Role of Heat Shock Protein 65/60 in the Pathogenesis of Atherosclerosis

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Abstract

Investigations in rabbits and humans have provided experimental evidence that autoimmune reactions play a major role in the initial stages of the development of atherosclerosis. These involve the infiltration of the arterial intima with T cells reacting with heat shock protein (hsp) 65/60 and the occurrence of anti-hsp 65/60 antibodies. This early immunologically mediated stage of atherosclerosis is still reversible but if additional risk factors, such as high cholesterol levels, come into effect, severe mostly irreversible lesions develop.

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The interest in autoimmune reactions possibly contributing to the pathogenesis of atherosclerosis emerged from our previous work on the role of an altered lipid metabolism for the declining immune response in the elderly on the one hand [1] and on spontaneous and experimentally induced animal models for organ-specific and systemic autoimmune disease on the other [2]. Trying to develop an experimental autoimmune model for atherosclerosis in normo-cholesterolaemic rabbits, we made the observation that immunization of this species with mycobacterial heat shock protein (hsp)-65-containing material, such as complete Freund's adjuvant (CFA), led to the development of inflammatory reactions at those sites of the aorta that are known to be predisposed for the occurrence of atherosclerotic lesions, e.g. after feeding a high-cholesterol diet [3]. A combination of hsp 65 immunization with a high-cholesterol-containing diet entailed the most severe lesions exactly resembling those found in humans, including foam cells. In addition to the production of anti-hsp 65 antibodies, these rabbits also showed the expected high numbers of hsp-65-reactive T cells in their peripheral blood. However, the frequency of the latter was significantly increased in lesion-derived T cell preparations [4]. Interestingly, T cells derived from lesions of rabbits that were not immunized but only fed a high-cholesterol diet also showed an increased frequency of hsp 65 responders as compared to the peripheral blood. Rats, immunized

with hsp 65 only developed adjuvant arthritis but no atherosclerosis, while the reverse was true for rabbits. These findings pointed to species-dependent differences in immune reactivity against different epitopes of hsp 65 which have now been corroborated in our laboratory.

Immunohistological studies in arterial human specimens showed activated, mostly CD4+ T cells to be among the first to accumulate in the intima at predilection sites for atherosclerosis, their numbers even exceeding monocytes [5]. As a matter of fact, such T cell accumulation had already been found in the development of fatty streaks in children.

Subsequent immunohistological analyses revealed an unexpectedly high percentage of the lesion-infiltrating T cells to express the T cell receptor (TCR) $\gamma\delta$, further supporting the notion of the possible involvement of stress proteins in this initial inflammatory stage of atherosclerosis [6]. Furthermore, it was shown that adhesion molecules [in-tracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)] were expressed by endo-thelial cells at the very same sites that demonstrated hsp 60,

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i.e. at regions of major haemodynamic stress, such as the aortic arch and arterial bifurcations.

Application of various stress factors (temperature, H₂O₂, cytokines, oxidized low-density lipoproteins) on monolayers of human venous and aortic endothelial cells not only led to the expression of hsp 60, but, concomitantly, also to that of ICAM-1 and VCAM-1 in a coordinated fashion. Thus, the prerequisites for interaction of hsp-65/60-specific T cells with stressed endothelial cells were fulfilled.

In an investigation of 867 clinically healthy volunteers with sonographic assessment of their carotid arteries for atherosclerotic lesions, we found significantly increased anti-hsp 65 serum antibody titres in affected persons as compared to the group without such lesions [7]. These antibodies cross-react with recombinant human hsp 60 [8]. They not only seem to be of diagnostic value but also of possible pathogenetic relevance since they were able to destroy stressed endothelial cells via complement-mediated lysis or antibody-dependent cellular cytotoxicity.

In summary, we postulate that the earliest stage of atherosclerosis consists of an autoimmune reaction against hsp 60 that is expressed by endothelial cells following the action of various forms of stress [9,10]. On the basis of results from animal experiments, this stage is still reversible. If additional risk factors, such as high blood cholesterol levels, come into play, the lesions become more severe and irreversible. At present we do not yet know if humoral or cellular immune reactions are the initiating factors of the disease.

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