The Immune Basis of Vascular Disease

Atherosclerosis and its associated complications, such as coronary artery disease, peripheral vascular disease, and stroke, are the leading causes of morbidity and mortality in all racial groups of most westernized societies. A better understanding of the events that lead to the induction and progression of atherosclerosis and the development of strategies that control these processes would have a significant impact on human health.

Atherosclerosis is a chronic inflammatory condition that usually begins at an early age in the absence of lipid accumulation, with fatty streaks composed of lipid-laden macrophages (foam cells) developing at later stages. T lymphocytes, predominantly of the CD4+ phenotype, are associated with the intima layer of the vessel. The inflammatory process at atherogenic sites leads to the production of cytokines and other inflammatory mediators, resulting in cell migration, proliferation, extracellular matrix production, and plaque development.1–3 Monocytes and macrophages appear intimately involved with the development of atherosclerosis; mice with the osteopetrotic (op) mutation in the macrophage colony-stimulating factor (M-CSF) gene, which results in a complete absence of M-CSF in the serum and tissues and a marked reduction in the number of circulating monocytes, exhibit significantly less atherosclerosis than control littermates when bred into an apolipoprotein (apo) E–deficient background.4

The precise role of T and B cells in atherosclerosis remains unclear. Although activated T cells are a dominant feature of atherosclerotic lesions,5,6 studies demonstrating that atherosclerosis can be induced by hypercholesterolemia in mice deficient in T and B cells suggest that these cells are not essential.7–9 However, the cholesterol levels in these studies far exceeded those present in human subjects without any genetic defect, and the findings should therefore be interpreted with caution. Indeed, other evidence indicates that T cells may attenuate atherogenesis by some means, because the elimination of T lymphocytes with monoclonal antibodies increases proliferative lesions in rats.10 The T-cell immunosuppressant cyclosporin has been reported to both increase and decrease atherosclerosis in hypercholesterolemic mouse and rabbit models.11–13 The atherosclerosis-promoting effects of cyclosporin A may be due in part to an inhibition of T-cell–mediated immunoregulation, the presence of which has been reported.10

In contrast to studies that suggest that atherosclerosis is not dependent on T cells,7–9 fatty streak lesions are smaller in immunodeficient apoE knockout mice. CD4+ cells transferred from immunocompetent mice aggravate atherosclerosis in immunodeficient apoE knockout animals.14 and treatment with immunoglobulin can inhibit atherosclerosis in LDL receptor and apoE knockout mice.15,16 These latter findings suggest that immunotherapeutic approaches may be of value in the treatment of atherosclerosis.

If T-cell reactivity is involved in atherosclerosis, then it is essential to identify candidate molecules/autoantigens that drive the response and to characterize the specificity and functional phenotype (proinflammatory or regulatory) of the T cells that infiltrate atherosclerotic lesions. A number of molecules have been proposed, including members of the heat shock protein (hsp) families.2,17

Hsps and Their Induction

Hsps or stress proteins are highly conserved molecules that fulfill a range of functions, including cytoprotection and the intracellular assembly, folding, and translocation of oligomeric proteins.18 These proteins are present and can be induced in all species, and they are categorized into several families that are named on the basis of their approximate molecular weight (eg, the 70-kDa hsp70). In addition to being constitutively expressed (making up 5% to 10% of the total protein content under normal growth conditions), the synthesis of these proteins can be markedly induced (up to 15% of the total cellular protein content) by a range of cellular insults that cause protein unfolding, misfolding, or aggregation and a flux of newly synthesized non-native proteins, the function of which is to stabilize and refold proteins. Such insults include not only an elevation in temperature but also a range of other conditions such as oxidative stress, viral infection, nutritional deficiencies, some chemicals, and exposure to cytokines.19

Hsp gene transcription in response to stress is regulated by the interaction of heat shock factor (HSF) transcription factors (of which the principal one in vertebrates is HSF1) with heat shock elements in the hsp gene promoter regions.20,21 The stress response is only transient, because a prolonged and inappropriate presence of protein-binding...
Heat Shock Proteins in Cardiovascular Disease

Pockley

Heat Shock Proteins in Cardiovascular Disease

1013

molecules would adversely influence protein homeostasis and a variety of intracellular functions. One mechanism by which HSF1 activity is negatively regulated is by hsp70 binding to its transactivation domain and the resultant repression of heat shock gene transcription.\(^{22}\) A second mechanism involves an interaction between hsp binding factor 1 (HSBP1) with the active trimeric form of HSF1 and hsp70, thereby inhibiting the capacity of HSF1 to bind to DNA.\(^{23}\)

