CHAPTER TWO

Heat Shock Proteins and Cardiovascular Disease

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Abstract

Atherosclerosis is the leading global cause of mortality, morbidity, and disability. Heat shock proteins (HSPs) are a highly conserved family of proteins with diverse functions expressed by all cells exposed to environmental stress. Studies have reported that several HSPs may be potential risk markers of atherosclerosis and related cardiovascular diseases, or may be directly involved in the atherogenic process itself. HSPs are expressed by cells in atherosclerotic plaque and anti-HSP has been reported to be

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increased in patients with vascular disease. Autoimmune responses may be generated against antigens present within the atherosclerotic plaque, including HSP and may lead to a cycle of ongoing vascular injury. It has been suggested that by inducing a state of tolerance to these antigens, the atherogenic process may be limited and thus provide a potential therapeutic approach. It has been suggested that anti-HSPs are independent predictors of risk of vascular disease. In this review, we summarize the current understanding of HSP in cardiovascular disease and highlight their potential role as diagnostic agents and therapeutic targets.

ABBREVIATIONS

Apaf-1 apoptotic protease activation factor 1
CP *Chlamydia pneumoniae*
CRP C-reactive protein
CVD cardiovascular disease
DC dendritic cells
GGA geranylgeranylacetone
hHSP60 human HSP60
HSPB1 HSP-binding factor 1
HSPs heat shock proteins
IL interleukin
LDL low-density lipoprotein
mHSP65 microbial HSP65
MI myocardial infarction
SMCs smooth muscle cells
TNF tumor necrosis factor

1. INTRODUCTION

Heat shock proteins (HSPs) are highly conserved families of proteins first discovered in 1962 [1]. Recent studies suggest that HSPs and an autoimmune response directed against them may be involved in the pathogenesis of atherosclerosis [2]. Some HSPs are expressed by cells within the atherosclerotic plaque and plasma anti-HSP titers have been reported to be increased in patients with vascular disease. There is a relationship between the serum concentration of some anti-HSP, particularly, anti-HSP27, -60/65, and -70 and the manifestations of atherosclerosis and its progression [3]. The strong association between HSP and cardiovascular disease (CVD) suggests that a better understanding of this relationship may allow the development of new therapeutic approaches [4].

1.1. Cardiovascular disease

CVD is a common and major cause of mortality and morbidity [5]. Established risk factors for CVD include age, male gender, family history of CVD, hypertension, hypercholesterolemia, smoking, diabetes mellitus, socioeconomic status, and obesity. Emerging risk factors include oxidative stress, inflammation, and autoimmunity [6].

1.1.1 Atherosclerosis

Atherosclerosis is associated with the accumulation of lipids including modified low-density lipoprotein (LDL) and extracellular matrix in the intima of arteries [7]. Extensive mononuclear cell infiltration and smooth muscle proliferation are characteristics of most atherosclerotic plaques [8]. Atherogenesis is a multistep process that requires an ordered sequence of events in which progress along the disease pathway is driven by risk factors that differ with disease stage [9]. Progression to the fibrofatty plaque is characterized by an enriched content of smooth muscle cells (SMCs) that migrate to the intima from the tunica media. The advanced plaque that forms may subsequently be stable or unstable dependent on its mechanical configuration and cell composition. An unstable plaque is associated with a thin smooth muscle cap and a large lipid pool with a large number of inflammatory cells in the shoulder region [10]. Atherosclerosis starts early in life and slowly progresses for decades. It remains the major cause of most forms of acute coronary syndrome (ACS) including myocardial infarction (MI) and angina, sudden death, and stroke. Inflammation and autoimmunity appear to have a pivotal role in the progression of disease [11] and will be described below.

1.1.1.1 Atherosclerosis and inflammation

The role of inflammation in atherosclerosis was first suggested in the 1850s [12]. More recently immunocytochemical techniques have allowed the cellular composition of atherosclerotic plaques to be determined [13]. These studies have shown that inflammatory cells are present at all stages of development [14]. The earliest lesions in atherogenesis are fatty streaks which have been observed in infants and young children [15]. They are characterized by a relative paucity of lipids and abundance of intimal inflammatory cells including activated T lymphocytes, mast cells, macrophages, and dendritic cells (DC) [16]. Granulocytes and natural killer cells, however, are less common [17].

Lesions of atherosclerosis also contain large numbers of T lymphocytes [18], most of which are T helper type 1 cells bearing α/β receptors [17]. Activated T cells bearing γ/δ receptors are also abundant at the earliest stages of atherogenesis [19] which can be inhibited in experimental animal models by T lymphocyte depletion [20]. The activation of T cells within atherosclerotic plaques indicates that an autoimmune mechanism may be involved. This response is characterized by production of Th1 cytokines and development of a dysfunctional endothelium. The latter occurs as an early event in atherogenesis with subsequent formation of the fatty streak containing lipid-laden foam cells and T cells [21]. Xu *et al*. reported that CD4+ cells predominate within the T-cell population in early lesions [13], while an increased CD8/CD4 ratio in both early and late lesions was reported by Van der Wal and coworkers [22]. Serum C-reactive protein (CRP) has been reported to be a stronger independent predictor of coronary events compared to LDL or total cholesterol [23–25]. It has been observed that increased soluble intercellular adhesion molecule, a marker of endothelial cell activation, was related to increased coronary risk [26]. Its expression in atherosclerotic lesions was also increased [27]. The activation of complement [28] may have a role in endothelial injury during atherogenesis and may be a consequence of autoimmune responses to modified LDL or denatured HSP [29]. The expression of human lymphocytic antigen class II antigen and several cytokines within atherosclerotic lesions supports the involvement of inflammation in atherosclerosis [18].

Blood-derived monocytes enter the intima of the arterial wall and differentiate into macrophages and DC. Modified lipoproteins are taken up by macrophages that are transformed into lipid-laden foam cells. Monocyte-derived macrophages appear to be involved at all stages of the disease process [30,31] and DC play a crucial role in directing innate or adaptive immunity against potential autoantigens involved in atherosclerotic disease [32].

Some studies have suggested that serum levels of proinflammatory, T helper-1-related cytokines are positively associated with the severity of atherosclerotic disease, or its risk of development [33,34]. For example, increased serum tumor necrosis factor (TNF)-α and interleukin (IL)-6 were reported to be predictors of coronary and CVD. Increased anti-inflammatory cytokine IL-10 was related to a significantly improved outcome of patients with ACS. It has also been reported that there is an association between serum IL-18 and IL-2 and intima-media (I-M) thickness in

carotid atherosclerosis [35,36]. The relationship between atherosclerosis and inflammation is summarized (Fig. 2.1).

