Molecular chaperones and heat shock proteins in atherosclerosis

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Xu Q, Metzler B, Jahangiri M, Mandal K. Molecular chaperones and heat shock proteins in atherosclerosis. Am J Physiol Heart Circ Physiol 302: H506–H514, 2012. First published November 4, 2011; doi:10.1152/ajpheart.00646.2011.—In response to stress stimuli, mammalian cells activate an ancient signaling pathway leading to the transient expression of heat shock proteins (HSPs). HSPs are a family of proteins serving as molecular chaperones that prevent the formation of nonspecific protein aggregates and assist proteins in the acquisition of their native structures. Physiologically, HSPs play a protective role in the homeostasis of the vessel wall but have an impact on immunoinflammatory processes in pathological conditions involved in the development of atherosclerosis. For instance, some members of HSPs have been shown to have immunoregulatory properties and modification of innate and adaptive response to HSPs, and can protect the vessel wall from the disease. On the other hand, a high degree of sequence homology between microbial and mammalian HSPs, due to evolutionary conservation, carries a risk of misdirected autoimmunity against HSPs expressed on the stressed cells of vascular endothelium. Furthermore, HSPs and anti-HSP antibodies have been shown to elicit production of proinflammatory cytokines. Potential therapeutic use of HSP in prevention of atherosclerosis involves achieving optimal balance between protective and immunogenic effects of HSPs and in the progress of research on vaccination. In this review, we update the progress of studies on HSPs and the integrity of the vessel wall, discuss the mechanism by which HSPs exert their role in the disease development, and highlight the potential clinic translation in the research field.

heat shock transcriptional factors; autoimmunity; inflammation

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Heat shock proteins (HSPs) are a group of evolutionarily conserved proteins that show high sequence homology between different species, from bacteria to humans (51), and are involved in maintaining various cellular proteins in their correctly folded functional forms (43). HSPs or molecular chaperones are classified into various families based on their molecular weight: small HSPs, HSP10, HSP40, HSP60, HSP70, HSP90, and HSP110 (Table 1). Organisms must survive a variety of stressful conditions, including sudden temperature increases that damage important cellular structures and interfere with essential functions (59). HSPs exhibit sophisticated protection mechanisms of molecular chaperones. For instance, there is a constant need for chaperone assistance during de novo protein folding and refolding of nonnative polypeptide chains, because the stability of cellular proteins is low and aggregation competes with productive folding even at physiological temperatures (59). All molecular chaperones interact promiscuously with a broad range of unfolded proteins. Generally, molecular chaperones do not contribute structural information for folding but prevent unwanted intermolecular interactions (17). Different types of HSPs seem to have different roles in protein folding/unfolding (Table 1). For example, HSP60, also called chaperonin, is a ring-shaped chaperone that encapsulates nonnative proteins in an ATP-dependent manner, whereas HSP70 is involved in the de novo folding of proteins, and under stress they prevent the aggregation of unfolding proteins and can even refold aggregated proteins (28). Thus HSPs have basic roles in maintenance of protein structure and function in all types of cells.

The arterial wall is an integrated functional component of the circulatory system that is continually remodeling in response to various stressors, including localized injury, toxins, smoking, and hypercholesterolemia (77). These stimuli directly or indirectly cause changes in blood pressure and damage to the vessel wall, and eventually induce arterial stiffness and obstruction, i.e., atherosclerosis (78). To maintain the homeostasis of the vessel wall, the vascular cells produce a high level of HSPs, which protect against damage during hemodynamic stress (85). However, accumulating evidence indicates that an immune reaction to HSP60 might contribute to the development of atherosclerosis (23, 72, 79). These findings suggest...
that the induction of HSPs is beneficial in the arterial wall’s response to stress but is harmful in certain other circumstances (85). The present review aims to update the studies on molecular chaperones or HSPs displaying either protective roles or pathological involvement in the arterial wall, emphasizing the autoimmune response to HSP60 in atherosclerosis.

