Inflammatory and Immune Aspects of Atherosclerosis

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Atherosclerosis is a multifactorial disorder characterized by the unregulated accumulation of lipids in the vessel wall. Although the consequences of atherosclerosis (myocardial infarctions, leg ischemia and cerebrovascular accidents) impose a heavy financial burden on developed countries, there is no treatment strategy — including lipid-lowering medications — that provides optimal amelioration of these complications [1].

The evolvement of the atherosclerotic lesions follows an orderly chain of events, initiated by the accumulation of activated T lymphocytes and lipid-laden macrophages at areas of predominant sheer turbulent stress. Subsequently the plaques undergo maturation manifested by the recruitment of additional cellular constituents such as smooth muscle cells [1,2].

Atherosclerosis as an Inflammation

The presence of macrophages, endothelial cells and activated lymphocytes in the atherosclerotic plaque has recently raised the concept of atherosclerosis as an inflammatory disorder [1,2]. Similar to an inflammatory process, there is an interaction between effector cells of the immune response and the production of mediators by leukocytes and macrophages (cytokines, chemokines, soluble adhesion molecules, etc). In this context, R. Ross compares atherosclerosis with 'classic' chronic inflammatory conditions, such as cirrhosis and rheumatoid arthritis [2]. The essence of this paradigm is that the initial triggers impose stress on the arterial wall, leading to a 'physiological' response to injury. Subsequently, the two opposing forces act to determine a relatively constant equilibrium in which the stress and the arterial response to the stress set the rate of progression of the atherosclerotic lesion

Cytokines, chemokines and the effector cells

The immune system is actively involved in the evolvement of the atherosclerotic lesion; evidence of its participation is presented in Table 1 and reviewed in refs. 3–5. The byproduct of the presence of immunopotent cells in the vicinity of the atherosclerotic plaque is the production and secretion of mediators that attract additional cells into the growing lesion. In this respect, the study of murine models of atherosclerosis has contributed much to the understanding of the role of inflammatory mediators.

Until recently, atherosclerosis was studied mainly in larger animals (the most popular being non-human primates and rabbits). However, the drawback of these models is the limited spectrum of transgenic large animals. More recently, several transgenic knockout mice have been developed that render the mice susceptible to the development of atherosclerosis [6–8]. The apolipoprotein E knockout mouse is the most popular, owing to its spontaneously developing hypercholesterolemia, with concomitant progression of atherosclerosis [6,7]. The LDL[1] receptor-deficient mouse develops significant atherosclerosis only when fed a high fat diet. In parallel, methods of assessing the extent and nature of lesions in these murine models have advanced considerably [9].

The construction of the transgenic mouse models represents a breakthrough in the study of the immunology of atherosclerosis, since it provides the means for studying the effects of distinct parameters on atherosclerosis. This is intensified by the findings of immunopotent cells (CD4 and CD8 lymphocytes) within the lesions of these mice [10]. Examples of these applications include:

- Crossing the apoE[2] mouse with interferon gamma knockout mice yields a double knockout mouse that is relatively protected from atherosclerosis, suggesting that IFN-γ[3] plays a pro-atherogenic role [reviewed in ref. 2].
- Treating mice with blocking monoclonal antibodies to CD4 lymphocytes or to tumor necrosis factor-alpha reduces atherosclerosis [rev. in ref. 5].
- Crossing apoE mice with mice whose lymphocyte maturation is defective results in decreased early, but not advanced, atherosclerosis [rev. in ref. 2].
- Mice deficient in intracellular adhesion molecule I or P selectin develop smaller atherosclerotic lesions [rev. in ref. 2]

A summary of the mediators involved in atherosclerosis is presented in Table 1.

[1] LDL = low density lipoprotein
[2] apoE = apolipoprotein E
[3] IFNλ = interferon gamma

Table 1. Evidence for the involvement of the immune system in atherogenesis

Presence of activated infiltrating T cells expressing MHC class II, HLA DR and IL-2 receptor in early stages of atherosclerosis
• Production of cytokines by the cellular components in the plaques: IL-1, IL-2, IL-6, IL-8, IL-12/IL10, TNF-α, MCP-1, IFN-γ, PDGF
• Immunosuppression by cytotoxic agents results in accelerated atherosclerosis in mice and rabbits
• Disruption of T and B lymphocyte maturation reduces early atherosclerotic lesions in apoE- deficient mice.
• Antibodies to epitopes of oxLDL present within atherosclerotic lesions
• Anti-oxLDL antibodies correlate with carotid occlusion
• Anti-HSP65 antibodies correlate with carotid atherosclerosis
• Anti-oxLDL antibodies have dual effects on the uptake of modified LDL to macrophage *in vitro*
• Anti-HSP65 antibodies mediate endothelial cells and macrophage cytotoxicity
• Immunization against oxLDL is associated with reduced atherosclerosis in animals.

