the presence of immune cells and immune cell products in the human and/or experimental atherosclerotic lesion is taken to indicate their likely participation in the lesion biology. Millonig, Malcom, and Wick (12) have described a "vascular associated lymphoid tissue" in vascular regions susceptible to atherosclerosis. Macrophages and T-cells are detectable in this tissue even before the plaque develops. Among the immune cells and molecules detected in human atherosclerotic plaques are macrophages, dendritic cells (13), CD4+ T-cells, CD8+ T-cells, MHC class II, CD40, and CD40L, the cytokines IL-1, IL-2, IFN-γ, IL-7, IL-10, IL-12, IL-18, TNF-α, transforming growth factor-β (TGFβ), as well as immunoglobulin (4, 12). The cytokines of Th2 cells are present at much lower levels (7). The presence of both proinflammatory and anti-inflammatory cytokines speaks to the possibility of the coexistence of proatherogenic and antiatherogenic influences in lesions. B-cells are relatively rare among the cells of the human atherosclerotic plaque, although they may be found in the neighboring adventitia.

Second, more compelling evidence for the role of the immune system in atherogenesis derives from specific gene deletion or overexpression in mice. The knockout of scavenger receptors (SR-A, CD36), mediators of monocyte chemotaxis (MCP-1, CCR_2), IFN-γ or its receptor, costimulatory molecules, CD40L, and the RAG genes, resulting in global immunodeficiency, all lead to a reduction in atherosclerosis in mouse models (3, 4). On the other hand, the knockout of the Th1 inhibitory cytokine, IL-10, results in an increase in lesions. The pattern of cell and cytokine involvement suggests a Th1 dominance in atherosclerotic lesions. This dominance has been reported to reverse in the face of marked hypercholesterolemia, at least in the apoE-deficient mouse (14). The Th2 cytokine IL-4 was thought to afford protection, but its influence may be more complex, because IL-4 deficiency in the background of either apoE deficiency or LDL receptor deficiency leads to a reduction in lesion size (15, 16). This argues that cytokine effects might not be simply explained by resorting to the Th1/Th2 paradigm. Non-T-cell-dependent effects of IL-4 may be at work (16). Recently, IL-5 has been reported to play a protective role (17). Cytokine effects on atherogenesis indeed seem to be quite complex. The knockout of immunoglobin μ results in a B-cell deficiency. Transplantation of bone marrow cells from B-cell-deficient mice into LDL-deficient mice results in an increase in atherosclerosis (18). This indicates that B-cells may have a protective effect on atherosclerosis, despite their relatively rare appearance within the plaque. This suggests that their effects may be mediated by the antibodies they secrete, or perhaps by some other immunoregulatory role.

A third basis for implicating the immune system in atherogenesis is provided by the direct transfer of immunological mediators. IFN-γ, IL-12, or IL-18 injection all in-

crease atherosclerosis (3). The administration of antibodies to CD40L reduces lesion formation in LDL receptor-deficient mice, whereas the use of antibodies to TGFβ in the apoE-deficient mouse increases atherosclerosis, emphasizing the atheroprotective influence of this cytokine.

The fourth type of evidence directing attention to the role of the immune system involves immune cell transfer or vaccination. When CD4+ cells from apoE-deficient mice in which the Th1 cell subtype is dominant are transferred to immunodeficient apoE-deficient mice, an increase in atherosclerosis is noted (19). On the other hand, the transfer of B-cells from apoE-deficient mice with or without T-cells reduces atherosclerosis (20). The two major antigens to which autoantibodies are detected are OxLDL and heat shock proteins. Vaccination with the former decreases lesion formation, whereas in the case of the latter, atherosclerosis is increased (3).

In the last 18–24 months (i.e., since the publication of the two reviews briefly summarized above), many additional studies have been published that bear on the topic of this overview.