HSP 10

It was believed that human mitochondrial HSP10 acts as a cofactor for HSP60. Structurally, the chaperonins exist as closely linked rings comprising seven HSP10 and/or HSP60 molecules in each (54). Genetically, each of these chaperonins are colocalized on chromosome 2 separated by a bidirectional promoter (26). Functionally, HSP10 displayed a protective role in response to stress stimuli in cardiovascular system. For instance, adenosine-mediated transfection of cardiomyocytes with either HSP10 or HSP60 reduced doxorubicin-mediated apoptosis and also resulted in overexpression of antiapoptotic Bcl-x-L (B-cell lymphoma-extra large) and Bcl-2 (63). Bcl-x-L is a transmembrane molecule in the mitochondria and is involved in signal transduction pathways mediating apoptosis and T- and natural killer-cell immune responses. Both Bcl-xL and Bcl-2 are involved in endothelial activation, and their expression has been associated with absence of inflammation, apoptosis, and atherosclerosis in long-term surviving xenografts, whereas rejecting xenografts do not show their endothelial cell expression (5). Given the fact that HSP10 serves as cofactor for HSP60, studies assessing the prevalence of anti-HSP10 antibodies in patients with atherosclerosis (e.g., coronary artery disease) demonstrate a positive correlation with prevalence of anti-HSP60 antibody levels (16), which is discussed in detail in HSP60.

HSP 27

HSP27, also called the small HSP, has a molecular mass ranging between 15 and 30 kDa and, apart from sequence homologies, shares biochemical properties such as oligomerization and phosphorylation (58) (Table 1). HSP27 oligomerization is a dynamic process dependent on the phosphorylation status of the protein and exposure to stress (11). HSP27 can oligomerize into large aggregates up to 800 kDa, but it can also form heteromeric structures with other small HSP family members (e.g., HSP20) and complexes with additional proteins such as p38 mitogen-activated protein kinase, mitogen-activated protein kinase (MAPK)-activated protein kinase 2 (MK2), and Akt. Oligomerization is regulated by phosphorylation (12, 20). Unphosphorylated HSP27 forms large multimers, whereas phosphorylation results in conformational changes, alteration of the direct interaction with other HSP, and dimer interaction with actin. HSP27 can be detected in most cells examined, although the expression levels seem to vary with some cells expressing undetectable or relatively low levels, whereas other cells express HSP27 abundantly (33). Synthesis of HSP27 can be induced by different conditions, including heat shock and other stress conditions, oxidative LDL, injury, and differentiation (20).

Importantly, HSP27 has also been identified as an estrogen receptor-β (ERβ)-associated protein and has been noted to be a biomarker for atherosclerosis (15). ERβ is expressed in endothelial and vascular smooth muscle cells of many arteries, including the coronary circulation. Estrogen activated receptors have implications in cardiovascular physiology (69). Estrogen treatment is antiatherogenic and has been shown to lower LDL oxidation in animal models. Stimulation of ERβ in vivo can reproduce the predicted HSP 27 atheroprotective effect, and vice versa (50). Extracellular HSP27 decreases

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Table 1. Heat shock protein families