1.1.1.2 The potential role of infection in atherogenesis

The potential contribution of chronic infection to the induction and progression of atherosclerosis and plaque rupture has been controversial [37]. The "infection hypothesis" of atherosclerosis has been investigated by epidemiologic and histopathologic studies, and clinical eradication trials [12]. Animal models and histopathological studies have also addressed the "infection hypothesis" directly. Recent studies on apoE knockout mice have been inconsistent; some studies indicate a potential causal relationship between infection with multiple pathogens and progression of atherosclerosis [38,39] while in another study no relationship between atherosclerosis progression and infection was found [40]. Bacterial DNA of several periodontal pathogens has been found in coronary atherosclerotic plaques, for example, *Porphyromonas gingivalis* [41]. *Chlamydia pneumoniae* was also found in the atherosclerotic arterial wall [42,43]; an analysis of 43 histopathological studies has reported that *C. pneumoniae* was present in 46% of atheromatous arteries and in less than 1% of healthy arteries [44]. A number of large clinical trial including the Weekly Intervention with Zithromax for Atherosclerosis and its Related Disorders (WIZARD), the Azithromycin in Acute Coronary Syndromes (AZACS), the Azithromycin and Coronary Events Study (ACES), and the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction (PROVE-IT-TIMI22) have investigated the effectiveness of different antibiotics and therapeutic

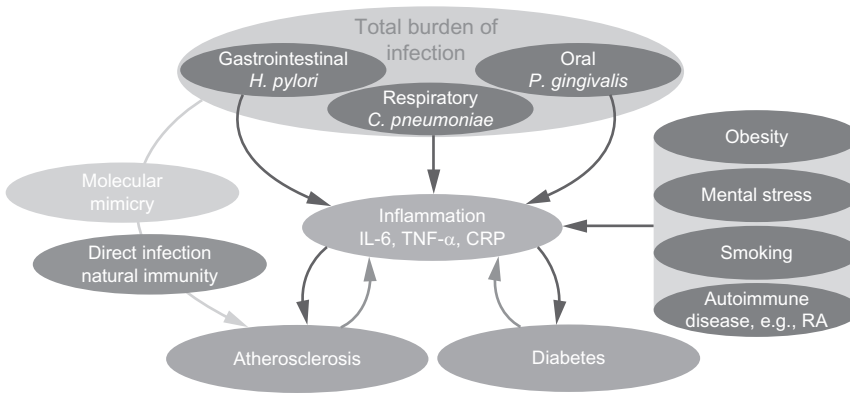

Figure 2.1 Atherosclerosis and inflammation.

regimens on the risk of CVD in patients with ischemic heart disease with or without serological evidence of *C. pneumoniae* infection. The evidence of a short-term reduction in the risk of CVD was weak and there was no evidence of a long-term reduction in these trials [21,45–47]. Antibiotic treatment for *Helicobacter pylori* infection was again inconsistent [48]. A decrease in cardiovascular risk markers has been reported in some studies; for example, plasma cholesterol, LDL cholesterol, fibrinogen, and IL-8; and a reduction of restenosis rates after percutaneous transluminal coronary angioplasty [48–50]. Other studies have reported no significant effect [51]. Pesonen and coworkers [52] reported that the presence of antibodies to several microorganisms was positively associated with carotid intimal thickening, an atherosclerotic marker, in young children. It has been suggested that infection acquired during childhood may lead to atherosclerosis in later life [53]. A positive correlation between CVD extent and infectious organism exposure has been reported [54].

1.1.1.3 Autoimmunity in atherogenesis

It has been widely reported that inflammation and immunity play an important role in the atherosclerotic process [55]. Several potential autoantigens have been identified including modified LDL (oxidized LDL and malondialdehyde-modified LDL) and β-2-glycoprotein I antigen presenting cells such as macrophages and DC present within atherosclerotic lesions, and hence it has been proposed that an autoimmune reaction may be initiated within atherosclerotic plaques [56].

HSPs may be also involved in an autoimmune response predisposing to atherogenesis. Investigation of HSP may provide a further insight into the prevention, prediction, diagnosis, and treatment of CVD.

1.2. Heat shock proteins

HSPs were originally found to be expressed in response to heat, as their name suggests [57]. "Stress proteins" may be more appropriate than "heat shock proteins." However, for historical reasons, the name endures. The discovery of genes expressed in stressful states was first made in fruit fly salivary glands following exposure to extreme heat, that is, chromosome "puffing" [58]. These highly conserved proteins display high sequence homology between prokaryotes and eukaryotes and between different species, a characteristic likely associated with their protective role against stressful environmental conditions [59]. Other stress stimuli include exposure to heavy metals, inflammatory cytokines, amino acid analogues, oxidative

stress, nutritional deficiency, ultraviolet radiation, chemicals, viruses, and ischemia–reperfusion injury [11]. Several risk factors for CVD can also induce HSP, for example, inflammation, ischemia, oxidized LDL, oxidative stress, and hypertension [11].

HSPs have been classified into seven major families on the basis of their molecular weight [3]. These include HSP10 (HSPE), small HSP (15–30 kDa, of which HSP27 (HSPB1) is a member), HSP40 (DNAJ), HSP60 (HSPD), HSP70 (HSPA), HSP90 (HSPC), and HSP100. HSPs have various functions apart from the stress response. Under physiologic conditions, HSPs play an important main role as molecular chaperones by promoting the correct protein folding. Some HSPs are also involved in the transportation of proteins across intracellular membranes and repair of denatured proteins. Although HSPs are protective proteins expressed at high levels in cells under stress, they are also expressed at low concentrations basally [60]. Functions of HSP include the regulation of essential cell functions, such as protein translocation, refolding, assembly and the recognition, and renaturation of misfolded proteins [61,62].

HSPs can therefore prevent the aggregation of denatured proteins, inhibit apoptosis [63], and maintain cellular integrity by stabilization of the cytoskeleton [64]. These functions of HSP are evident in the human arterial wall where HSPs have been shown to be important mediators of protective pathways as well as targets for autoimmunity that may exacerbate atherosclerosis [65,66]. HSPs are normally intracellular proteins, but upon release may induce an autoimmune response [52,67]. Some HSPs have been shown to have immune regulatory responses and modify innate and adaptive immune responses [68] and stimulate the production of proinflammatory cytokines [68].

The cell expression, functions, and reported utility of HSPs as circulating biomarkers are summarized in Table 2.1. Wick *et al.* proposed that the close sequence homology between microbial and human HSP60 (hHSP60) may lead to endothelial cell damage. Early atherosclerosis may involve an immune response mounted against microorganisms that may ultimately lead to an autoimmune response [102]. When HSPs are present on the cell surface or released into the extracellular environment during conditions such as necrotic cell death or viral infection, they may have an immune-stimulatory effect [3]. Surface expression of HSPs as cryptic antigens allows the immune system to recognize them as foreign. Immune responses mounted against microbial HSP may also cross-react with homologous host proteins in a form of molecular mimicry [103]. The autoimmune responses targeted to HSP60

Table 2.1 HSP: cell expression, intra/extracellular functions, and circulating biomarkers

HSP	Other nomenclature	Prokaryotic homologue	Pathological involvement	Cardiovascular expression	Cell functions	Reported potential as biomarkers	
						Antigen	Antibody
HSP 20	HSP B6	–	–	Skeletal muscle and heart muscle [69]	Vasorelaxation [70] Suppresses platelet aggregation [71]	–	–
HSP 27	HSP B [72]	Mycobacterial 18-kDa antigen [73]	–	Smooth muscle cells [74] Endothelial cells [75] Cardiac myocytes [76] Monocytes/ macrophages [77]	Actin stabilization – Muscle contraction – Cell migration – Cell survival [78]	Atherosclerosis – Acute coronary syndrome (ACS) [79]	MI in patients with ACS relative to unstable angina – Acute chest pain [80]
HSP 40	–	–	–	–	Chaperone functions [70]	–	–
HSP 60	HSP D [72]	GroEL (*E. coli*) Mycobacterial 65-kDa antigen [73]	Adjuvant arthritis, rheumatoid arthritis, atherosclerosis, diabetes mellitus, systemic sclerosis, schizophrenia [81]	Ubiquitously expressed [72]	Cell survival [82] Apoptosis [83] – Protein trafficking [84] – Peptide hormone signaling [85]	In carotid atherosclerosis [86] – Associated with IMT in borderline hypertension [87] – Associated with severity of CVD – Infection, stress, myocardial necrosis [88]	In carotid atherosclerosis – Associated with severity of CVD [89] – MI compared to CVD – Predicative of 5-year mortality in carotid atherosclerosis [90] – Higher risk of new CV event [91]

