Role of the Immunological System in the Initiation and Progression of Atherosclerosis

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Summary

Atherosclerosis is a chronic inflammatory disease that is considered as major cause of death in the western world. Many studies and observations suggest that it could be caused by immunological reactions against autoantigens at the endothelial level, the most relevant of which are oxidized low-density lipoproteins and heat shock proteins 60/65. Experimental and clinical observations confirm the pathological role of these antigens during atherogenesis. Both innate and adaptive immunity and impaired regulatory mechanisms of the autoimmune reaction are involved in the atherogenic development. The aim of the present work was to examine the immunological mechanisms that contribute with the initiation and progression of atherosclerosis and also offer an overview of the recent advances in this field.

Keywords: Atherosclerosis, immunological system, autoimmune disease, autoantigens.

Introduction

Atherosclerosis is a chronic inflammatory disease that affects the intima of arteries (1,2). The origin of this inflammation is still under discussion. However, according to many investigators, one hypothesis prevails: that it is an autoimmune disease (3-5). Research over the last decades has found that the atherosclerotic lesion is characterized not only by cell proliferation and cholesterol deposition but also by infiltration of immune cells, mostly of monocyte derived macrophages and T cells (6-8).

Atherosclerosis lesions start when low-density lipoproteins (LDL) are trapped in the arterial wall, the first step in the evolution of atheroma being the generation of a fatty streak due to the retention of LDL in the subendothelial extracellular matrix (2). At this stage lymphocytic T cells are already present in the lesion, before development of the plaque (9).

Later on, an abundant subendothelial infiltration of mononuclear cells takes place: mostly T-helper cell 1 (Th1) activated lymphocytes (mainly $CD4^+$ and but also $CD8^+$), together with macrophages, monocytes and mast cells (10). Complexes of antibodies, antigens and complement are also present in the subendothelial space, while B cells can be found in the more external layer and the draining lymph nodes (11,12). Thus, the most precocious phase of atherosclerosis is characterized by an immune-mediated reaction presumably caused by local autoantigens.

According with several Authors, the presence of an autoimmune disease could be confirmed when it fits the so-called "Koch´s postulates" derived from microbiology. Major criteria for considering an inflammation as being autoimmune origin are: (a) presence of autoantibodies or evidence of cellular reactivity to self; (b) the presence of autoantigens (c) documentation of lymphocytic infiltrate in the pathological lesion; (d) demonstration that the autoantibodies or activated T cells can cause tissue damage. Supportive evidences are: (a) reasonable animal models; (b) beneficial effects from immunosuppressive agents; (c) association with other evidences of autoimmunity. Since this point of view, atherosclerosis can be considered as an autoimmune disease, and the main immunological mechanisms involved in the pathogenesis of the disease will be analyze in the present work.

Early events during atherogenesis.

Although the underlying events that initiate the formation of fatty streak, the earliest stage of an atherosclerotic plaque, remain poorly understood, the expression of vascular cell-adhesion molecule 1 (VCAM 1) on endothelium is an early sign of the aberrant immune response characterizing atherogenesis (13). VCAM 1 expression rises at sites of low shear stress and/or as a response to bioactive lipids such as oxidized-LDL (ox-LDL) that have been trapped within the arterial wall or of locally-acting cytokines (14). Some data suggest early arrival of platelets at sites of lesion formation where they may activate further the endothelium and thus promote leukocyte influx (15). Monocytes and T cells bind to VCAM 1-expressing endothelial cells and migrate into the arterial tissue in response to locally produced chemokines (16). Monocytes then differentiate into macrophages and augment expression of many pattern-recognition receptors, including scavenger receptors (SR) (17). The macrophages accumulate cholesterol via SR and become foam cells. During this time T cells become activated in response to antigens and contribute to disease progression by producing pro-inflammatory mediators such as interferon gamma (IFN-γ) which further amplifies the inflammatory response (18).

Mechanisms of activation of the immunological response during atherogenesis.