<table>
<thead>
<tr>
<th>Family</th>
<th>Members/Other Names</th>
<th>Physiological Function</th>
<th>Pathological Involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSP10</td>
<td>HSP10, HSP17</td>
<td>Promotes substrate release with HSP60; cofactor for HSP60</td>
<td>Heart disease</td>
</tr>
<tr>
<td>HSP27</td>
<td>HSP27, HSP28</td>
<td>ERβ-associated protein; F-actin assembly; molecular chaperones</td>
<td>Atherosclerosis</td>
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<tr>
<td></td>
<td>HSP22, α-crystallin</td>
<td></td>
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<tr>
<td></td>
<td>HSP23, αβ-crystallin</td>
<td></td>
<td></td>
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<tr>
<td>HSP40</td>
<td>HSP32, HO-1</td>
<td>Guide protein folding; binding and transport of collagen</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HSP40, Hdj-1</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>HSP47</td>
<td></td>
<td></td>
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<tr>
<td>HSP60</td>
<td>HSP58, GroEL</td>
<td>Assemble polypeptides; translocate proteins across membranes; accelerate protein folding and unfolding.</td>
<td>Adjuvant arthritis, rheumatoid arthritis, atherosclerosis, diabetes mellitus, systemic sclerosis, schizophrenia</td>
</tr>
<tr>
<td></td>
<td>HSP60, HSP65</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Grp 95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSP70</td>
<td>HSP68, Dnak</td>
<td>Molecular chaperone; assemble and transport newly synthesized proteins; fold or unfold polypeptides; remove denatured proteins; bind to specific polypeptides (e.g., p53); ATPase activity</td>
<td>Tuberculosis, leprosy, filariasis, atherosclerosis</td>
</tr>
<tr>
<td></td>
<td>Hsc70, Hxs70</td>
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<tr>
<td></td>
<td>HSP72</td>
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<td></td>
<td>HSP73</td>
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<td></td>
<td>HSP75, Grp75</td>
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<td></td>
<td>HSP78, Grp78</td>
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<tr>
<td>HSP90</td>
<td>HSP93, Hsp70</td>
<td>Bind to specific polypeptide, receptors (e.g., glucocorticoid receptor)</td>
<td>Atherosclerosis, systemic lupus erythematosus</td>
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<tr>
<td></td>
<td>Grp94</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>HSP90-α</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>HSP90-β</td>
<td></td>
<td></td>
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<tr>
<td>HSP110</td>
<td>HSP94, HSP104, HSP105</td>
<td>Only in yeast (?)</td>
<td></td>
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<tr>
<td></td>
<td>HSP110, Apg-1</td>
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<td>Grp170</td>
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HSP, heat shock protein; HO-1, heme oxygenase-1; ERβ, estrogen receptor-β.
formation of acetylated human LDL, which in turn accentuates release of proinflammatory cytokine IL-1β. Hence, HSP27 is postulated to exert its atheroprotective effect, probably by virtue of its ability to compete with the uptake of atherogenic lipids or by attenuating inflammation (Fig. 1) (58). Immuno-histochemical analysis of atherosclerotic plaques has demonstrated that apoptotic cells are localized in the core/shoulder, whereas HSP27 are mainly expressed in smooth muscle cells of the cap and media regions (50). Martin-Ventura et al. (40, 41) have shown in vitro that plasmin is able to degrade HSP27 present in conditioned medium of normal arteries or cultured smooth muscle cells. Furthermore, when HSP27-rich conditioned media were incubated with culprit atherosclerotic plaques in culture (as a source of proteases), similar bands of degradation were observed, supporting the fact that proteases secreted by atherosclerotic plaques could be responsible for the degradation of soluble HSP27 in vivo. These data suggest that reduced HSP27 levels could reflect proteolytic imbalance occurring during pathological vascular remodeling process and may thus provide an index of plaque instability and rupture (40, 41). HSP27 is also one of the major phosphoproteins involved in actin filament dynamics, focal adhesions, and cell migration, all of which are important events in atherosclerotic plaque formation and progression (33).