HSP 70	HSP A [72]	DnaK (*E. coli*) [73]	Tuberculosis, leprosy, filariasis, atherosclerosis [81]	Smooth muscle cells [92] Cardiac myocytes [93] Monocytes/ macrophages [92]	Anti-inflammatory [92] – Antiapoptotic – Antioxidant [94]	Levels associated with decreased IMT in hypertensive patients [95] – Levels associated with low CVD risk – Carotid atherosclerosis – Inversely correlated with neutrophil activation – ACS [96]	CVD [97] – Not related with prevalence of CVD [95] and high risk of ACS [98]
HSP 90	HSP C [72]	C62.5 (*E. coli*) [73]	Schistosomiasis, systemic lupus, erythematosus [81]	Macrophages [92] – Smooth muscle cells [92]	Antioxidant – Antiapoptotic – Proangiogenic [99]	Atherosclerosis [100]	Atherosclerosis [101]

appear a consistent feature of atherosclerosis suggesting that this HSPs may play a role in the pathogenesis of atherosclerosis [104]. The expression of some HSPs is increased in regions of the artery wall containing atheromatous plaques [81,105]. Following cerebral ischemia, protein synthesis in the brain is generally suppressed. However, some specific genes are expressed in increased levels, and these include the genes for HSPs and amyloid precursor protein [106].

Most HSPs facilitate the correct folding of denatured proteins. These complex interactions between chaperones, co-chaperones, and their client proteins lead to either a new folding attempt or protein ubiquitination and subsequent degradation via the proteasome pathway [107].

Some HSPs can inhibit apoptosis [107]. Apoptosis involves the release of cytochrome *c* from mitochondria. It is binding to apoptotic protease activation factor 1 (Apaf-1) triggers oligomerization. This complex then attracts the inactive unprocessed pro-form of the proteolytic enzyme caspase-9, which is cleaved to its active form, thereby initiating the apoptotic process. HSP90 binds Apaf-1 thereby preventing its binding to cytochrome *c*, whereas HSP70 prevents oligomerized Apaf-1 from recruiting pro-caspase-9 [107].

HSP90 has also been shown to bind to endothelial nitric oxide synthetase in the heart and increase its activity [108]. With increasing age, the induction of HSP by stressful stimuli appears to diminish in a variety of tissues including the heart due to impaired activation of heat shock transcription factor 1 (HSF-1) by stress [108]. This phenomenon was associated with a reduction in the protective effect of mild heat shock or ischemia against subsequent severe ischemic stress in the aged hearts cells in animal studies [108,109].

Individuals with chronic infection have high serum HSP60. These individuals also tend to have poorer diet and living conditions that could potentially confound this observation [47]. A positive relationship between the immune responses to HSP following immunization with bacillus Calmette–Guerin (BCG), an attenuated strain of tuberculosis, and subsequent atherosclerosis has been reported in a rabbit model of atherosclerosis [110]. This finding suggested that exposure to specific microorganisms may promote atherogenesis.

1.2.1 Production of HSPs

Upregulation of HSPs is mediated by the release of HSF1 in response to stressful stimuli such as increased temperature [111] or ischemia [112].

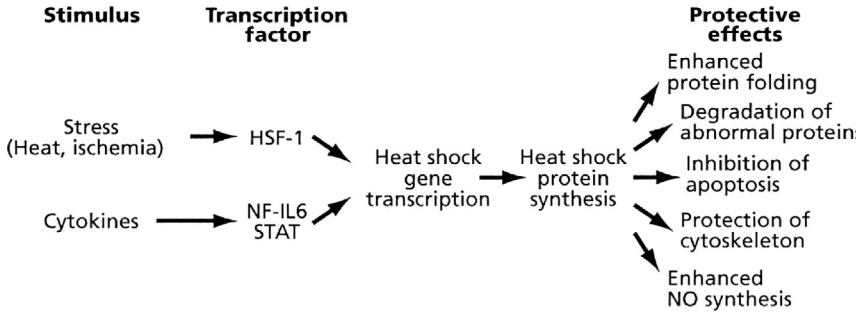

Figure 2.2 Activation of the HSP by specific stimuli and their protective effect [73]. Chronic inflammatory conditions such as smoking, stress, obesity, and rheumatoid arthritis may contribute to the total burden of inflammation and hence to atherosclerosis. *Ref. [89]. From P.J. Ford, G.J. Seymourm, K. Yamazaki, Cardiovascular and oral disease interactions: what is the evidence? Prim. Dent. Care 14(2) (2007) 59–66.*

Monomeric cytoplasmic HSF1 forms a trimer and translocates to the nucleus where it binds to its target sites (heat-shock elements) in the regulatory region of the HSP genes (Fig. 2.2) [30]. The activity of HSF1 is negatively regulated by the binding of HSP70 to its transactivation domain and the resultant repression of heat shock gene transcription [100]. There is also an interaction between HSP-binding factor 1 (HSPB1) with the active trimeric HSF1 and HSP70 thereby inhibiting the capacity of HSF-1 to bind DNA [113].

1.2.2 HSP in the extracellular space

The presence of increased plasma HSP concentrations is usually associated with stress including inflammation, and bacterial, and viral infection [114]. HSP exit the cell and enter the extracellular milieu by two mechanisms: passive release usually followed cell damage and active release involving exosomes [115,116] or lysozome-like vesicles [117]. Exosomes are small membrane vesicles that numerous cell types secrete. These contribute to HSP70 release from human peripheral blood mononuclear cells in both basal and stress-induced states [115]. Infections may represent a nonendogenous source of HSP in the extracellular compartment [118,119].

2. ATHEROGENESIS AND HSP

The importance of HSPs in atherogenesis is unclear. HSP expression and reactivity thereof appear to be associated with the inflammatory

response that is characteristic of atherogenesis [120]. The intensity of HSP expression is positively associated with atherosclerotic severity. Immunization with recombinant mycobacterial HSP65-induced atherosclerotic lesions in normocholesterolemic rabbits [121], normal C57BL/6J mice fed a high-fat diet [122], and LDL receptor-deficient mice [123]. In early atherosclerotic lesions, only DC were found to express HSPs [124]. Furthermore, in advanced atherosclerotic lesions, HSPs were found to be expressed by several cell types including SMC, DC, and monocytes/macrophages.

Inflammation and immunologic reaction, including the autoimmune response against several HSPs, appear to play an important role in atherosclerotic pathogenesis [125]. Both HSPs and anti-HSPs have been shown to stimulate the production of proinflammatory cytokines [48].

Wick and colleagues [124] have hypothesized that an immune response to HSPs, either endogenously derived from cells involved in atherogenesis or exogenously from microorganisms, may lead to complement-mediated endothelial injury and subsequent atherosclerosis. Mayr *et al.* reported that serum anti-HSP directed against *Escherichia coli* and *C. pneumoniae*-mediated lysis of stressed, but not unstressed endothelial cells [126]. Alternately, these immune responses may be induced following exposure of HSPs on the surface of infected endothelial cells [11]. SMC can be induced to express HSPs as part of a survival mechanism following exposure to a variety of stressors, that is, high blood pressure [127].