Infections, inflammation and atherosclerosis

The idea that infections and immune-mediated inflammation may contribute to the progression of atherosclerosis has gained renewed interest in recent years [11,12]. Several studies have found an association between viral and bacterial agents and atherosclerosis.

Two of the more widely investigated agents in this respect are *Chlamydia* and the herpes viruses (mainly cytomegalovirus). Evidence has been presented for the presence of both microorganisms in different organs as well as the atheromas in autopsies. Indirect data associating both organisms with atherosclerosis have also been reported. Accordingly, titers of antibodies to herpes viruses and *Chlamydia* were found to be elevated in atherosclerosis-related conditions and predictive of future atherosclerosis-related events [11,12]. However, despite the resurgence of interest in the association between infectious agents and atherosclerosis, no direct evidence has been provided to firmly establish a causative role for these agents. This can be accomplished. By introducing the given agent, it is possible to directly induce atherosclerotic lesions.

If atherosclerosis is viewed as an inflammatory condition, it is conceivable that acute-phase reactants will be elevated to an extent that is proportional to the magnitude of the lesion size. C-reactive protein, a marker of inflammation, appears to predict cardiovascular events in healthy men. For example, high levels of CRP[4] were associated with a twofold increase in the risk of stroke, a threefold increase in myocardial infarction, and a fourfold increase in the risk of developing peripheral artery disease [reviewed in 13]. CRP has also been shown to predict the risk of fatal coronary events among high risk male smokers, as well as recurrent coronary episodes in patients with preexisting coronary vascular disease.

The Autoimmune View of Atherosclerosis

The idea that a self-mediated immune attack takes place in the vicinity of the atherosclerotic plaques stems directly from the inflammatory nature of the lesion. The presence of inflammatory cells bearing activation markers raises the question of possible target and trigger mechanisms. The presence in the lesions of modified or cross-reactive antigens that are capable of mounting an autoimmune response supports the autoimmune concept. To exemplify the concept, we describe here three autoantigens suggested to influence atherosclerosis.

Modified low density lipoproteins

LDL can undergo several forms of modification following exposure to glucose (glycation), oxidizing agents (oxidation), or aggregation [14,15]. Oxidized LDL has been studied extensively in recent years because it displays functional properties different from LDL. These properties (activation of endothelial cells, mitogenicity to smooth muscle cells, etc) constitute the base of the hypothesis that states that oxLDL[5] is the dominant player in the initiation and progression of atherosclerosis [14]. Several observations regarding oxLDL bear special relevance to autoimmunity:

- LDL undergoes oxidative modification *in vivo*.
- OxLDL is immunogenic, since autoantibodies to its modified targets — associated with the extent of carotid atherosclerosis and peripheral vascular disease — predict future myocardial infarctions and subsequent restenosis [16–19]. T cells recognizing LDL have been isolated from atherosclerotic lesions in humans [20].
- *In vitro* oxLDL — but not unmodified LDL — induces secretion of pro-inflammatory cytokines by lymphocytes.

Particularly complex is the issue of the immune response against oxLDL. As mentioned, antibodies to oxLDL correlate with atherosclerosis [16]. On the other hand, experimental data suggest that generation of antibodies to oxLDL, by deliberate immunization with the lipoprotein, protects against atherosclerosis in animals [21–23]. In this respect it is possible that anti-oxLDL antibodies play a dual role; or alternatively, that other components of the immune system take part in the protective role.

Heat shock protein 60/65

The HSP[6] family consists of approximately 25 proteins discerned by their molecular weights [24,25]. These proteins play an important role in protecting the cell against stressful insults such as heat, radiation and mechanical