INNATE IMMUNITY

In relation to the innate immunity network, the potential proatherogenic role of NK cells has received renewed attention (21, 22). Dendritic cells seem to be concentrated in the rupture-prone areas of vulnerable human carotid artery plaques (23). There has of course been continued study of CRP and its role as a marker of or a participant in atherosclerosis. The involvement of the Toll-like receptors in signaling and the initiation of atherosclerosis has also been studied recently. Among the hepatic products that participate in the anti-inflammatory response is the complement system, which can be activated by CRP (24). The complement peptides C3a and C5a may be chemotactic for monocytes. The lack of complement component 5 in apoE-deficient mice apparently has little impact on atherosclerosis. On the other hand, C3 deficiency studied in the model that is lacking both apoE and the LDL receptor resulted in an increase in aortic lesions, although cross-sectional analyses of the aortic root showed no difference in the size of the lesions (25). The C3-deficient mice had increased triglyceride and LDL cholesterol levels. Aortic atherosclerosis in C3-deficient mice crossed with the LDL receptor-deficient model was also increased (26).

Most of the emphasis in atherogenesis has been on the recruitment of monocytes into the lesion-prone areas, where they become macrophage foam cells. The fact that lesions regress, leaving fewer macrophages, implies that these cells must have the capacity to migrate out of the lesion. In an elegant transplant model, Llodra and colleagues (27) have demonstrated the outward migration of macrophages and dendritic cells from the lesion-filled aortic arch of an apoE-deficient mouse when the arch was transplanted into a wild-type mouse. The migration appears to be limited by lipids, such as platelet-activating factor and lysophosphatidic acid, which are likely to be enriched in progressing lesions.

ADAPTIVE IMMUNITY

With respect to the adaptive immune system, NK-T cells have entered the atherosclerosis arena. As they recognize lipid antigen in the context of CD1, their involvement is worthy of consideration. Their activation increases atherosclerosis in the apoE-deficient model (28). It has recently been shown that regulatory T-cell subtype 1 upon transfer to apoE-deficient mice reduces atherosclerosis. This is associated with a reduction in the IFN-γ-to-IL-10 ratio (29).

The role of costimulatory molecules has been emphasized recently (30). It has been known for some time that the CD40-CD40L interaction is implicated in atherosclerosis (31). These molecules are members of the TNF superfamily. An alternative name for CD40 is TNF receptor superfamily 5 (TNFRSF 5), and the CD40 is designated TNFSF 5 (31, 32). Although the expression of this pair was thought to be restricted to B-cells, dendritic cells, and activated T-cells, it is now clear that they are more widely expressed in the cells present in atherosclerotic plaques. Interdiction of their interaction results in a reduction in lesion development, while also modifying the composition of the lesion toward a less inflammatory and more fibrogenic lesion phenotype (31). Another pair of costimulatory molecules that belong to the TNF superfamily is LIGHT (TNFSF 14) (32) and its receptor, either HVEM (TNFRSF 14) expressed on lymphocytes and NK cells or lymphotoxin β expressed on stromal cells and monocytes (33). These molecules have been noted in human atherosclerotic plaques clustered in macrophage-rich regions, and their properties suggest that their interaction is proinflammatory (34, 35). The other costimulatory pair is B7-1 (CD80) and B7-2 (CD86). They are members of the immunoglobulin superfamily, are expressed in antigen-presenting cells, and bind to CD28 on resting T-cells. B7-1 and B7-2 overlap in function. When their coupled absence is combined with LDL receptor deficiency, atherosclerosis is reduced and the lesions have fewer T-cells, smooth muscle cells, and less collagen (36). Thus, like the other pairs of costimulatory molecules, they are proinflammatory.

CYTOKINES

As described above, cytokines play important roles in modulating atherosclerosis. Lymphotoxin α also functions as a proinflammatory cytokine in murine atherosclerosis (37). Either the absence of IL-1 or a reduced gene dosage of IL-1 receptor antagonist in the apoE-deficient background suggests an influence of IL-1 in promoting atherogenic cell signaling (38, 39). The larger lesions of the IL-1 receptor antagonist-deficient mice are enriched in macrophages relative to smooth muscle cells.