HSPs are immunodominant molecules. A significant element of the immune response to pathogenic microorganisms is directed toward HSP-derived peptides. HSPs are highly conserved molecules with phylogenetic similarity between microbial and mammalian forms (50–60% identical residues for HSP60). This finding has led to a debate as to whether HSPs might act as potentially harmful autoantigens [128]. Immunologic recognition of cross-reactive HSP epitopes might provide a link between infection and autoimmunity [129]. This premise has been supported by studies implicating immunity to HSPs in arthritis [130,131], multiple sclerosis [132,133], and diabetes [134,135].

Some studies have shown that prior induction of the HSPs by a mild stress has a protective effect against more severe stress. Increased expression of HSPs in cardiac cells in culture or intact heart produced a protective effect similar to antioxidant enzymes [73].

Most previous studies of HSP and CVD have focused on HSP60, 65, and 70 [11]. However, there has been recent interest in HSP27.

2.1. HSPs and CVD in animal models

Reduced atherosclerotic lesion size after subcutaneous immunization with mycobacterial HSP65 has been demonstrated in apoE knockout mice [136]. HSPs released into the circulation from the infarcted heart tissue bind to circulating anti-HSPs. This antigen–antibody complex may then subsequently be removed by the reticuloendothelial system [120].

Reactivity to conserved HSP60 was reported to induce a regulatory T-cell phenotype in animal models of arthritis [137,138]. These findings suggested that altered immune response in the atherosclerotic lesion may influence disease pathogenesis. In fact, a reduction of Th1 polarization of CD4− T cells in apoE knockout mice by pentoxifylline appeared quite effective in reducing atherosclerosis [139]. The data support the possibility that HSP 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 [140]. The induction of HSP70 expression in rat aortic tissue by heat and stannous chloride treatment was associated with an anti-inflammatory state [141]. The roles of HSP in animal model studies are summarized in Table 2.2.

2.2. HSPs and CVD in human studies

2.2.1 Atherogenesis and HSP20

Kozawa *et al.* reported that serum HSP20 (HSPB6) was increased in cardiomyopathy in hamsters, though its tissue of origin was unidentified. Myocardial angiogenesis was enhanced in hearts that overexpressed HSP20 [71]. HSP20 appeared to act as a cardiokine, regulating myocardial angiogenesis through activation of the VEGFR signaling cascade. HSP20 has also been reported to affect platelet aggregation [147]. HSP20 was found to inhibit thrombin-induced calcium influx without affecting release from calcium stores. Also, HSP20 inhibited the increase in cytoplasmic free calcium triggered by collagen, but not that induced by the calcium ionophoreA-23187 in platelets *in vitro* [148].

The maintenance of redox status is a constant challenge in normal hearts, but even more so under pathological conditions. Small HSPs, in particular, may play a protective role against redox imbalance, that is, when excess production of reactive oxygen species exceeds scavenging mechanisms [149].

Zhang *et al.* observed that circulating HSP20 was increased in a transgenic mouse model with cardiac-specific overexpression [150]. HSP20 was secreted via exosomes independent of the endoplasmic reticulum–Golgi

Table 2.2 Immunomodularity effects of HSP in cardiovascular disease in animal models

Study	Subjects	Finding	References
ApoE knockout mice were infected with MCMV and CP	ApoE knockout mice	Infection with MCMV alone, CP alone, and both MCMV and CP increased lesion size of atherosclerosis	[38]
Effect of HSP70 administration on the size of infract	Rat and rabbit	HSP 70 administration limited infarct size following the exposure of the heart to ischemia–reperfusion injury	[142]
Immunization with HSP65 or mycobacterial HSP65	Wild-type C57BL/6J mice	Enhanced the lesion formation	[143]
Effects of antibodies directed against, and lymphocytes reactive to HSP65 on fatty-streak formation	LDL-RD mice	Promote fatty-streak formation	[144]
Immunization with HSP65	Normocholesterolemic rabbits	Promotes atherosclerotic lesion formation	[121]
Immunization with the bacillus Calmette–Guerin (BCG)	Cholesterol-fed rabbit	Enhance atherogenesis	[145]
Effect of high-cholesterol diet on anti-HSP and von Willbrand factor (vWF)	Rabbit	Induce the expression of anti-HSP60, 65, and 70 and thus increasing concentrations of von Willbrand factor (vWF), a marker of endothelial injury	[146]
Effect of immunization with HSP60 on T lymphocytes	Rabbit	Depletion of peripheral blood T lymphocytes	[20]

pathway. HSP20 was involved in regulating myocardial angiogenesis via VEGFR signaling cascade activation.

HSP20 expression and its phosphorylation at its serine16 residue was increased following experimental MI. Mouse models of cardiac-specific overexpression of HSP20 subjected to ischemic/reperfusion showed smaller MI and improved recovery of contractile performance during the reperfusion phase [151]. This observation is likely to be due to the ability of HSP20 to protect against cardiomyocyte necrosis and apoptosis. As such, HSP20 may be a new therapeutic target for the prevention and treatment of myocardial remodeling and ischemic injury.

Li *et al*. reported on a proteomic study of cardiac ventricle isolated from failing and nonfailing human hearts [152]. Altered proteins included those associated with metabolic, cytoskeletal, and stress response function. Of the latter, HSP27 and 20 were identified.

HSP20 and its phosphorylation have been implicated in multiple physiological and pathophysiological processes including smooth muscle relaxation, platelet aggregation, exercise training, MI, and insulin resistance. HSP20 has an important role in contractile function and cardioprotection [153].

It has recently been shown that HSP20 triggers apoptosis by interacting with key proapoptotic signaling proteins [154], and its overexpression was shown to protect cultured rat cardiac myocytes from β-agonist-induced apoptosis [155].

Qian *et al*. have generated a cardiac-specific overexpression model in which HSP20 serine 16 was substituted with alanine [156]. This model was subjected to ischemia/reperfusion to ascertain if the cardioprotective effects of HSP20 were associated with serine16 phosphorylation. Interestingly, prevention of phosphorylation influenced the balance between cell death and autophagy. The HSP20 aggregation pattern was also altered. Prevention of phosphorylation attenuated cardioprotection against ischemia/reperfusion injury via suppressed autophagy and increased cell death.

HSP20 has been shown to enhance myocardial contractile function and protect against doxorubicin-induced cardiotoxicity [157]. Wang *et al*. investigated the role of HSP20 in sepsis-mediated cardiac injury by examining the expression profiles of five major HSP in response to lipopolysaccharide challenge [158]. HSP20 expression was downregulated in lipopolysaccharide-treated myocardium suggesting that this decrease might contribute to cardiovascular defects. In contrast, overexpression of HSP20 significantly enhanced cardiomyocyte contractility following lipopolysaccharide treatment. This study indicated that HSP20 overexpression prevented

endotoxin-induced myocardial dysfunction and apoptosis via inhibition of NF-κB activation.

β-Adrenergic neurohormonal axis stimulation contributes to the progression of heart failure and mortality in animal models and patients. Activation of the β-adrenergic pathway in cardiomyocytes has been shown to result in transiently increased cardiac HSP20 expression [159]. Cardiac HSP20 overexpression may protect the heart against β-agonist-induced cardiac remodeling. HSP20 attenuated the cardiac hypertrophic response, markedly reduced interstitial fibrosis and decreased apoptosis.