The immunological system consists in two components: the innate and adaptive response, and acts in two phases: identification of *non-self* and reaction against it (12).

The innate component represents the defensive outpost. It is germline encoded (i.e.: coded by cells with genetic material that can be passed to offspring), and has been found unmodified all along the phylogenetic line, from insects to mammals (19). As it is the most ancient form of immune surveillance, it is not specific and it is rapid. It is based on the recognition of *non-self* molecular structures associated with a large number of pathogen agents called "PAMP" (Pathogen-associated molecular patterns) (20). Both heat shock proteins (HSP) and ox-LDL are part of PAMP (21).

These molecular structures are recognized by particular receptors (SR and Toll-like receptors (TLR)) present on the surface of the antigen presenting cells (APC), like macrophages, dendritic cells and the endothelial cells themselves.

Inflammation can induce expression of class I and II of the major histocompatibility complex (MHC) on the surface of endothelial cells that become APC (5). Through TLR, endothelial cells present the pathogen antigen to the effector T cell, activating it and stimulating antibody production (see below) (22,23).

The adaptive immune response evolved later and is found only in vertebrates. It is activated more slowly, requiring days or even weeks, reacts only with highly specific molecular structures and utilizes clonally distributed receptors on T and B lymphocytes. The antigens are presented by the APC to the naive T cells which are activated and have two alternative destinations: effector Th cells or regulatory T cells (see below) (24).

Autoantigens considered responsible for the autoimmune reaction at the origin of atherosclerosis.

The main autoantigens associated to the development of atherosclerosis are HSP and ox-LDL. They are not only present in atherogenesis, but also the secondary autoantigens such as β2 glycoprotein and the structural components of some microorganisms (25).

Nature and physiological role of HSPs.

The HSP or chaperonins, as they were previously called, are a group of evolutionarily conserved proteins that show high sequence homology between different species, from bacteria to human (10). Their name is derived from their isolation in a small fly, the *Drosophila*, which was utilized for pioneer research on chromosomes. After exposure to heat, the induction of genes for the synthesis of HSP was observed in the *Drosophila* salivary glands (26). These stress proteins are present in all living organism. Many of them, like HSP60, are localized in the mitochondria (of which they are considered "bodyguards" as they play an important role in their preservation). Others are located in the cytosol, in the nucleus and the endoplasmic reticulum of the cells (27). They are present in nature in about 24 molecules, grouped in five families, according to their molecular weight: HSP 100, 90, 70, 60, 40 kDa and "low molecular weight" (28). The 60 family is of major interest for the pathogenesis of atherosclerosis (4).

HSP function both inside and outside the cells. In the first case, they act as a "chaperon" for the neo-synthesized proteins. They help them to reach a correct configuration during the phase of folding, of assembling their sub-units and during their translocation across the subcellular membranes toward different cellular compartments (29). Moreover they are involved in repairing or clearing the proteins that are improperly folded, totally unfolded or that have become unstable (30). This is why HSP are synthesized in larger amounts in sites of cellular stress, such as damaged tissues (31), including arterial endothelium (32).

The function of HSP outside the cells takes place on their surface. Here, in response to stress (such as shear stress -a major cause of atheromatous lesion -and the stress caused by the other atherosclerotic risk factors), large amounts of HSP move from mitochondria, nucleus and endoplasmic reticulum (33). They can also be released into the outer space in a soluble form (22). Presumably, they signal to the immune system (whose purpose is to guarantee the homeostasis and the functional integrity of the organism) (34), the dangerous situation into which the stressed cell has fallen ("danger theory") (23). This enables the cell to be identified, and, if necessary, to be eliminated, so as to protect the rest of the cellular community (35).

The antigenic role of HSP in the pathogenesis of atherosclerosis.

It has been amply demonstrated in the literature that HSP can play a primary antigenic role in the autoimmune pathogenesis of atherosclerosis (23,25,36-38).