Information on the role of the proinflammatory cyto-

kine IFN-γ produced mainly by Th1 and NK cells has been extended in several experiments. The knockout of IFN-γ in the LDL receptor-deficient background substantially reduced lesion size in several regions of the aorta, with a relative loss of macrophages and smooth muscle cells in the early lesions (40). IFN-γ, which has previously been reported to downregulate the SRA and CD36 scavenger receptors, is thought to be important for the uptake of modified lipoproteins and foam cell formation. IFN-γ has now been shown to upregulate another scavenger receptor, SR-PSOX, which recognizes phosphatidylserine and OxLDL. SR-PSOX is identical to the transmembrane protein CXCL16, a chemokine receptor involved in T-cell migration. SR-PSOX and its receptor are present in atherosclerotic lesions (41). The dominance of Th1 and their secreted products within the plaque is balanced by counterinflammatory influences, mediated in part by IL-10, expressed in macrophages and Th2 lymphocytes in the plaque. When LDL receptor-deficient mice are transplanted with bone marrow overexpressing IL-10 in T-cells, atherosclerosis is reduced (42). In contrast, the transplantation of IL-10-deficient bone marrow into LDL receptor-deficient mice led to a marked increase in lesion development at several sites, and these lesions were rich in macrophages and lymphocytes (43). Nuclear factor κB (NF-κB) mediates many proinflammatory effects. The recent observation that selective attenuation of NF-κB signaling in macrophages results in an increase in atherosclerosis in the LDL receptor-deficient background is somewhat surprising (44). This could be attributed to the substantial reduction in IL-10 production. A most interesting recent finding hypothesizes that IL-5 influences the stimulation of the production of anti-oxidized phosphatidylcholine antibodies after immunization with malondialdehyde LDL (17). The immunization caused a preferential expansion of cognate Th2 cells that secrete IL-5, which then stimulates B1 cells. This provides a link between natural and adaptive immunity and will be fully discussed in the thematic review by Binder, Witztum, and colleagues on natural antibodies.

The atheroprotective cytokine TGFβ has also received considerable attention. It functions as an anti-inflammatory cytokine, limiting the recruitment of leukocytes and promoting the synthesis of collagen and extracellular matrix (45, 46). TGFβ-deficient mice die early, so alternative strategies are used to attenuate its function. Most have used either a soluble TGFβ receptor that inhibits signaling (47) or a dominant negative (dn) mutant receptor driven by a T-cell-specific promoter (CD2 or CD4) expressed as a transgene (48, 49). These studies noted some similarities and some discrepancies. In the Robertson study (48), T-cell dn receptor-expressing mice were crossed with apoE-deficient mice, whereas in the Gojova study (49), bone marrow was transplanted from dn TGF receptor-expressing mice into LDL receptor-deficient mice. Both groups of investigators noted lesions that exhibited increased T-cells, macrophages, and reduced collagen, an inflammatory phenotype. The Robertson study noted increased lesion size, whereas a modest reduction in aortic root lesions was noted in the Gojova study. These experimented models are significantly distinct, so that further investigation is required to resolve the discrepancy.

THIS SERIES

There will be eight substantial reviews in this series, all of which deal with currently active topics. Four of these will be devoted largely to the innate immune system, and the others will deal with the bridge between innate immunity and the adaptive immune response. Because macrophages are the hallmark of the atherosclerotic lesion, they will be heavily represented in at least half of the reviews. The first of these will be devoted to the increasing variety of scavenger receptors expressed on the macrophage and dendritic cell surfaces. David Greaves and Siamon Gordon will discuss how these receptors might be involved in atherogenesis, but they will also address other aspects of the biology of these receptors. This review will be followed by an overview of the currently very active field of the role of acute phase proteins, such as CRP, SAA, and other members of this family. This will be reviewed by Alan Chait, Jack Oram, and Jay Heinecke. One of the major issues to be addressed relates to the possibility that these proteins are directly involved in atherosclerosis, rather than "simply" serving as markers of the inflammatory state. Much work has been done on the possible proatherogenic influence of microorganisms such as *Chlamydia*. The recognition of the cell surface molecules of these organisms is via the so-called Toll-like receptors, which are expressed on such cells as macrophages, neutrophils, NK cells, dendritic cells, and endothelial cells. These receptors may signal to the transcriptional machinery of the cell to promote a proinflammatory state. There are at least 10 such receptors with differing specificity and cell distribution. Recent papers have implicated some of these receptors in atherogenesis (50, 51). This subject will be reviewed by Linda Curtiss.

I will then comment on the bridge between innate and adaptive immunity in an extended editorial. This will be followed by a review of the natural antibodies recognizing modified lipoproteins and apoptotic cells as well as molecules that mimic these antigens. This is an exciting field with continuing surprises pointing in new directions of substantial potential clinical significance. The recent observations in this area will be reviewed by Peter Shaw, Christoph Binder, and Joseph Witztum and colleagues, who have contributed greatly to this field.