2.2.2 Atherogenesis and HSP27

HSP27 is a 27-kDa protein ubiquitously expressed by many cell types including vascular cells. HSP27 is expressed at high levels in a variety of tumors and normal tissues including the heart [160]. HSP27 has several roles, including regulating apoptosis and is involved in vascular SMC migration and proliferation, embryogenesis, cardioprotection, resistance to oxidative stress, and modulation of inflammation [161,162]. These roles may have an important bearing on atherogenesis. HSP27 may have cardioprotective effects by mechanisms that include increasing myocardial resistance to oxidative stress and its effects on apoptosis [163]. Some studies have indicated that HSP27 overexpression protected cardiac myocytes against ischemic injury [161,164] and promoted cell protection and survival during inflammation by regulating anti-inflammatory gene expression [165]. Reports have suggested that HSP27 was associated with acute cardiac chest pain [166] and ACS [167]. Increased HSP27 expression was related to improved right ventricle function and systemic perfusion [168]. Park *et al.* found that serum HSP27 was increased in the early hours following ACS, but fell to near normal levels about 12 h from chest pain onset [169].

HSP27 may be a putative autoantigen involved in atherogenesis [170]. It was reported that high antibody titers against HSP27 were associated with cardiovascular events [171]. Although HSP27 IgG was significantly increased in patients with chest pain versus healthy control subjects, antibody levels were similar in both troponin I positive (MI) and negative patients [167].

Anti-HSP27 has been detected in plasma of patients with CVD. Although increased serum anti-HSP27 has been found in patients with AMI, the evidence for its association with CVD has not been fully established [80]. Shams *et al.* found increased anti-HSP27 in patients with chest pain [153]. Anti-HSP27 was increased during the first 12 h following

an ACS event, then fell to near normal after 12 h. Ghayour-Mobarhan *et al.* reported that serum antibody titers of HSP27 increased and decreased rapidly after onset of ACS [154], suggesting that anti-HSP27 may be an early marker of MI and unstable angina.

2.2.3 Atherogenesis and HSP60/65

The HSP60 family of proteins is involved in development of several diseases including arthritis in rats, rheumatoid arthritis in humans, insulin-dependent diabetes mellitus in mice, and systemic sclerosis in humans [172]. Several studies have reported that increased anti-HSP60 was associated with CVD. Recent studies indicate that high titers of anti-HSP60 were associated with coronary atherosclerosis [89,173]. Children with increased anti-HSP60 had an ~10-fold chance of being from a high-risk CVD family versus children with normal anti-HSP60 concentration [174]. Anti-HSP may initiate complement activation [175] thereby increasing vessel wall inflammation, that is, a process that plays an essential role in atherosclerosis [91,176]. Some studies suggested that plasma HSP60 was increased early in heart failure [177].

Significantly increased serum anti-HSP65 was observed in patients with CVD [90] and carotid atherosclerosis [178]. A strong correlation was observed between increased anti-HSP65 and restenosis after percutaneous transluminal coronary angioplasty [179]. In patients with CVD, anti-HSP60 and anti-HSP65 were significantly associated with the presence and severity of disease. The concentration of HSP60 and 65 has been related to more severe CVD forms [180]. Some conditions, such as cardiac failure, result from heart damage caused by a variety of factors [181]. Heart damage due to stress factors can lead to sudden death because of cardiac dysfunction [182]. Serum anti-HSP60/65 concentration was associated with CVD severity and progression [89,98]. One study investigated the effect of cardiac rehabilitation therapy, statin treatment, or combination of both on anti-HSP in patients with CVD after percutaneous coronary intervention [178]. This study showed that reduced anti-HSP was accompanied by greater improvement in blood biochemical variables including lipids, high-sensitivity CRP (hsCRP), and IL-6. HSP and anti-HSP elicited the production of proinflammatory cytokines by macrophages and adhesion molecules [183]. There was no significant relationship between antibody titers to HSP and other inflammatory factors (hsCRP and IL-6), suggesting they are independent risk factors for CVD [184]. HSP60 expression was localized to atherosclerotic lesions as opposed to nonatherosclerotic regions of the arterial wall [185].

In animal studies, anti-HSP60, 65, and 70 were correlated to the extent of atherosclerosis as well as markers of endothelial dysfunction [146]. Furthermore, HSPs were potential targets for the immune response, may be altered during the stress response, and thereby directly contribute to the inflammatory process [11].

These immune responses may initially be directed against antigens present on pathogenic organisms and then cross-react with homologous host HSP elaborated by cells of the vascular wall including endothelial cells [186]. It has been reported that a cellular and humoral response to HSP65 was present in subjects with carotid and coronary atherosclerosis [90,187].

Kleindienst *et al.* demonstrated that HSP60 was present on endothelial, smooth muscle, and mononuclear cells of carotid and aortic specimens [17]. Increased plasma anti-HSP was associated with the presence and progression of vascular disease. Increased antibody against mycobacterial HSP65 was reported in patients with carotid atherosclerosis [187], coronary heart disease [90], and borderline hypertension [188]. Serum anti-HSP60 was increased in subjects with peripheral vascular disease [150]. Anti-HSP65 might be prognostic because its concentration appeared to predict 5-year mortality in patients with carotid atherosclerosis [98]. Wysocki *et al.* showed that anti-HSP60 was significantly higher in ACS versus controls [151]. Giuseppina *et al.* showed that HSP60 may be an independent predictor of post-MI adverse cardiovascular events [152].

2.2.4 Atherogenesis and HSP70

HSP70 (prokaryotic homologue: DnaK (*E. coli*)) is involved in protein folding [3]. In the rat, serum HSP70 was related to the extent of experimentally induced MI [155]. Larger infarct after focal ischemia and more abundant apoptotic cell death after transient focal ischemia were associated with lower HSP expression. Previous studies have shown a strong positive correlation between plasma anti-HSP60, -65, -70, and -72 with CVD [167]. HSP70 expression was associated with the extent (thickness) of the atherosclerotic plaque. HSP70 distribution in the human aorta showed a homogeneous staining pattern in "normal-appearing" regions, but a heterogeneous pattern in areas of atherosclerosis. It was reported that the heterogeneous pattern of HSP70 distribution in atherosclerotic lesions may be due to the leakage of HSP70 from damaged cells into the plaque [126]. Synthesis of HSP normally protects cells from death, but it has been hypothesized that insufficient HSP70 in SMC of the aorta leads to their death and further promotes plaque

rupture and thromboembolic complications [189]. Although HSP70 expression was increased in advanced atherosclerotic lesions and several cell types (monocytes, macrophages, DC, and SMC), only DC expressed high levels of HSP70 in early lesions [70].

Pockley *et al*. reported that HSP70 expression was localized to the center of thickened atheromatous plaques and that staining intensity correlated with atherosclerotic plaque thickness [95]. Several cross-sectional studies [95,156] have reported that HSP70 may have an atheroprotective role, a finding that may be mediated by its effect on SMC survival. It was subsequently shown that location of HSP70 expression changed during plaque evolution and was positively associated with severity of atherosclerosis and altered protein distribution [158].

Although increased serum HSP70 was associated with a lower risk of CVD independent of established CVD risk factors, some induced HSP70 expression by endothelial cells and SMC. Zhu *et al*. have also reported that the severity of coronary disease (number of diseased vessels) was inversely related to serum HSP70 [157]. Increased HSP70 was reported in patients with chronic heart failure [159]. Because HSP represented vessel wall cellular response to stress-inducing factors, including several classical atherosclerosis risk factors, these may have roles in atherogenesis [190]. Interestingly, increased HSP70 was associated with low CVD risk, suggesting a complex role for these proteins in atherosclerosis [180].