When rabbits are fed a cholesterol-rich diet, the atherosclerotic lesions that consequently arise contain the "HSP factor-1" (the transcription factor of HSP) in significantly higher levels than in normal vessel walls (39). In these animals, titres of antibodies against HSP60, 65 and 70 increase rapidly, soon after starting the high fat diet, probably in association with the initial endothelial injury (40).

In a young population, the presence of circulating anti-HSP antibodies and of T lymphocytes which are highly reactive towards HSP60 are significant predictors of a precocious thickening of the vessel wall (41) and of future cardiovascular events (10). In healthy male teenagers, the risk of developing precocious atherosclerotic lesions increases in parallel with the level of reactivity of T lymphocytes to human HSP60, a risk almost identical to the rise in the number of daily smoked cigarettes (40). Also in adults, a high level of anti-HSP60/65 antibodies is often associated with diffused atherosclerosis, involves a high risk of cardiovascular diseases and can be used as an indicator of the seriousness of the prognosis (10).

Cell demise by apoptosis (programmed cell death) or necrosis are conditions that can generate auto-epitopes (23). As a matter of fact, many of the common autoantigens are released from dead or dying cells including endothelial cells (42). HSP, abundantly present inside such cells are released into the bloodstream triggering (also in this way) the autoimmune reaction (27). A large number of apoptotic cells accumulate in the atherosclerotic lesion as it progresses, because they are not sufficiently cleared from the site (43). Circulating anti-HSP60 antibodies have been associated with the severity of cardiovascular disease (27).

One hypothesis, based on different observations, suggests that the inflammatory first phase of atherosclerosis might be the consequence of the autoimmune reaction against the HSP60 produced by endothelial cells stressed by different pathological factors (risk factors). In this way, HSP60-expressed by the endothelial cells becomes the target of an immune system which is already sensitized to the HSP60 originated by different causes (23). Also, it has been demonstrated that HSP60 and its epitopes play an important pathogenic role also in other auto-immune diseases, such as Crohn's disease, type 1 diabetes, multiple sclerosis, rheumatoid arthritis, psoriasis, etc. (44).

Nature and role of oxLDL

Ox-LDLs are the product of oxidation of their polyunsaturated fatty acid component due to the increased concentration of reactive oxygen species (ROS) at the subendothelial level (45-47). Two aldehydes with strong immunogenic properties (immunogenic epitopes) are formed: malondialdehyde and 4-hydroxynonenal with anionic valence (48,49). By such alteration of their chemical structure these self-proteins are transformed into auto-antigens (5). The uptake of ox-LDL by macrophages activates immune mechanisms; this might represent another danger signal for the immune system arising from the arterial wall (50). Moreover, ox-LDL themselves might be a further stress factor inducing the production of HSP by the endothelial cells (51). On the other hand, ox-LDL interacts with an endogenous plasma protein, β2-glycoprotein I, to form complexes. The binding of autoantibodies to these complexes at endothelial level has been reported to be responsible for the endothelial activation and the immune-mediated inflammation (27).

Monocytes recruitment and foam cell formation.

It has been appreciated that atherosclerosis is a chronic immune-inflammatory disease in which the interaction of monocytes with activated luminal endothelium is a crucial event leading to atherosclerotic damage of the arterial intima (52,53). Monocytes migrate into the subendothelial layer of the intima where they differentiate into macrophages or dendritic cells (54). According to the modified "response to injury" hypothesis of atherosclerosis (55), endothelial injury or endothelial stress leads to the adherence of monocytes to the endothelial cells, followed by their migration to the intima. In the subendothelial space enriched with atherogenic lipoproteins, most macrophages transform into foam cells (56). Foam cells aggregate to form the atheromatous core and as this process progresses, the atheromatous centers of plaques become necrotic, consisting of lipids, cholesterol crystals and cell debris (57).