**Hsp and the Immune Response**

In addition to being molecular chaperones, hsps are immunodominant molecules, and a significant element of the immune response to pathogenic microorganisms is directed toward hsp-derived peptides.\(^{24,25}\) This is intriguing given the phylogenetic similarity between microbial and mammalian forms of these molecules (\(\approx 50\% \) to \(60\%\) identical residues in the case of the hsp60 family), and it has prompted debate as to whether hsps might also act as potentially harmful autoantigens.\(^{24}\) The proposition that immunologic recognition of cross-reactive hsp epitopes might provide a link between infection and autoimmunity\(^{26}\) has been supported by studies implicating immunity to hsps in arthritis,\(^{27-29}\) multiple sclerosis,\(^{30-32}\) and diabetes.\(^{33-35}\)

**Hsp Expression and Hsp Reactivity in Vascular Disease**

Although the precise influence of hsps on atherogenesis and atherosclerosis is unclear, an association between expression of and reactivity to hsps and induction of the inflammatory response that characterizes the development of atherosclerosis has arisen from a number of studies. The intensity of hsp expression positively correlates with the severity of atherosclerosis; there is a localized enrichment of \(\gamma/\delta\) T cells, which have a predisposition to respond to hsps, in the lesion\(^{36}\); and immunization with recombinant mycobacterial hsp65 can induce atherosclerotic lesions in normcholesterolemic rabbits.\(^{37}\) Normal C57BL/6J mice fed a high-fat diet and LDL-receptor–deficient mice.\(^{38}\)

Raised levels of anti-hsp antibodies have also been associated with the presence and progression of vascular disease. Elevated levels of circulating antibody to the mycobacterial 65-kDa hsp have been reported in carotid atherosclerosis,\(^{40}\) coronary heart disease,\(^{41}\) and borderline hypertension,\(^{42,43}\) and levels of antibodies to human hsp60 are increased in peripheral vascular disease.\(^{44}\) Levels of anti-hsp65 antibodies might have some diagnostic value, because titers appear to predict the 5-year mortality of patients with carotid atherosclerosis.\(^{45}\)

The in vivo physiological significance of such antibodies has yet to be established, especially given that they are present, albeit at lower levels, in the sera of normal individuals.\(^{43,46}\) The finding that hsp60–specific monoclonal antibodies and antibodies to the hsp60 family isolated from human serum mediate the cytotoxicity of endothelial cells on whose surface hsp60 expression had been induced by tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) or heat treatment\(^{47-49}\) suggests that such antibodies might play a role in vascular injury and the pathogenesis of atherosclerosis. This involvement might be via an autoimmune-type mechanism, because anti-hsp65/60 antibodies in individuals with atherosclerosis recognize 3 distinct, conserved (self) sequences.\(^{50}\)

**Hsps as Inducers and Mediators of Vascular Disease**

The potential nature and temporal involvement of hsps in the establishment and progression of the atherosclerotic lesion are complex. They may be involved in the initiation of atherosclerosis via nonspecific inflammatory events and/or its progression via the induction of adaptive immunity either to themselves or to homologous molecules derived from infective organisms. Hsp60 and hsp70 are present in the serum of normal individuals,\(^{46,51,52}\) serum hsp60 levels correlate with the presence of early atherosclerosis,\(^{43,52}\) and levels of hsp70 are raised in patients with peripheral and renal vascular disease.\(^{54}\)

The expression of hsps in the early stages of atherosclerosis might result from one or a combination of factors. Risk factors for atherosclerosis such as hyperlipidemia, diabetes, smoking, and hypertension cause oxidative stress, and oxidative stress leads to the induction of hsp expression in vascular smooth muscle cells.\(^{55}\) Hemodynamic factors such as raised blood pressure have direct effects on the vasculature, and vessels subjected to greater mechanical and shear stress express hsp60 and are more prone to the development of atherosclerosis.\(^{36,48,54,55}\) In addition, shear stress induces hsp60 expression in cultured human endothelial cells, and hsp60 expression is present at sites in the rat common carotid artery subjected to increased wall shear stress.\(^{56}\)