2.3. Molecular mimicry

The potential for cross-reactivity of the immune response to bacterial HSP with hHSP60 expressed on stressed endothelial cells has been proposed as a possible mechanism for the early atherogenic involvement of HSP60/65 in atherogenesis. Infections have been implicated in CVD [136]. The fact that microorganisms produce HSP homologous to human HSP has given rise to the concept of molecular mimicry [61]. A high degree of sequence identity between human and microorganism HSP is one potential mechanism to explain its association with infection, high HSP expression, autoimmunity, and CVD [126]. GroEL (a bacterial HSP) is highly immunogenic [128]. It has been proposed that GroEL immune response cross-reacted with hHSP60 expressed on endothelial cells leading to endothelial dysfunction and atherogenesis [126]. The immune system may not be able to differentiate HSP of human and bacteria origin and thus molecular mimicry may provide a mechanism for autoimmunity (Fig. 2.3).

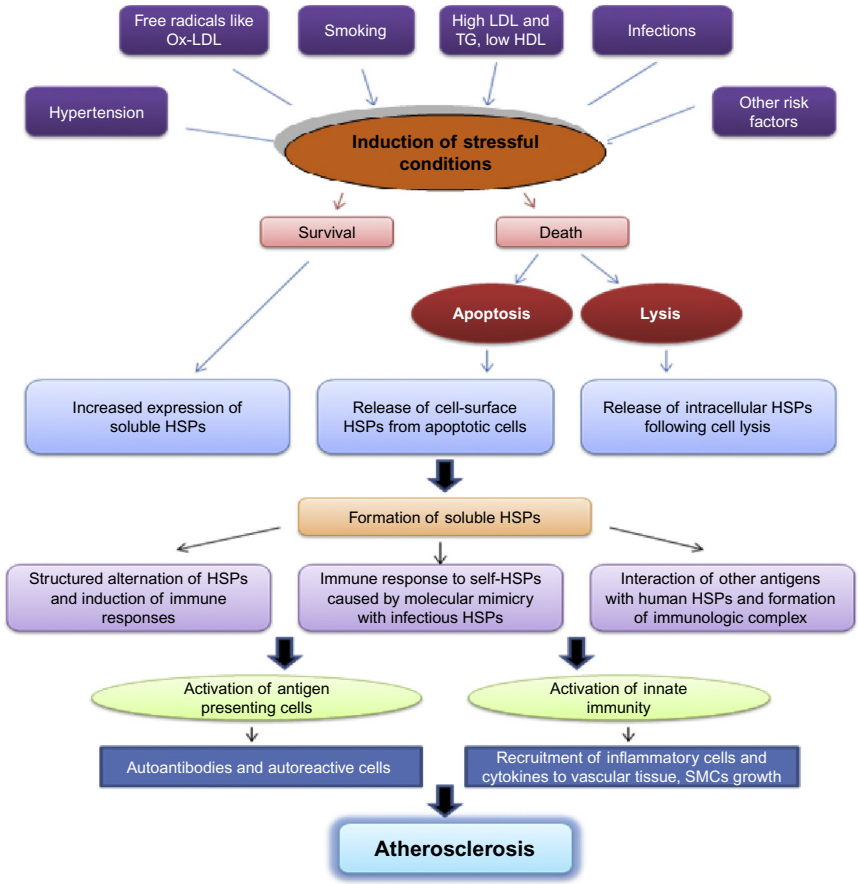

Figure 2.3 Possible mechanisms of involvement of HSP in atherosclerosis.

It has been reported that cellular immunity against HSP60 is related to I-M thickness in young male individuals but not in the elderly, suggesting a possible role of specific cellular immunity to HSP60 in early stages of atherosclerosis [191]. However, these results were not consistent with those of the Bruneck study [192] which showed no relationship between circulating HSP60-specific T cells and late stages of atherosclerosis. Moreover, Ramage *et al.* have reported that the proliferative response of human T lymphocytes to highly purified hHSP60 was confined to the adult CD45RA− RO+ naïve subset, whereas both memory and naïve T-cell populations proliferated in response to bacterial HSP60 [193]. In patients with different autoimmune conditions and those with established atherosclerosis, increased

T cell response to microbial HSP65 (mHSP65) as well as increased circulating anti-mHSP65 and anti-HSP60 have been reported [194]. These immune responses may initially be directed against antigens of pathogenic organisms and then cross-react with homologous host HSP elaborated by vascular wall cells, including endothelial cells [186]. Anti-HSP may lead to endothelial injury by antibody-dependent complement-mediated cellular cytotoxicity, whereas anti-HSP, probably produced for the primary purpose of eliminating infectious organisms, may lead to endothelial injury. Mayr *et al.* have reported that serum anti-HSP to *E. coli* and *C. pneumoniae* can mediate endothelial cell lysis of stressed, but not unstressed endothelial cells [126]. Alternatively, these immune responses may be induced following exposure of HSP on the surface of infected endothelial cells [61]. In atherogenesis, SMC have an important role and can be induced to express HSP as part of a survival mechanism following exposure to a variety of stressors including high blood pressure. HSP have an important role in the function of cells involved in the immune system. Because of their distribution and homologous structure in different species, they may serve as autoantigens. A positive relationship of HSP immune response with atherosclerosis has been observed in rabbit models [110]. The possible mechanisms by which HSP may be involved in atherosclerosis are summarized in the figure (Fig. 2.3). Anti-HSP60 and 65 were positively related to cardiovascular morbidity and mortality [136]. Lenzi and coworkers reported that increased anti-HSP60 may constitute a marker of CVD [12]. Studies have shown that CVD risk in patients with a systemic immune response was associated with increased HSP [195].

2.4. HSP and risk factors of atherosclerosis

Although it was hypothesized that increased anti-HSP developed relatively early in the atherogenic process, a prospective study would be required to confirm this. However, increased anti-HSP appears associated with CVD risk factors [196].

In a large community-based study, increased soluble HSP60 correlated with LDL cholesterol [197]. Anti-HSP60 was positively associated with indices of obesity, more so than anti-HSP65 and 70 [58]. A positive association was found between serum HSP60 and various psychosocial measures including low socioeconomic status, social isolation, and psychological distress [198].

HSP27 and its antibody concentration were related to cardiovascular complications in patients with glucose intolerance [199]. It was reported that

anti-HSP27 IgG was strongly associated with age, gender, and hypertension, but weakly with diabetics with ACS [196]. Increased anti-HSP70 was also reported in hypertensive men versus normotensive controls [200].

Figueredo *et al.* measured class-specific antibodies to mycobacterial HSP70 in patients with diabetes mellitus with or without vascular complications [201]. IgG and IgM in diabetic patients did not significantly differ from healthy control subjects or between types of diabetes, regardless of gender, disease duration, hemoglobin A1c, or type of vascular complication. Mean serum IgA in type 2 diabetics was, however, significantly higher than matched controls. This result suggested a possible role of anti-HSP70 IgA in the pathogenesis of the vascular complications in diabetes mellitus [202].

Pockley *et al.* determined HSP and anti-HSP in subjects with established hypertension [203]. HSP60 and 70 antigen and anti-HSP60 were similar in hypertensive and normotensive controls, whereas anti-HSP70 and anti-HSP65 were increased. HSP60 was not associated with atherosclerosis. Anti-HSP70 and anti-HSP65 were both associated with hypertension, independent of age, smoking status, and blood lipids.