The adherence of monocytes to the endothelium and their subsequent migration into the arterial wall are facilitated by the presence of cellular adhesion molecules on the surface of endothelial cells (58,59). The rolling of monocytes along the endothelial monolayer is mediated by the selectin family of adhesion proteins (59,60). L-selectin is expressed on the surface of monocytes while P-selectin and E-selectin are expressed on the luminal surface of activated endothelium. The major ligands for all three selectins are highly fucosylated and sialylated carbohydrates. (59,61). The interactions between selectins and their ligands do not lead to the firm adhesion of monocytes to the luminal surface of the endothelium unless the initial attachment is followed by the engagement of integrins. The strong attachment of monocytes to the luminal surface of the endothelium is mediated by the interactions of the integrins (β1 and β2) with ligands that belong to the immunoglobulin superfamily, in particular, intercellular adhesion molecule-1 (ICAM-1) and VCAM-1 (58,59,61). In the bloodstream, the integrins expressed by monocytes are characterized by a low affinity for the ligands unless they undergo activation, receiving a chemokine signal which mediates the firm adhesion of monocytes to the endothelium (58,59,62). The selectins and integrins are intensely expressed by the activated endothelium that covers developing atherosclerotic lesions but their expression is low in arterial areas not affected by atherosclerosis (57). Studies in humans and experimental animals have found that an increased expression of VCAM-1 and ICAM-1 is associated with an increased intimal leukocyte accumulation and that there is an abundance of adhesion molecules on arterial sites prone to the development of atherosclerotic lesions (63). Studies in mice have found that genetic deficiencies of adhesion molecules are associated with significantly delayed atherosclerosis (59,61) and it is known that activated monocytes express Fc receptors and complement receptors which facilitate their adhesion to the endothelial cells (53).

In developing atherosclerotic lesions, many chemotactic factors for monocytes are produced. These include monocyte chemo-attractant protein-1 (MCP-1), macrophage colony stimulating factor (M-CSF), granulocyte/macrophage colony stimulating factor (GM-CSF), migratory inflammatory protein-1 (MIP-1), tumor necrosis factor (TNF-α), transforming growth factor-beta (TGF-β), RANTES and endothelin-1 (ET-1). On the other hand, circulating blood monocytes are activated by a variety of pro-inflammatory cytokines, including IL-1, IL-6, Il-8, IL-10, IL-12 and TNF-α, produced by intimal cells in response to infiltrating ox-LDL (64,65).

In the arterial wall, macrophages react to the plaque microenvironment by internalizing and metabolizing a variety of subendothelial components (66). Some lipoproteins infiltrating the arterial wall become trapped in the intima by matrix components and become modified (67).

Ox-LDLs are formed through enzymatic and nonenzymatic oxidation (56). It is commonly accepted that modified lipoproteins and lipoprotein aggregates are internalized by macrophages through a SR pathway. SRs were initially described in cultured macrophages where they mediate cholesterol uptake from modified lipoproteins, determining the formation of lipid-loaded macrophages that resemble foam cells present in atherosclerotic lesions (68,69). The internalizing-mediated SR-A and CD36 constitute the major pathways for foam cell formation in vivo and that lipid uptake by either receptors is considered as a proatherogenic event (53).

From the early stages of atherosclerosis, macrophage foam cells form clusters. Elucidating how foam cells selectively adhere to each other is an important issue for understanding lipid core formation. The mechanisms that regulate the formation of foam cell aggregates are poorly understood. It has been noted that scattered foam cells intensely express E-cadherin but when foam cell aggregates form, E-cadherin expression is downregulated (70). Studies on embryogenesis and in vitro experiments indicate that the switching on and off of E-cadherin expression correlates with a variety of morphological events involving cell aggregation or disaggregation and that closely aggregating cells show greater E-cadherin expression than non-aggregating cells (53). The intensive aggregation of foam cells leads to the formation of an atheromatous core. In the central portion of the atheromatous core, the destruction of foam cells occurs and this is accompanied by the extracellular accumulation of lipids and cellular debris. Also, take place the recruitment of more immune-competent cells and contribute to the plaque destabilization (71).