Interestingly, O’Brien’s group provided the direct evidence that HSP27 protects the vessel wall from atherosclerosis in an apolipoprotein E-deficient (apoE−/−) mouse model (57). They showed that overexpression of human HSP27 (HSP27°/e) in atherosclerosis-prone apoE−/− mice results in a 35% reduction in aortic atherosclerotic burden in female (but not male) mice (57). In addition, they demonstrated that baseline serum levels of HSP27 were virtually undetectable in all mice, after 4 wk of feeding a cholesterol-enriched diet, whereas there was a >10-fold increase in serum HSP27 levels in female apoE−/− mice with little change in the male levels. There was a remarkable inverse correlation between HSP27 levels and extent of atherosclerotic burden. The rise in serum HSP27 levels closely paralleled the development of atherosclerosis and occurred in the absence of significant changes in the lipoprotein profile. In vitro study indicates that HSP27 binds to scavenger receptor-A, which is involved in cholesterol uptake, and inhibits the uptake of modified LDL (57). Therefore, HSP27 is crucial for atheroprotective in the vessel wall via interaction with proteins related to lipid metabolisms (Fig. 1).

HSP27 is constitutively expressed in smooth muscles at relatively high concentrations of 2–8 μg HSP27/mg total protein (61). HSP27 has been colocalized to contractile proteins in freshly dispersed intestinal smooth muscle cells stimulated with ceramide, and it coprecipitates with actin, tropomyosin, and caldesmon, suggesting some molecular association with contractile proteins (30, 65). In smooth muscle, the small HSPs HSP27 and HSP20 probably regulate actin cytoskeleton structure and may modulate the interaction of actin and myosin. Thus these HSPs have also been shown to play an important role in smooth muscle contraction, migration, and survival, which are key events in atherosclerotic plaque development (61). In parallel, unstable plaques have been shown to have reduced expression of HSP20 and HSP27 compared with stable plaques, suggesting they might have a functional role in inflammation and resultant plaque stability (35).

HSP70

All HSP70 proteins have a two-domain structure, an NH2-terminal ATPase domain (45 kDa, nucleotide binding domain), and a 25-kDa COOH-terminal polypeptide binding domain. An additional EEVD motif at the COOH terminus augments its interaction with other chaperones (10). HSP70 functions in a myriad of biological processes, modulating polypeptide folding, degradation and translocation across membranes, and protein-protein interactions. This multitude of roles is not easily reconciled with the universality of the activity of HSP70s in ATP-dependent client protein binding and release cycles. An early report indicated that HSP70 is expressed within human atherosclerotic lesions (25), and its localization within the aortic atheromas changes temporally as the atheroma evolves, despite the total aortic HSP70 content remaining unchanged (91). Induction of HSP70 expression has been documented in cultured human endothelial (91) and smooth muscle cells (92) in response to oxidized LDL. In advanced atherosclerotic lesions, macrophages, dendritic cells, and smooth muscle cells have been found to overexpress HSP70 (9), which causes macrophages to express proinflammatory cytokines. Cytokines such as TNF-α released from necrotic cells from within atheromas may stimulate the innate immune response, promoting inflammation during atherogenesis (31). Binding of oxidized LDL to CD36 can inhibit HSP70 expression in monocytes through peroxisome proliferator-activated receptor γ (PPARγ)-dependent pathway. The HSP70 forms complex with inhibitory κBα and attenuates NF-κB activation. Because NF-κB is an important transcription factor responsible for the expression of proinflammatory genes, these pathways may be responsible for HSP70s anti-inflammatory activity (34, 64).