As mentioned above, a number of lymphocyte subclasses have come into sharp focus as influencing atherogenesis. This includes B1 cells, NK cells, and NK-T cells. These unusual suspects as well as dendritic cells and their contribution to atherogenesis will be reviewed by Catherine Reardon and Paul VanderLaan.

It is clear from the above overview that the atherosclerotic plaque is a "soup" of cytokines, produced by many cell types within the inflammatory plaque and acting upon many of their neighbors. Knockout experiments and other manipulation of expression levels of these molecules clearly

demonstrate their influence on the atherogenic process. We have asked Elaine Raines and Alan Daugherty to focus our attention on the role of these cytokines on target cells within the plaque. Elaine Raines will do this for endothelial cells and smooth muscle cells. Alan Daugherty will inform us about the macrophage and leukocytes as targets.

We will conclude the series by a return to the central role of the monocyte, which must be recruited into the evolving lesion to fulfill its critical participation. Thus, Oswald Quehenberger will review monocyte chemotaxis and atherogenesis.

ADDITIONAL COMMENTS

In summary, the prevailing evidence suggests that atherosclerosis progression represents a chronic inflammatory reaction involving the participation of the innate immune system and modulated by the adaptive immune system. In the latter case, the proinflammatory pattern dominates as a result of Th1 cells secreting IFN-γ, a situation stimulated by IL-12 and IL-18. However, it seems clear that developing atherosclerotic plaques represent a balance between proinflammatory and anti-inflammatory influences, with the former being dominant during early plaque evolution. That there are anti-inflammatory influences at work is indicated by the experimental manipulation of IL-4, IL-10, and TGFβ as discussed above. Also, B-cells and their secreted antibodies recognizing relevant antigens may contribute to some degree of atheroprotection. However, the adaptive immune system is not required for the development of atherosclerosis. Quite substantial lesions develop in the absence of mature T- and B-cells in mouse models in which the function of the RAG gene(s) is eliminated.

Our current conception of experimental atherosclerosis derives predominantly from a limited examination of atherosclerosis either by the measurement of aortic root lesions, not a characteristic site for human atherosclerosis, or en face measurement of the extent of aortic lipid lesions. Sometimes both methods are used. This examination may be undertaken early in atherosclerosis, when the predominant lesion is a fatty streak (xanthoma), or later, when more complex lesions are seen. It should not be a surprise that influences on early lesions and more complex lesions may differ. In the early lesion, composed mostly of foam cells, the cellular composition is reasonably homogeneous. On the other hand, as the lesion increases in complexity, the potential for cellular interactions may be very different. The microarchitecture of the lesion, with the stromal separation of clusters of cells of different function, could influence the measured outcome of an intervention. Seldom does a single investigator examine lesions at two different times, and this can be misleading, because the selection of a particular time point as readout may bias the results in a lesion that evolves over time. For example, in apoE/IL-12-deficient mice, lesion area is reduced at 30 weeks of age but not at 45 weeks of age (16). Also, the major atherosclerosis parameter may rest solely or largely on the size of the lesions, often with little attention to the lesion phenotype or detailed morphology. In modeling lesions of clinical relevance, the quality and composition of the lesion is probably of more importance. In accepting the general view of the mechanisms of atherogenesis, one should bear in mind the potential limitations in the evidence that supports this view.

Some interventions have modest effects on the size of the lesion but do influence the lesion phenotype. This is exemplified in recent murine atherosclerosis studies on IFN-γ deficiency in atherosclerosis in the LDL receptor-deficient mouse (40). Lesion phenotype is dramatically changed with modulation of IL-10 signaling and attenuation or alteration of TGFβ signaling (42, 43, 47, 49).

We have previously drawn attention to the differential responses of various vascular regions to immune modulation (52). We suggest that this is attributable to the way regional differences in hemodynamic profiles prime the endothelial phenotype to respond to such modulations. An alternative explanation could be that differences in the microenvironment in the subintimal space in which lesions develop condition the atherosclerotic response. We have seen this in comparing the response of the aortic root and the innominate artery to immune deficiency in the LDL receptor-deficient model (53). Other examples of the differential response of aortic regions to immune modulators include IL-4 deficiency combined with LDL receptor deficiency, in which there was no impact on the aortic root lesion size but substantially reduced lesion size in the aortic arch and thoracic aorta (15). A second example involves immunization of LDL receptor-deficient mice with OxLDL, which reduced aortic root lesion size but had no influence on lesions in the remainder of the aorta (54). The majority of studies of atherosclerosis in the mouse have examined lesions in the aortic root or in the whole aorta by en face analysis. Given the hitherto reported differences in response according to the vascular region examined, it seems that our understanding of the interplay of the immune system on murine atherosclerosis would be greatly enhanced by measuring the atherosclerosis response at more than one vascular site. A dramatic example of such a difference is shown by the surprising increase in abdominal aortic atherosclerosis seen in female apoE-deficient mice also lacking CD4+ cells (55).