[4] CRP = C-reactive protein

[5] oxLDL = oxidized LDL

[6] HSP = heat shock protein

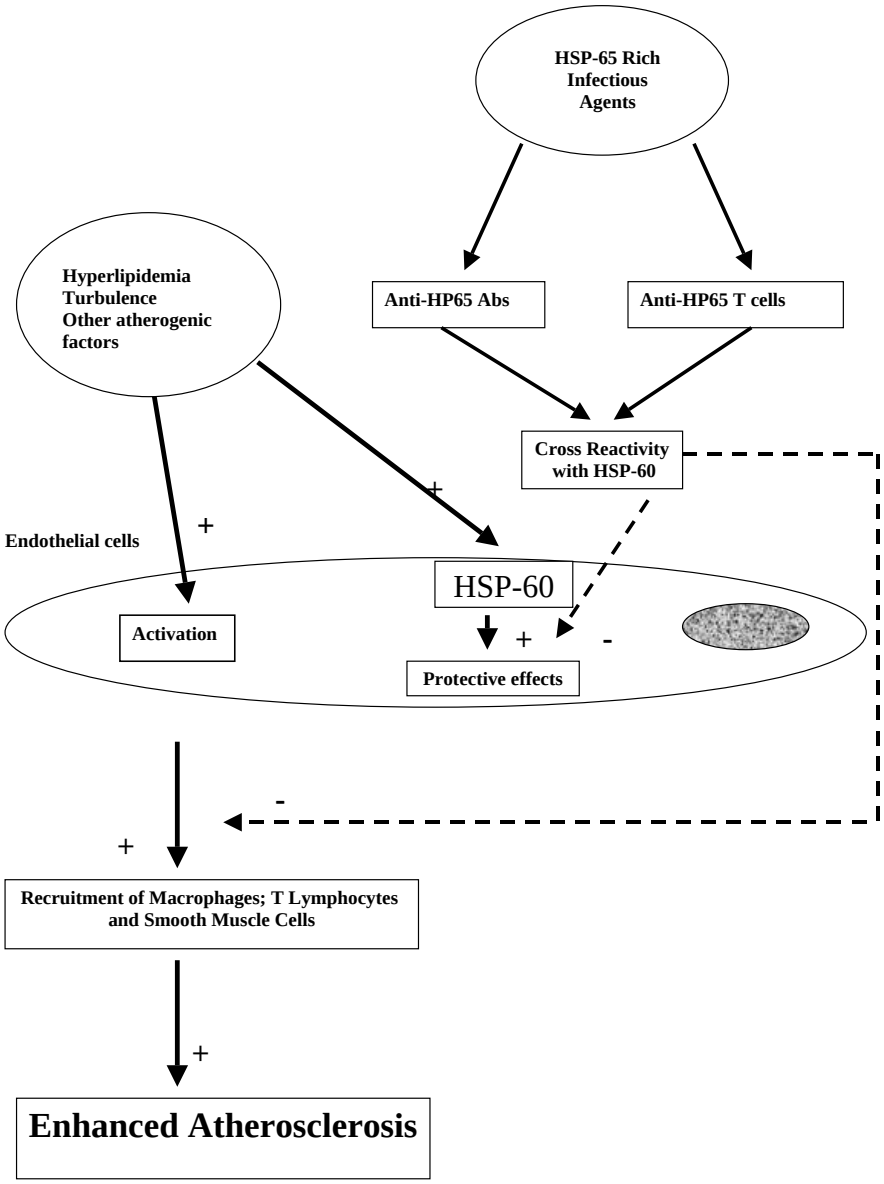

Figure 1. Infectious agents containing HSP65 invade the host and lead to an immune response against this protein. HSP60, the mammalian equivalent protein, is expressed at areas of bifurcation in the arterial tree, following 'physiological' insults such as hyperlipidemia, oxLDL, or mechanical forces derived from local turbulent flow. Mammalian HSP60 and bacterial HSP65 are very similar, and the effectors of the immune response to HSP65 do not 'discriminate' between the bacteria and host tissue. Thus, an autoimmune attack is initiated by means of anti-HSP65 T cells and antibodies on tissues expressing HSP60 (i.e., within the atherosclerotic plaque). The autoimmune attack culminates in a cytokine imbalance and secretion of additional mediators that, together, promote the progression of atherosclerosis.

trauma. They act by stabilizing the internal proteins and minimizing unfolding during damage induction.

Heat shock proteins are relatively unchanged during evolution. This means that throughout evolution the proteins are highly conserved, a property that is relevant to the study of autoimmunity. HSP60 — the mammalian form, and HSP65 — the bacterial form, are thus similar and cross-reactive. Since several organisms possess HSP65, when they invade the host an immune response to HSP65 can potentially be produced (i.e., due to the presence of anti-HSP65 antibodies and T cells). Interestingly, it has been shown that levels of anti-HSP65 are correlated with carotid artery atherosclerosis [26]. This observation led to the hypothesis that an HSP65-induced immune response (towards bacteria) is eventually turned on against our own self-expressed HSP60 [Figure 1]. Indeed, heat shock proteins are expressed in atherosclerotic plaques, probably as a result of the local stress imposed by hyperlipidemia and turbulence; hence they provide the required target for the cross-reactive response. This hypothesis is supported by experimental models showing that induction of an immune reaction against HSP65 in rabbits [27] and mice [28] — by immunization against the protein — results in enhanced atherosclerotic plaques.