The TLRs as contributors of the immunological response during atherogenesis.

The Toll receptor was initially described in the dorsoventral development of the *Drosophila* (72). In 1996, Toll was described as a crucial receptor for the *Drosophila*'s defense against fungal infection, restricted to the innate immune response (73). One year later, a mammalian homologue of the Toll receptor, TLR4, was discovered (74). Since then, 13 different TLRs have been identified in mammals and 10 in the human species, with different endogenous and exogenous ligands. Some TLRs (1, 2, 4, 5, 6, 10) are found on the cell surface, whereas other TLRs (3, 7, 8, 9) are intracellular localized. These TLRs are part of the innate immune system, which in contrast to the adaptive immune system is activated in a non-specific manner and forms the first line of defense against invading pathogens in mammalians (75).

Toll like receptors are type I transmembrane receptors characterized by an extracellular domain with leucine-rich repeats and a carboxy terminal intracellular domain, similar to the intracellular domain of the interleukine-1 receptor. This domain, designated the Toll/IL-1R (TIR) domain, contains about two hundred amino acids and consists of three conserved regions essential for the signaling cascade to downstream adapter molecules (76).

The TLR of endothelial cells play a very important role in mediating the autoimmune reaction which is assumed to give rise to atherosclerosis (23). The subtypes TLR4 and TLR2 recognize the bacterial and human HSP60 as well as the ox-LDL (77).

Mullick et al. reported that complete deficiency of TLR2 led to a reduction in atherosclerosis in atherosclerosis-susceptible low-density lipoprotein receptor-deficient mice, indicating that host-derived endogenous TLR2 agonists might play a crucial role in treating atherosclerotic disease (78). The critical role of TLR2 in the progression of atherosclerosis seems to be associated with MCP-1 level and macrophage recruitment to atherosclerotic lesion (79). On a related article, Epstein-Barr virus can specifically activate human monocytes via TLR2-dependent signaling, a process that may contribute to the secretion of MCP-1 (80).

In apolipoprotein E deficient ($ApoE^{-/-}$) mice, inactivation of TLR2 reduced lipid accumulation and decreased macrophage recruitment to the aortic sinus, as well as down-regulating MCP-1 levels. TLR2 deficiency has also been associated with reduced progression of atherosclerosis. These findings implicate that TLR2 expression on monocytes might participate in atherogenesis as a risk factor (79).

The mechanism underlying the modulation of TLR2 expression on monocytes remains unknown. In an ex-vivo study that IFN-γ and TNF-α potently upregulate TLR2 levels on human monocytes, while IL-4 downregulates TLR2 (80). TLR2 level is probably modulated in vivo by not only its ligands but also by the integration of cytokines (81). It also remains unclear what effect the enhanced TLR2 level has on the monocytes. Hadley et al. showed that the reciprocal upregulation of TLR2 and TLR4 monocyte-surface expression could increase the cell's sensitivity to other ligands, leading to enhanced intracellular signaling and proinflammatory-cytokine release, including TNF-α (82). On the other hand, proinflammatory cytokines are the established participants in atherosclerosis, and therefore, the association between TLR2 upregulation and some cytokines might synergize the atheroma development (81).

On the other hand, TLR4 is expressed in a number of different cell types present in the atherosclerotic plaque. TLR4 is expressed at low levels in endothelial cells in non-atherosclerotic arteries, but expression is up regulated in human atherosclerotic lesions (17). Michelsen et al. were the first to suggest a direct link between TLR4 and atherosclerosis formation in $TLR4^{-/-}/ApoE^{-/-}$ mice which developed less atherosclerosis compared to $ApoE^{-/-}$ controls (83).