![Fig. 1. A possible model of the protective role of heat shock protein 27 (HSP27) in atherosclerosis. Modified LDL can bind to its receptor to mediate lipid accumulation in the macrophages, in which proinflammatory cytokines are released and foam cells may be formed. In this process, HSP27 released by estrogen stimulation could block it and thus retard the development of atherosclerosis. Further information and illustration about the role of HSP27 can be found in another review (56).](image-url)
The regulation of HSP70 expression was recently studied, and the main finding is the role of heat shock transcriptional factors (HSFs) (1). HSFs are essential for all organisms to survive exposures to acute stress. They are best known as inducible transcriptional regulators of genes encoding molecular chaperones and HSP70 (1). Four members of the HSF family were found, which are critical for normal development and lifespan-enhancing pathways. HSFs integrate the metabolic state of the cell with stress biology and in doing so control fundamental aspects of the vessel protection (48). HSFs interact with a specific regulatory element, the heat shock element, present in the promoters of HSP genes (56, 74). In the cell, HSFs are constitutively present in a non-DNA-binding state; they are activated in response to various stresses to a DNA-binding form. This activation process appears to involve the oligomerization of HSF from a monomeric to a trimeric state and is associated with HSF hyperphosphorylation (Fig. 2) (56, 74). In atherosclerotic vessels, increased HSF1 levels were found, but not HSF2, that were mainly localized in the nuclei, and the molecular weight of HSF1 from lesional extracts was larger than that of normal vessels (48). This indicated that HSF1 in lesions was phosphorylated (modified) and activated. Usually, HSF1 proteins in cultured cells do not markedly change in response to stress, e.g., heat shock or mechanical stress. Surprisingly, it was demonstrated that protein levels of HSF1 in atherosclerotic lesions were much higher compared with normal vessels (48), suggesting different mechanisms of HSF1 activation and regulation of HSP expression in vivo from in vitro cultured cells.

Functionally, reports in support of protective role of HSP70 show that its immunodominant epitope, peptide aa111–125, stimulates production of anti-inflammatory cytokine IL-10 (71). Higher serum levels of HSP70 are associated with reduced atherosclerotic intimal thickening and lower risk of coronary artery disease (90). Thermal treatment of rats increased arterial wall HSP70 expression and attenuated intimal thickening after injury (53). Recent studies have also shown a role for HSP70 in the extracellular milieu and provide evidence of specific pathways for stressed cells to actively release extracellular HSP70 (3). HSP70 and HSP90 both mediate enhanced ApoB degradation in mammalian cell lines (21, 24). HSP70 (GRP78) has been observed in early and late stages of atherosclerosis in apoE-knockout mice (88). GRP78 provides numerous protective effects to minimize stress through unfolded protein signaling as well as antiapoptotic and antiinflammatory signaling (19). Furthermore, it was found that extracellular HSP70 has a role in carotid intimal thickening of mice exposed to cigarette smoke (42). Cigarette smoke exposure decreased arterial HSP70 expression and significantly increased intimal thickening compared with mice exposed to air. Treatment of mice exposed to cigarette smoke with intravenous recombinant HSP70 attenuated intimal thickening through reduced phosphorylated extracellular signal-regulated kinase expression in the arterial wall (42). The data suggest that decreased expression of arterial HSP70 is an important mechanism by which exposure to cigarette smoke augments intimal thickening.

On the other hand, a recent study reported that HSP70 was identified as a novel matrix Gla-protein-binding protein (87). The interaction between matrix Gla protein and HSP70 was confirmed by coimmunoprecipitation and chemical cross-linking, and blocked the interaction between matrix Gla protein and bone morphogenetic protein (BMP)-4 (87). In endothelial cells, HSP70 enhanced BMP-4-induced proliferation and tube formation, and in calcifying vascular cells, HSP70 enhanced BMP-induced calcium deposition. In addition, HSP70 mediated the procalcific effect of IL-6 on calcifying vascular cells. In apoE-null mice, levels of BMP-4, HSP70, and IL-6 were elevated in the aortic wall. Levels of BMP-4, HSP70, and IL-6 were also elevated in serum. The investigators concluded that HSP70 binds matrix Gla protein and enhances BMP activity, thereby functioning as a potential link between cellular stress, inflammation, and BMP signaling (87).