Antiphospholipid antibodies, β_2-glycoprotein I and atherosclerosis

Antiphospholipid determination is widely used in clinical practice to evaluate the prothrombotic tendency of the individual. aPLs[7] are heterogeneous in their binding targets: some react with phospholipids (e.g., cardiolipin and phoshatidylserine), whereas others are bound to 'co factors' that associate with these phospholipids [29]. β_2GPI[8] is a phospholipid-binding protein that is considered the most likely target of aPLs [reviewed in 30]. It has recently been observed that the antibodies that bind phospholipids are mainly nonspecific (generated in response to infections), whereas antibodies that bind β_2GPI are of an autoimmune nature and are thus associated with the prothrombotic predisposition [29]. The recently coined term "antiphosholipid syndrome" encompasses the combination of the tendency towards thrombotic events with the presence of autoimmune-type aPLs [30,31]. The most likely disease associated with antiphospholipid syndrome is systemic lupus erythematosus. Patients with SLE[9] experience premature atherosclerosis that is more extensive than would be anticipated from their predisposing factors (namely, dyslipidemia and use of corticosteroids) [reviewed in 32]. Thus, we have raised the hypothesis that aPLs — known to impose a thrombotic tendency — also possess pro-atherogenic properties [32]. This assumption appears to be logical since these antibodies have been shown to display endothelial and platelet-activating properties *in vitro* [33–35].

We recently addressed this question experimentally by generating anti-β_2GPI antibodies (by immunizing with β_2GPI) in LDL-receptor deficient mice [36]. Indeed, early atherosclerosis was enhanced with no apparent change in the lipid profile of the mice. These results were also reproduced in more advanced atherosclerotic lesions in apoE-deficient mice [37]. Next, we wished to test the presumption that β_2GPI is present in atherosclerotic plaques and can serve as a target of the immune-mediated attack. We found that β_2GPI is expressed in subendothelial regions of human atherosclerotic plaques [38]. Interestingly, it co-localized with CD4 lymphocytes, supporting its role as a target of immune-mediated inflammatory effector cells. These observations led us to assume that the immune response against β_2GPI is involved in boosting the inflammation within the atherosclerotic plaques.

We also explored the possibility that aPLs lacking β_2GPI reactivity are also involved in atherosclerosis. This was achieved by immunizing LDL-RD[10] mice with non-β_2GPI reactive aPLs [39]. It has been shown that immunization with antibodies can induce a sequential chain of events that result in induction of host antibodies bearing similarities with the initially introduced antibodies. When we used human aPLs for immunization, the mice developed 'self'-murine aPLs that were similarly nonreactive with β_2GPI. The immunized mice developed increased atherosclerotic lesions [39].

Conclusions

The realization that autoimmune reactions can be involved in the progression of atherosclerosis has been gathering support in recent years. In this review we presented three candidate antigens that influence the progression of the atherosclerotic plaque. As we have shown, these autoantigens act in counter directions: oxLDL promotes the inflammatory process within the lesions, whereas HSP60 and β_2GPI reduce the process. However, the immune response against oxLDL appears protective, while the one mounted against the protective proteins (HSP60 and β_2GPI) seems pro-atherogenic.

In a very recent study [40] we evaluated the presence of antibodies to each of the candidate antigens in SLE patients. We found that these patients, who are at increased risk to develop atherosclerosis, displayed elevated levels of anti-oxLDL, anti-HSP65 and anti-β2GPI antibodies, pointing to the possible multifactorial role of autoimmune factors in atherogenesis (40).

These findings of target autoantigens in atherosclerosis bear more than mere academic interest. If the destiny of the atherosclerotic plaque can be anticipated by determining the relative role of these antigens, selective and targeted immunomodulation strategies can be formulated that will enable better treatment for these patients.

[7] aPLs = antiphospholipid antibodies
[8] β_2 GPI = β_2 glycoprotein 1
[9] SLE = systemic lupus erythematosus
[10] LDL-RD = LDL receptor deficient

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