Kervinen *et al.* studied middle-aged men with MI or coronary death during an 8.5-year follow-up [204]. Control individuals were subjects without coronary events and matched for drug treatment and geographic region. Although anti-HSP60 IgA predicted coronary risk, the effect was modest without simultaneous occurrence of other classical risk factors.

The relationship between HSP and risk factors of CVD is summarized (Table 2.3).

Table 2.3 HSP and risk factors of CVD

Subjects	Findings	References
Subjects with dyslipidemia	Anti-HSP60, 65, and 70 are significantly increased in dyslipidemic patients	[205]
Diabetic patients	Positive relationship between anti-HSP70 and type-2 diabetes	[202]
Hypertensive patients	Positive relationship between anti-HSP65 and 70 and hypertension	[203]
Patients with CVD	Positive relationship between anti-HSP27 and total cholesterol	[169]
Dyslipidemic middle-aged men	Negative relationship between anti-HSP60 and HDL cholesterol	[206]

2.5. The role of HSPs in other common CVDs

2.5.1 Cardiac hypertrophy

During the development of cardiac hypertrophy, cardiac chaperones such as HSP70 and 22 are found to have increased expression. Angiotensin II infusion, isoproterenol infusion, and swimming were identified as hypertrophic stimuli of HSP70 in an animal model study [207]. Cardiac hypertrophy is a response to a number of stress stimuli. In cardiac hypertrophy, a general increase in cardiac chaperone expression was not surprising, but the reasons for this finding are poorly understood [208].

It appears that HSP70 is necessary for the induction of cardiac hypertrophy. Overexpression of a dominant-negative form of HSP70 or decreased histone deacetylase 2 with small interfering RNA blunted the hypertrophic response in the heart [207]. In mice, cardiac hypertrophy induced by isoproterenol infusion or aortic banding resulted in a blunted histone deacetylase 2 activity suggesting a role for HSP70 in the induction of cardiac hypertrophy possibly by stabilization of histone deacetylase 2.

The histone deacetylases (HDACs) are key enzymes in the regulation of hypertrophic gene expression [209]. Class II HDAC (HDAC4, HDAC6, HDAC7, and HDAC9) appear to negatively regulate hypertrophy by repressing myocyte enhancer factor/GATA/NFAT-mediated gene transcription [210].

During development of cardiac hypertrophy, HSP20 was increased, and this may lead to activation of signaling pathways involved in survival and cell growth, including PI3K/Akt, AMP kinase, protein kinase C, nitric oxide, and mTOR [201]. These appear associated with several cardioprotective mechanisms including preconditioning, growth, and protection against apoptosis [201].

In a variety of animal models, HSP22 was increased during development of cardiac hypertrophy and in cultured cardiomyocytes and intact mouse heart [211,212]. Increased HSP22 expression resulted in the development of a spontaneous hypertrophy characterized by reexpression of the fetal gene program.

2.5.2 Heart failure

Knowlton *et al.* investigated HSP90, 72, 70, 27, and 60 expression in cardiomyopathy and ischemic cardiomyopathy patients [213]. HSP72, 70, and 90 were unchanged versus normal controls. A twofold increase in heart HSP27 expression was, however, noted in cardiomyopathy.

HSP60 contains NF-κB binding elements. HSP72 does not. This may explain why HSP60 expression was increased in heart failure, whereas other HSP were not [214].

Using a rat model of heart failure, increased HSP72 and 27 expression was noted [215]. HSP60 expression was not changed.

Using the same model, Tanonaka *et al.* reported that increased myocardial HSP60 expression was associated with subsequent development of heart failure [204].

The presence of HSP60 in the blood of normal individuals was first reported in 1999 [216]. HSP60 distribution in the myocardial cells changes in dilated cardiomyopathy and ischemic heart disease by translocation from the cytosol to the mitochondria [217].

In explanted failing hearts, HSP60 was localized to the plasma membrane where it was associated with increased apoptosis [110]. Its interaction with other cells may trigger the innate immune response resulting in release of proinflammatory cytokines such as TNF. This finding may imply that HSP60 is an early effect or molecule that induces myocyte loss and contributes to heart failure. The role of circulating HSP60 in the pathophysiology of heart failure has not been clearly delineated and more research is needed [218].

It has been hypothesized that circulating HSP60 induces inflammation and autoimmune responses that play a role in atherosclerosis [219]. Niizeki *et al.* showed that serum HSP60 was increased in patients with congestive heart failure [220].

2.5.3 Ischemic cardiac injury

A cardioprotective effect for HSPs in ischemia/reperfusion injury has been reported. Ischemia/reperfusion injury resulted in increased HSP70 and HSP90 mRNA [221]. The increase in HSP70 was substantially higher than HSP90. Increased expression was most likely due to a concurrent increase in HSF1 driven by an accumulation of reactive oxygen species [211].

Depre *et al.* investigated the differential expression of genes following ischemia/reperfusion injury in a pig model and found that HSP22 increased significantly after 1 h of reperfusion [212].

In animal studies, increased HSP70 and 72 expression was beneficial following cardiac ischemia/reperfusion injury [222]. HSP72, induced by successive bouts of endurance exercise, led to improved ischemia/reperfusion injury outcome as demonstrated by decreased infarct size and cardiac apoptosis.

In mouse models, cardiac-specific HSP20 overexpression of HSP20 protected against ischemia/reperfusion injury [70]. Improved contractile performance, decreased myocyte cell death, and significantly decreased infarct size were noted.

One of the mechanisms for the protective effect of HSP20 appears to be in activating autophagy, a mechanism critical for ischemia/reperfusion injury [213]. Interestingly, transgenic mice in which serine 16 on HSP20 is mutated, that is, nonphosphorylatable, were more susceptible to ischemia/reperfusion injury than wild-type mice.

3. THERAPEUTIC IMPLICATIONS

HSP may act as an autoantigen that results in vascular injury cycle. As such, tolerization may limit atherogenesis [61]. Bacterial HSP60 is immunologically cross-reactive with its mammalian counterparts. The finding that immune responses to HSP are associated with both experimentally induced and spontaneous autoimmune diseases in animals has prompted intense research to assess the role of bacterial HSP as the etiological agents involved in the development of autoimmune disease [223].

Mucosal administration of HSP has been used to induce a state of immune tolerance. Harats and colleagues have shown that HSP65 tolerization led to reduced plaque formation in a murine model of atherosclerosis. Tolerization was also associated with reduced macrophage and T-cell infiltration and increased expression of the anti-inflammatory cytokine, IL-10 [215]. Some HSPs have been used as carriers for delivering epitopes to the immune system in the absence of adjuvant [224]. Whole pathogen vaccines such as BCG contains a high concentration of potentially immunogenic HSP. While reducing morbidity and mortality, these vaccines may also stimulate proatherogenic mechanisms [225]. Indeed, immunization with BCG vaccine increased atherosclerosis in the cholesterol-fed rabbit. Anti-HSP60 was related to plaque formation suggesting that the specific immune response to BCG-associated HSP might be proatherogenic [110,145]. In atherosclerotic lesions induced by BCG immunization alone in the absence of traditional risk factors, the inflammatory response to HSP60 was insufficient to drive atherogenesis over prolonged period of time [226]. HSP27 may also be a potential therapeutic target. Wolfgang *et al.* suggested that HSP27 had a protective effect against simulated ischemia. They proposed a model in which proteins, not in their final folding state, bind to oligomeric HSP complexes that act as a shelter. After resolution

of ischemia, these proteins are released in their final folded state thereby assuming normal activity in cells recovering from ischemic injury [227]. It has been suggested that serum HSP27 may be a predictive marker of atherogenes which helps to identify individuals who may benefit from estrogen therapy [221]. Targeted HSP27 overexpression in tissues with ischemic injury may also be beneficial. The use of pharmacologic inducers or enhancers of the endogenous HSP expression in the target cells can be used. For example, herbimycin A and geranylgeranylacetone (GGA), a cyclic polyisoprenoid, induced an acytoprotective potential in simulated ischemia studies. Oral administration of GGA rapidly upregulated HSP expression in response to a variety of stressors, while its effect was attenuated under non-stress conditions [228]. Oxidative stress, inflammation, and apoptosis were involved in the initiation, development, and rupture of atherosclerotic plaques. The role of HSP in treatment of CVD continues to receive much attention [3].