It is well known that mouse strains differ in their susceptibility to atherosclerosis, with the C57BL/6 strain being the most sensitive. Many of the earlier experiments used mouse strains that were not fully backcrossed into this genetic background. Often, strain 129 was originally used for the generation of the knockout mice. This was the case for the knockout of the RAG genes. The initial work on the apoE-deficient strains lacking the RAG genes used animals of modestly mixed background (56–58). We have shown that the effect of immune deficiency on innominate artery atherosclerosis in LDL receptor-deficient mice is quite sensitive to the genetic background (53). An interesting illustration of this complexity is shown in studies of peroxiredoxin 6 knockouts fed atherogenic diets. Strains were either 129 or C57BL/6 or a mixed strain, 129:BL/6. 129 animals were equally resistant whether or not the per-

oxiredoxin function was present. Similarly, the BL/6 strain was equally sensitive with this gene functioning or not. A difference between knockout and control mice was seen with the mixed strain. The caveat is that all lesions were very early and were quite small (59).

Hypercholesterolemia is a requisite for the development of atherosclerosis in the mouse. The effects of immune modulators could operate on the level of hypercholesterolemia or upon the responding blood vessel wall or perhaps at both levels. There are clearly some immune modulators that affect atherosclerosis without influencing the level of plasma lipoproteins. On the other hand, some immune modulators (e.g., IFN-γ signaling) do influence lipoprotein metabolism (60). The role of cytokines on lipoprotein metabolism has mostly focused on the response to the cytokines of the acute phase response to infection (i.e., TNF-α, IL-1, and IL-6) (61). However, global deficiency of the adaptive immune system generally results in a reduction in plasma cholesterol and triglyceride, especially in the VLDL fraction in both LDL receptor-deficient and apoE-deficient mice (53, 58). It has been reported that in the face of marked hypercholesterolemia in the apoE-deficient mouse, global immunodeficiency has no effect on atherosclerosis (57). We have recently shown that when apoE-deficient mice backcrossed to BL/6 mice for 10 generations are used and the plasma cholesterol levels are matched with chow as the diet, there is no obvious effect of global immunodeficiency on atherosclerosis at the aortic root up to 7 months of age (C. Reardon and G. S. Getz, unpublished data). We take this to imply that in this model the proinflammatory and anti-inflammatory influences on atherogenesis are similarly balanced in the presence or absence of an adaptive immune system. When the whole population of apoE-deficient mice that are immune competent or incompetent is considered, the plasma lipids are notably reduced in the incompetent mice. This is also the case for the LDL receptor-deficient model. The mechanisms that account for the influence of the immune system on plasma lipoprotein metabolism are largely unknown and little explored.

CONCLUSION

I have summarized above the current information on the immune modulation of atherosclerosis. Most of the mechanistic understanding derives from the study of different strains of mice, either overexpressing engineered genes at substantially increased levels or lacking the function of a particular gene. These are relatively blunt genetic instruments that take little account of the adaptive responses to the overexpression or lack of a gene. We have seen in some small measure the effects of genetic background on these modulations. Most of the available data, at least in murine experimental atherosclerosis, describe the potentialities or capacity of the immune network, rather than its finer tuning, which is probably involved in spontaneous atherosclerosis in humans. The single genes that are generally targeted are participants in complex networks, leading some investigators to adopt system-wide approaches (62). In spontaneous atherosclerosis that is so prevalent in the human population, the modulation of gene function is probably much more subtle and complex. The challenge for future studies of the role of the immune system in atherosclerosis is to model this complexity. This will require the selective and layered reconstitution of the innate and adaptive immune systems, the manipulation of more than one cell type or more than one cytokine or metabolite in particular models, and more subtle modification of gene function in particular cell components of the atherosclerotic plaque.

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