**HSP90**

HSP90 is a chaperonin, with essential functions in several organisms ranging from bacteria, protozoa, and higher eukaryotes (Table 1). It has many homologs with its endoplasmic reticulum, mitochondrial isoforms being among the more prominent ones. LDL receptor-related protein 1 is the first identified as HSP90 receptor (8). LDL receptor-related protein 1 is an endocytic receptor, and it binds and internalizes various ligands such as apoE and activated α2-macroglobulin. It promotes intracellular signaling, which downregulates cellular proliferation and migration of different cell types, including macrophages, and plays a central role in atherogenesis (8). HSP90 and HSP70 have been shown to bind to the cytoplasmic domain of macrophage scavenger receptor, a trimeric membrane protein that binds to LDL (52). Thus it seems that HSP90 and HSP70 exert their effects on atherosclerosis by influencing LDL metabolisms.

In human atherosclerotic plaques, HSP90 immunostaining was increased in inflammatory regions and in plaques characterized by lower cap thickness (37). In cultured human macrophages and vascular smooth muscle cells, treatment with
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HSP90 inhibitors increased HSP70 expression and reduced transcription factor STAT and NF-κB activation and chemokine expression induced by proinflammatory cytokines (37). In vivo in hyperlipidemic apoE-deficient mice, atherosclerotic plaques of animals treated with HSP90 inhibitor displayed increased HSP70 expression and diminished NF-κB and STAT activation, along with decreased lesion, lipid, and macrophage content (37). HSP90 expression is associated with features of plaque instability in advanced human lesions. HSP90 inhibitors reduce inflammatory responses in atherosclerosis (37). Taken together, these data suggest that HSP90 could have a protective role in atherosclerosis.

HSP60

The HSP60 family comprises HSP60 in mammals, mycobacterial homolog HSP65, chlamydial HSP60, and the Escherichia coli homolog GroEL (Table 1). HSP60 are expressed in the cytoplasm, mitochondria, endoplasmic reticulum, and nucleus, with each location varying depending on the particular protein (60). HSP60 is normally found inside the cells, and when located outside, it is an indication of cell death and one of the important signals for the immune system to activate the macrophages and other immune cells to clear off the dead cells (70). Endothelial and other cells in the vessel wall respond to various stressful stimuli such as oxidized LDL, biomechanical stress, infections, oxidants, and cytokine stimulation by producing high levels of HSP60 to maintain viability faced with these unfavorable conditions (7, 85). Misdirected immune reactions, due to their antigenic homology across species, against HSP60s may be involved in the initiation of inflammatory processes, a hallmark of the earlier stages of atherosclerosis (73).

Anti-HSP60 antibodies in humans. Xu et al. (86) were the first to report the association between anti-HSP65 (mycobacterial homolog of human HSP60) antibodies and atherosclerosis in the early 1990s. Within the framework of the Bruneck study, a large prospective population-based survey was carried out on the pathogenesis of atherosclerosis. It was demonstrated that serum antibodies against HSP65 were significantly elevated in subjects aged 40–79 yr with carotid atherosclerosis (82). Schett et al. (62) have purified human anti-HSP65 antibodies and have shown that these antibodies from individuals with severe coronary artery disease were purified using affinity chromatography and injected into apoE-deficient mice. Atheromacular lesions in their aortas were significantly increased (22). Furthermore, administration of a specific mouse monoclonal antibody (II-13) recognizing amino acid residues 288–366 of HSP60 (αHSP60288–366) effectively induced atherosclerotic lesions in apoE-deficient mice. αHSP60288–366 injection resulted in endothelial cell damage followed by increased leukocyte attachment and accumulation of macrophages and smooth muscle cells in atheroma. Interestingly, αHSP60288–366-induced atherosclerosis was blocked by pretreatment of animals with F(ab)2 fragments derived from the antibody, but not mouse IgG F(ab)2 (22). Thus autoantibodies recognizing amino acid residues 288–366 of HSP60 induce atherosclerosis via the mechanisms of autoimmune reactions to HSP60 expressed on arterial endothelial cells, which can be prevented by F(ab)2 fragment derived from these antibodies.