Different roles for oxidized lipids have been reported in TLR4 expression and activation. Xu et al. showed that ox-LDL is able to increase TLR4 mRNA expression in cultured human monocytes-derived macrophages, in contrast to native LDL (84). Also, this interaction activates, through the nuclear transcription factor κappa B (NF-κB), the genes that give rise to the inflammatory and autoimmune responses (20). Interestingly, subjects characterized by anomalous TLR structures, by attenuating receptor signaling, run a significantly lower risk of developing precocious carotid plaques. For example, the polymorphism of the TLR4 could explain the different genetic susceptibility to atherosclerosis shown by some individuals (85).

The adaptive immunity in atherosclerosis.

The presence of activated T cell populations in human atherosclerotic plaques strongly suggests that they influence disease progression (18). In support of this concept, 10% of all T cells in human plaques recognize ox-LDL in an MHC class II-restricted manner (86). The occurrence of T cell-dependent antibodies recognizing antigens implicated in the pathogenesis of atherosclerosis also supports the involvement of T cells in disease progression (25). However, to prove formally that T cells play a role in atherogenesis and to dissect further the underlying mechanisms influencing disease progression investigators have turned to animal studies.

$CD4^+$ and to a lesser extent $CD8^+$ T cells localize in atherosclerotic plaques of both $ApoE^{-/-}$ and LDLR knockout ($LDLR^{-/-}$) mice (87). Several lines of evidence have directly demonstrated the importance of these T cells throughout atherogenesis. Both C57BL/6 mice receiving depleting anti-CD4 antibodies and CD4 deficient C57BL/6 mice fed an atherogenic diet exhibit reduced fatty streak formation (88). In agreement with this finding $ApoE^{-/-}$ and $LDLR^{-/-}$ lacking an adaptive immune system through loss-of-function mutations also exhibit reduced atherosclerosis (89,90).

In contrast, ApoE-deficient SCID mice reconstituted with $CD4^+$ T cells develop accelerated atherogenesis (89). Thus, the overall picture strongly suggests an overall pro-atherogenic role for $CD4^+$ T cells starting early during atherosclerotic disease progression, even though data conflict concerning the outcome of atherosclerotic disease in $ApoE^{-/-}CD8^{-/-}$ mice (90).

The role of $CD8^+$ T cells in atherogenesis is less clear. $ApoE^{-/-}CD8^{-/-}$ mice exhibit no change in plaque burden suggesting that $CD8^+$ T cells play a small if any role during atherogenesis (25). $CD8^+$ T cells can promote atherogenesis as demonstrated when the $CD8^+$ T cell activation was induced by the expression of a foreign antigen by vascular smooth muscle cells. Similarly, a CD8 T cell activation stimulus, CD137 ligation, increased lesion size in $ApoE^{-/-}$ mice (91). Thus $CD8^+$ T cells seem to play a minor role during atherogenesis under normal circumstances, but they might be triggered by intracellular infections and then contribute towards atherosclerotic plaque buildup (25), or might modulate later stages of atherosclerosis.

T cells responses are initiated by antigen presenting cells, which are usually dendritic cells in the case of naive responses, although B cells and macrophages can also sub-serve this function. Activation of T cells occurs through simultaneous engagement of the T cell receptor (TCR) with peptide antigen: MHC class complexes and costimulatory molecules with their ligands. Many questions remain concerning triggering of T cell responses during atherogenesis, but immature dendritic cells may ingest atherosclerosis-related antigens arising from the arterial intima and then process/present these antigens in draining lymph nodes (92). Many of the candidate antigens implicated in atherosclerosis appear to have a relatively wide distribution and T cell responses might therefore begin in distant lymph nodes. Antigen presentation also occurs within the atherosclerotic lesion, which contains macrophages, dendritic cells and non-classical antigen presenting cells such as smooth muscle cells and endothelial cells that all can express MHC class II (93).