A principal component of the atherosclerotic plaque is lipid-laden cells formed from the uptake of oxidized LDL via a scavenger receptor that does not recognize native LDL. Given that in vitro exposure to oxidized LDL induces hsp60 expression by monocytic cell lines\(^{57}\) and hsp70 expression by human endothelial and smooth muscle cells, it is likely that lipid-laden foam cells in the early atherosclerotic lesion express hsps.\(^{58,59}\)

Alternatively, the induction of hsps might be secondary to the inflammatory process in the early lesion in that the expression of cytokines both by the vascular endothelium and by infiltrating leukocyte populations drives the expression of hsps in the vessel wall. Human atherosclerotic plaques express a spectrum of cytokines, the profile of which is dominated by proinflammatory (Th1) cytokines, including interferon-\(\gamma\), interleukin (IL)-1\(\alpha\) and -\(\beta\), and TNF-\(\alpha\), of which induce hsp expression.

Once expressed, hsps can influence the development of nonspecific inflammatory and adaptive immune responses in a number of ways. Although typically regarded as being intracellular molecules, it is now apparent that these proteins can be released into the extracellular environment from viable cells, although the precise mechanisms by which this occurs have yet to be elucidated. Cells that have been shown to release hsps include cultured rat embryo cells,\(^{61}\) human islet cells,\(^{62}\) rat glial cells, and a human neuroblastoma cell line,\(^{63}\) as well as vascular smooth muscle cells exposed to reactive oxygen species.\(^{53}\)

It is now apparent that extracellular hsps are intercellular signaling molecules that can mediate and influence a range of
As well as being an intracellular protein, hsp60 is an intercellular signaling molecule with the capacity to induce cytokine secretion from and adhesion molecule expression on a range of cell types. ICAM-1 indicates intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.

inflammatory responses (Figure). Bacterial and mycobacterial hsps induce proinflammatory cytokine expression (IL-1, IL-6, and TNF-α), and bacterial hsps (DNAK and GroEL) induce E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 expression on human vascular endothelial cells. Bacterial and human hsp60 activate human vascular endothelial cells to express E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1, and they activate vascular endothelial cells, smooth muscle cells, and monocytes/macrophages to secrete IL-6 and TNF-α.

The finding that hsps could act as intercellular signaling molecules prompted the search for their receptors. The receptor for hsp60 on human peripheral blood mononuclear cells and monocytes has been identified as the CD14 antigen, which uses the signaling pathway also used by lipopolysaccharides. Signaling is also mediated by the Toll-like receptor 4, which is an important mediator of innate immunity and lipopolysaccharide signaling in murine cells. The CD14 molecule is also involved in hsp70-induced activation, leading to intracellular calcium fluxes and the induction of proinflammatory cytokines (IL-1β, IL-6, and TNF-α). A CD14-independent but calcium-dependent response that leads to TNF-α production has also been identified.

Taken together, these findings would appear to indicate that hsps induced in the early stages of atherogenesis might promote the nonspecific inflammatory response in the vessel wall and the recruitment of monocytes and T cells into the developing inflammatory lesion. However, the situation is more complex, because the induction of hsp70 expression in rat aortic tissue by heat and stannous chloride treatment induces an anti-inflammatory state characterized by an inhibition of in vivo leukocyte adhesion to the mesenteric endothelium after topical suffusion of formyl-methionyl-leucyl-phenylalanine (FMLP).

Hsps as Autoantigens: Friend or Foe?

In addition to being targets for proinflammatory anti-hsp antibody responses or acting as mediators of nonspecific inflammatory events, hsps may influence the progression of atherosclerosis by acting as target autoantigens for infiltrating self-hsp reactive T cells. However, despite the association of hsp expression and hsp reactivity with autoimmune, a number of observations question the proposition that self-hsp reactivity has a direct proinflammatory role in autoimmune disease, and the situation might also be the same in cardiovascular disease.

First, the normal T-cell repertoire includes cells reactive against autologous hsps and although it could be suggested that these molecules are intracellular and are therefore shielded from self-hsp reactive T cells, this is not necessarily so, because hsps can be released from a variety of cell types, hsp60 and hsp70 are present in the peripheral circulation of normal individuals, and hsp60 is transported to the surface of stressed cells.

Second, as opposed to being proinflammatory, T-cell reactivity to self-hsp60 and self-hsp70 can downregulate autoimmune disease, and the administration of self-hsp60 and peptides derived therefrom delays murine skin allograft rejection. It might be that the reactivity of T cells to self-hsps is part of a normal immunoregulatory T-cell response with the potential to control proinflammatory disease processes.