Molecular mimicry and immune reactions to HSP60/65. Because of high sequence homology between microbial and human HSP60 (51), which is present in a soluble form in the blood (83), it is plausible that cross-reactivity between antibodies and T cells against HSPs of microbes and human beings contributes to the development of atherosclerosis (Fig. 3). Serum anti-HSP65 antibodies have been shown to react with recombinant human HSP60 and homogenates from atherosclerotic plaques (82). Human anti-HSP65 antibodies react with HSP60 present in endothelial cells, macrophages, and smooth muscle cells in the atheroma (82). Schett et al. (62) have purified human anti-HSP65 antibodies and have shown that it is cytotoxic to endothelial cells. By performing Western blot analysis, they have shown that these antibodies from individuals with atherosclerosis react specifically with recombinant mycobacterial, human, chlamydial, and E. coli HSP60 (45). In the presence of complement (complement-mediated cytotoxicity) or peripheral blood mononuclear cells (antibody-dependent cellular cytotoxicity), these antibodies have been shown to lyse stressed endothelial cells (45). In addition, a population of T cells, responding to HSP60, within the atherosclerotic lesions may play a similar destructive role (Fig. 3) (81).

Toll-like receptor 4: a link between HSP and atherosclerosis. Innate immune repertoire is characterized by a group of evolutionarily selected, germline-encoded receptors that recognize highly conserved motifs in pathogens. One such family of pattern recognition receptors, involved in mediating the inflammatory effects of gram-negative endotoxemia (68), are Toll-like receptors, in particular the TLR4 subtype (2, 47, 66). TLR4 is expressed on cells such as endothelial cells, smooth muscle cells, and macrophages, all of which are known to be involved in atherogenesis (18). Its proinflammatory effects are possibly mediated via NF-κB pathway (68). Human (55) and chlamydial (13) HSP60 have been shown to require functional TLR4 to stimulate the production of TNF-α and nitric oxide and for activating macrophages. It has been proposed that HSPs act as an endogenous danger signal and link autoimmunity and infection (49) in the context of atherosclerosis. Extracellular serum HSPs mediate proinflammatory and proatherogenic effects (4), in which TLR4 may be a crucial link between...
It was also reported that HSP60 peptide aa253–268 was orally administrated to LDL receptor-deficient mice, resulting in a 80% reduction in plaque size in the carotid arteries and a 27% reduction in plaque size at the aortic root (67). The reduction in plaque size correlated with an increase in the number of T regulatory cells (67), although whether immune reactions against this epitope aa253–268 are involved in the pathogenesis of atherosclerosis remains to be elucidated. Thus these studies suggest that vaccination with HSP60, or more preferably with atheroprotective HSP60 peptides, is a promising idea for the prevention and treatment of atherosclerosis.

Based on recent data derived from animal models, we wonder whether a similar vaccination with the HSP60 peptides could be applied to humans in future. In fact, phase I/II studies have been conducted in melanoma patients with detectable tumor and in colorectal cancer patients rendered disease free by complete resection of liver metastasis. In these studies, each patient was vaccinated with GRP96-peptide complexes isolated from his or her own tumor, ensuring therefore an antigenic repertoire as large as possible, potentially also including unique tumor-specific antigens (6, 46). These studies indicate there is no major side effect in terms of the safety in humans. On the other hand, atherosclerosis is a chronic disease that is initiated in the early life of humans. If the vaccination against HSP60 could be applied, it could be considered in childhood. However, there is no report on human vaccination against atherosclerosis. There might be a long way to go for HSP therapy in clinic application.

**Summary and Perspectives**

The past several years have seen a dramatic increase in the number of studies of the protective role of HSP10, HSP27, HSP70, and HSP90 in cardiovascular disease such as hypertension, ischemic heart disease, and atherosclerosis (32). Conversely, the involvement of (auto)immune reactions to HSP60 in the pathogenesis of atherosclerosis is supported by accumulating experimental and clinical evidence (23). These findings could be significant for understanding the mechanism of atherogenesis and establishing new parameters for diagnosis of atherosclerosis, perhaps leading to efficient therapeutic intervention in this disease. However, there are several issues that need to be addressed, as discussed below.