Antigen presentation in the lesion may boost already established T cell responses rather than initiate responses of naive T cells. Several studies have demonstrated oligoclonal T cell populations in plaques from either $ApoE^{-/-}$ mice or humans (94). The TCR usage appears to become more limited with time, suggesting recruitment of effector T cells into the lesion and that a few specific clones then expand within the lesion. This hypothesis derives support from the observation that the T cell expansions evident in plaques do not accompany a similar expansion in peripheral blood (25).

$CD4^+$ T cell subsets in atherosclerosis.

Naive $CD4^+$ T cells have long been thought to irreversibly differentiate into distinct subsets that can be identified by their ability to produce specific cytokines. The Th1 and Th2 subsets were identified more than 20 years ago and are characterized by production of IFN-γ and IL-4, IL-5 and IL-13, respectively (95). Recent studies have demonstrated the presence of additional subsets including $CD4^+FoxP3^+$ T cells, $CXCR5^+$ follicular B helper T cells, Th9 cells and Th17 cells (25). To complicate the picture further it has become increasingly clear that T helper subsets are plastic rather than terminally differentiated and can undergo changes if needed.

Th1 cells and INF-γ

The principal Th1 cytokine, IFN-γ, is produced by a majority of all T cells in human atherosclerotic plaques (85). Several functional studies have demonstrated a deleterious role for IFN-γ during atherogenesis as $LDLR^{-/-}ApoE^{-/-}$ mice lacking either IFN-γ or IFN-γ receptors develop attenuated atherosclerosis (96,97).

In addition, injections of recombinant IFN-γ result in increased lesion size (97). Studies of human arteries transplanted into immunodeficient mice support a pro-arteriosclerotic role for IFN-γ (25).

Not only Th1 cells produce IFN-γ, as both NK T cells and NK cells can elaborate large amounts of IFN-γ, macrophages can also secrete smaller amounts of IFN-γ and even smooth muscle cells can under certain circumstance produce IFN-γ (98). NK T cells localize in atheroma in both humans and mice (99). Although not abundant in atherosclerotic lesions, NK cells may still promote pro-atherogenic reactions outside of the lesion via IFN-γ (100,101). IFN-γ may also "destabilize" plaques through prevention of smooth muscle cell infiltration and proliferation, reduction of collagen synthesis and increased production of extracellular matrix-degrading proteins potentially yielding rupture-prone plaques (101). A large number of both pro- and antiatherogenic features have been attributed to IFN-γ, including recruitment of T cells and macrophages to the plaques, augmented class II histocompatibility expression, increased macrophage uptake of lipids leading to the formation of foam cells, increased activation of APC and enhanced secretion of Th1-promoting cytokines. On the other hand, potential anti-atherogenic functions of IFN-γ include decreased concentrations of serum cholesterol, suppression of LDLR-related protein, scavenger receptor A, CD36 and inhibition of lipoprotein lipases (102).

Another line of evidence suggesting the pathogenicity of Th1 cells emerges from studies of T-bet deficient mice. The transcription factor T-bet is required for differentiation into the Th1 lineage and T-bet-deficient $LDLR^{-/-}$ mice develop reduced atherosclerosis (97). However, the interpretation of this experiment is complicated by the fact that T-bet is also required for T cell trafficking (103).

Th2 cells, IL-4, IL-5 and IL-13.

The role of Th2 cells in atherogenesis is poorly understood. At first glance it would be expected that Th2 cells would protect against atherogenesis as they oppose the differentiation of pro-atherogenic IFN-γ-producing Th1 cells. However, both $ApoE^{-/-}IL\text{-}4^{-/-}$ mice and $LDLR^{-/-}$ mice reconstituted with IL-4-deficient bone marrow develop less severe atherosclerosis (104,105). The potential proatherogenic effects of IL-4 include mast cell activation, which can result in apoptosis of smooth muscle cells (SMC), reduced collagen production, and increased production of proteases, in turn destabilizing plaques and leading to plaque rupture. IL-4 can also induce the metalloproteinase-12 (MMP-12) that can digest structural elements of the artery wall and promote aneurysm formation (106).