Because there appear to be many similarities between the inflammatory responses in atherosclerosis and those observed in rheumatoid arthritis, it might be that similar regulatory potential exists or can be induced in atherosclerosis. Evidence, albeit limited, for the presence of immunoregulatory T cells with the capacity to control atherogenesis has already been generated by observations that elimination of T cells increases proliferative lesions in rats, although the specificity of such putative regulatory populations is currently unknown. Evidence from experimental animal models of arthritis and patients with rheumatoid arthritis indicates that reactivity to conserved (self) hsp60 induces a regulatory T-cell phenotype, whereas reactivity to non–self-hsp60 induces a proinflammatory T-cell phenotype, and it is possible that hsp60 contains both proinflammatory atherogenic epitopes and anti-inflammatory epitopes, the immune response to which protects from atherosclerosis. These findings suggest that alteration of the polarization of the immune response in the atherosclerotic lesion may influence disease pathogenesis, and certainly, reduction of the Th1 polarization of CD4+ T cells in apoE knockout mice by use of pentoxifylline appears to be effective.

Infection, Hsps, and Cardiovascular Disease

Despite the fact that the events that influence atherosclerosis remain unclear, its development and progression appear to be a balance between proinflammatory and regulatory immune responses. One factor that may influence the overall phenotype of the immunologic response in atherosclerotic lesions, at least in the clinical situation, is the presence of concomitant infection. There is an increasing body of evidence to indicate that infective pathogens, particularly *Chlamydia pneumoniae,*
are involved in the pathogenesis of atherosclerosis. C pneumoniae has been found in atherosclerotic plaques,93,94 and the organism can induce foam cell formation in macrophages.95 C pneumoniae elicits T-cell–mediated immune responses,96 and T cells specific for C pneumoniae have been isolated from human atheromatous plaques.97 Hsp60 from C pneumoniae may be involved in atherogenesis and the induction of plaque instability, because it induces macrophage production of TNF-α and matrix metalloprotease activity.98 Although the role of infection in the pathogenesis of atherosclerosis is unclear, a recent population-based study99 reported a significant correlation between anti-mycobacterial hsp65 antibody levels and antibodies against C pneumoniae in the general community, and it was suggested that immune reactions to hsp60 in atherogenesis are due at least in part to bacterial infections. However, a cautionary note with regard to translating data that arise from animal studies into the clinical situation is that although C pneumoniae has been shown to exacerbate atherosclerosis in some animal models,100,101 it does not in others,102 for reasons that are currently unknown.

Given that hsps are immunodominant molecules and immune responses to bacterial hsps have been reported in a number of inflammatory conditions, one influence of coexistent infection might be to polarize localized immunity in atherosclerotic lesions toward a Th1 proinflammatory phenotype. Evidence supporting this proposition arises from work in autoimmune demonstrating that T cells isolated from the synovial fluid of patients with rheumatoid arthritis respond to self-hsp60 by predominantly producing regulatory Th2-type cytokine responses, whereas cells stimulated with bacterial hsp60 produce higher levels of interferon-γ, consistent with a proinflammatory Th1-type response.80 In the same study, T-cell lines generated in response to self-hsp60 but not mycobacterial hsp60 had the capacity to suppress TNF-α production by peripheral blood mononuclear cells.80 Coexistent infection can dominantly polarize hsp reactivity toward a Th1 phenotype in that immunization of mice with self-hsp60 induces lymphocytes that secrete high levels of IL-10 but do not proliferate in response to mouse hsp60, whereas coadministration of hsp60 from C trachomatis induces lymphocytes that proliferate strongly to mouse hsp60 and secrete markedly lower amounts of IL-10, thereby leading to a 12-fold increase in the interferon-γ/IL-10 production ratio.103

Conclusions

The immunologic responses that lead to the induction and progression of atherosclerosis are clearly complex, and additional insight is required to more fully understand the relationship between innate and adaptive immune components of atherogenesis and to establish roles for these in cardiovascular disease. Although the role of hsps in vascular disease remains unclear, the emerging evidence that reactivity to self-hsps confers an immunoregulatory phenotype on responding CD4+ T cells suggests that a new perspective be taken. It might be that it is the balance between self- and non–self-reactivity that is important rather than reactivity per se, and this would establish a more formal mechanistic link between infection and atherosclerosis. Certainly, future studies should consider the potential protective effects that these proteins and the immune response to them may have in atherosclerosis. A better understanding of these components might lead to new ways in which to modify the response by immunotherapeutic approaches.

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References


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