The protective role of HSPs has been widely studied, but the detailed mechanism by which HSPs may exert their effects on the cell remains unresolved. For instance, HSP27 can be released from the cells in response to estrogen, preferentially responding to ERβ modulation, which may be the mechanism of the protective role of sex hormone (58). The question is how estrogen-estrogen receptor binging results in HSP27 release from the cells. Is bypassing hormones altogether and directly administering recombinant HSP27 another therapeutic approach not only for menopausal women but also for men at risk of the ravages of atherosclerosis? For HSP70, there are many reports describing a general protective role for the cells but lacking the specific target for such a role. Nevertheless, studies

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**Fig. 3.** HSP60/65-regulated immune and inflammatory response in atherosclerosis. Soluble HSP60/65 can directly bind to Toll-like receptors on macrophages to promote inflammatory cytokine production. Meanwhile, macrophage-processed HSP60/65 may also result in activation of T and B cells via molecule mimicry of HSP60/65 between microbes and humans. Both innate and adaptive immune responses can contribute to the development of atherosclerosis.

**HSP vaccination.** In the last few years, several studies have provoked an increased interest in mucosal tolerization against HSP60 aiming to suppress atherogenesis (27, 39). Both of these studies could successfully demonstrate a reduction in aortic plaques by this approach. Developing tolerance toward HSP60/65 by mucosal administration of the autoantigen, resulting in a Th2 bias, may help in reducing atherogenesis and could be a therapeutic strategy. Both nasal (39) and oral (27) administration of mycobacterial HSP65 has been shown to decrease autoimmune responsiveness and was associated with significant reduction in the number of infiltrating macrophages and the size of atherosclerotic plaques in LDL receptor-deficient mice. These animals, compared with controls, also had higher levels of IL-10, a potent anti-inflammatory cytokine, and a reduced number of CD4+ T cells (39). All of these findings are compatible with the concept that a Th1-to-Th2 shift in the autoimmune reactivity could exert an atheroprotective effect. These data in conjunction with experimental evidence provided by us not only supports the role of HSP65 in atherosclerosis but also opens up a new possibility of modulating the progression of atherosclerosis.
on in vivo animal models provide some clues for the protective effects of HSP70 in atherosclerosis. For instance, a link between exposure to cigarette smoke and HSP70 expression in the vessel wall has been described (42). The effect of exogenous administration of HSP70 was found in the neointimal formation in animals, which is specific only to cells or subjects that have been exposed to cigarette smoke (42). What have been critically lacking are studies on the mechanisms of HSP70 action in vivo.

For the pathogenic role of HSP60, although the currently available data do not allow us to establish atherosclerosis as an autoimmune disease, immune reactions to HSP60 do contribute, at least in part, to atherogenesis (23). In the absence of risk factors for atherosclerosis (endothelial stressors), the endothelial cells do not express HSP60 on their surface and hence are not targets for autoimmune insult. In the presence of risk factors for atherosclerosis, cells overexpress HSP60 and the cross-reactive anti-HSP60 antibodies damage endothelial cells, laying a foundation for subsequent development of atherosclerosis (84). However, whether the data obtained in the mouse could be translated into humans is not known. As discussed above, researchers are now in the process of delineating atherogenic HSP60 peptides in the murine system to use for the development of an antiatherosclerosis vaccine via the induction of mucosal tolerance. Although the tolerizing approach in mice may form the basis for the subsequent development of such a vaccine in humans, it is rather improbable that the same HSP60 peptide candidates will emerge as atherogenic in both species. Currently, research on HSPs and atherosclerosis is highly topical. The mystery of the molecular mechanism in this process may be elucidated eventually, and thus a much greater reduction of mortality and morbidity due to atherosclerosis may be achieved in the near future.

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