While IL-13 has not been studied in atherosclerosis IL-5 appears to have an opposite effect to IL-

4 and plays a protective role during atherogenesis. Engraftment of IL-5-deficient bone marrow in $LDLR^{-/-}$ mice led to enhanced lesion formation. IL-5 may protect by promoting the development of B-1 cells. B-1 cells express immunoglobulin-M (IgM) in greater quantities than IgG, and their receptors show polyspecificity, meaning that they have low affinities for many different antigens, although they have a preference for other immunoglobulins, self-antigens, and common bacterial polysaccharides (107). Thus IL-5 may act in an antiatherogenic fashion by stimulating the production of protective antibodies (25).

Regulatory T cells.

$CD4^+FoxP3^+$ regulatory T cells comprise a separate lineage of T cells that can suppress immune responses in a dominant manner (108). They characteristically express the transcription factor FoxP3 and in most cases CD25, the IL-2 receptor α- subunit. The molecular mechanism(s) through which $CD4^+FoxP3^+$ regulatory T cells mediate their suppressive effects remains poorly understood. The presence of $FoxP3^+$ cells during atherogenesis was initially demonstrated in human atherosclerotic plaques using immunohistochemistry and later in the aorta of $ApoE^{-/-}$ mice using PCR (109). The mere presence of $FoxP3^+$ T cells does not directly prove that they play a role during disease development. Functional studies in which $CD4^+FoxP3^+$ T cells were depleted with anti-CD25 antibodies in $ApoE^{-/-}$ mice resulted in increased atherosclerotic plaque size. $ApoE^{-/-}$ mice with T cells that have an impaired response to TGF-β (ApoE CD4-dnTGF-RII) do not exhibit a similar increase in lesion size upon anti-CD25 depletion (110). A previous study using an animal model of colitis demonstrated that T cells that cannot respond to TGF-β from non $CD4^+FoxP3^+$ T cells are no longer suppressible by $CD4^+FoxP3^+$ T cells (111). Thus it is impossible to know if $CD4^+FoxP3^+$ regulatory T cells enforce their antiatherogenic effects directly through TGF-β, or through other immunosuppressive mechanisms $CD4^+FoxP3^+$ T cell-derived IL-10 limits infarct growth in a model of ischemic stroke (112).

Novel $CD4^+$ T cells subsets: Th9 and Th17.

Several novel $CD4^+$ T cells subsets have emerged recently including Th9 cells (113) and Th17 cells (114). It is not clear if either of these subsets plays a role during atherogenesis, but this question merits investigation if for no other reason that subset differentiation is a balancing act and induction of one subset may relate directly to impaired differentiation of another pro- or anti-atherogenic subset. Th9 cells produce abundant IL-9 and operate in immune responses during allergies and in defense against helminthes. Th17 cells elaborate IL-17 and contribute to defense against bacterial and fungal pathogens, but can also promote autoimmune diseases through heightened tissue damage partially due to the recruitment, activation and migration of neutrophils (25).

Both Th9 cells and Th17 cells require TGF-β for their differentiation, in combination with IL-4 and IL-6, respectively. Both subsets will oppose the induction of $CD4^+FoxP3^+$ T cells, which protect against atherogenesis. Recent data demonstrate IL-17 expression by T cells in human arteries and that IL-17 acts in combination with IFN-γ to induce a pro-inflammatory response from vascular smooth muscle cells. IL-17 also appears to promote atherosclerosis, at least when Th1 activity is reduced (115).

Conclusions

There are, at the moment, different theories concerning the nature of the inflammation at the origin of atherosclerosis. The autoimmune hypothesis appears to be one of the most realistic and attractive. Future studies may add other elements to the data illustrated in this review. Vaccination against atherosclerosis, through the induction of tolerance against self-antigens (or external epitopes) and amplification of the regulatory response, may hopefully pave the way to the prevention or even regression of the inflammation and to the stabilization of the plaques.

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