Oral administration of HSP65 and β2-glycoprotein inhibited development of atherosclerosis in experimental models [56].

Induction of immune tolerance through activation of the mucosal immune response, a novel immunomodulating therapy to prevent atherosclerosis, warrants further evaluation [229]. GGA protected the gastric mucosa by inducing HSF1 and HSP70 mRNA [222] and has recently been shown to be cardioprotective by inducing HSP72 [230].

Arimoclomol, a small molecule that induces HSF1, has been shown to increase HSP70 and 90. Interestingly, arimoclomol is currently in phase II/II clinical trials as a treatment for amyotrophic lateral sclerosis [231].

Celastrol is a triterpenoid compound with a retinoid skeleton used in traditional Chinese medicine. This drug potently induces HSF1 and HSP70 expression with antioxidant and anti-inflammatory properties [232].

Statins have many benefits including modulation of the immune system, reduction in apoptosis, and nitricoxide production. Simvastatin and lovastatin induced HSP27 in an osteoblast-like cell line [233].

4. HSPs AS PREDICTORS OF CVD

Although HSPs are present in the sera of healthy individuals and increased in early CVD, their value for predicting atherosclerotic risk has not been comprehensively evaluated.

Pockley *et al.* studied subjects with established hypertension over a 4-year follow-up [95]. Atherosclerosis was assessed by measuring common

carotid artery I-M thickness. Increased I-M thicknesses at follow-up was less prevalent in subjects with increased serum HSP70 (75th percentile) at the time of enrollment. A similar trend was observed for serum HSP60, but was not statistically significant. Anti-HSP was not associated with I-M thickness. The relationship between HSP70 and I-M thickness was independent of age, treatment with atenolol or lacidipine, smoking history, and blood lipids. These findings indicated that circulating HSP70 predicted development of atherosclerosis in established hypertension. An intriguing possibility is that HSP70 protected against or modified atherosclerotic progression [95].

Zhu *et al.* investigated whether combining some risk factors (pathogen burden, increased CRP, and anti-HSP60) enhanced CVD risk. Their results indicated that CVD risk prediction can be improved by combining these three risk factors [234].

Okada *et al.* analyzed the prevalence of the serum IgG antibodies against *H. pylori*-derived HSP60 or its peptide fragments in patients with CVD as compared to those in age- and gender-matched non-CVD patients. Results strongly suggested that IgG against *H. pylori*-derived HSP60 cross-reacted with hHSP60 and that these represented independent CVD markers [235].

Increased anti-HSP65 was associated with subsequent cardiovascular events in a cohort of men with documented coronary artery disease [236]. Xu *et al.* found that increased anti-HSP65 was sustained in persons with severe and progressive carotid disease and was an independent predictor of mortality at 5 years [187]. Gromadzka *et al.* found increased anti-HSP65 and anti- HSP70 after ischemic stroke and suggested that these antibodies were independent risk factors for stroke [237].

Jin *et al.* have reported that anti-HSP70 was a risk factor for ischemic stroke and may be a marker for neuroprotection at early stages [238]. Many mechanisms have been suggested for HSP70 including defense against apoptotic and necrotic cell death in cerebral ischemia [239].

5. IMMUNIZATION AND VACCINATION WITH HSP

Currently, there are several clinical trials evaluating autologous cancer-derived HSP–peptide complexes for treatment of carcinoma and melanoma [240]. Some human vaccines, such as BCG, contain HSP. Although vaccination programs are necessary for maintaining "herd" immunity and the prevention of serious infection, they may lead to increased

susceptibility to atherosclerosis. HSP-free vaccines could satisfy the goals of protection from infection and also reduced incidence of CVD [240].

BCG vaccines were first used against human tuberculosis in France in 1921, and have been widely used since the 1950s following recommendations by the World Health Organization [241]. BCG vaccination elicited an immune response to HSP, and in young people with preexisting atherosclerotic lesions, there may be an exacerbation of the disease process [242].

BCG vaccine immunization, which contains HSP, also increased the extent of atherosclerosis in the cholesterol-fed rabbit. Anti-HSP60 in BCG immunized rabbits was correlated with atherosclerotic plaque formation suggesting that the specific immune response to BCG-associated HSP might be proatherogenic [110,145]. Atherosclerotic lesions induced by BCG immunization in the absence of traditional risk factors, such as hypercholesterolemia, tend to regress with time, which suggest that the inflammatory response to HSP60 in the absence of other CVD risk factors, is not enough to drive atherogenesis over long period of time (Fig. 2.4) [226].

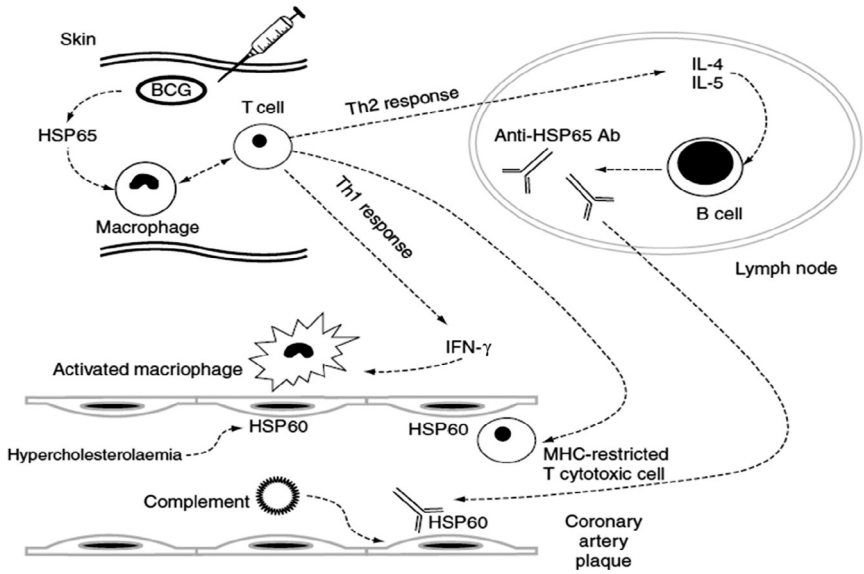

Figure 2.4 HSP65 from BCG is taken up by tissue macrophages within the dermis which present HSP65-derived peptides with class II MHC molecules to either type 1 (Th1) or type 2 (Th2) [186]. *Publisher and year of copyright: Elsevier, 2002. Permission for reproduction/adaptation was granted by the copyright holder.*

6. EFFECTS OF THERAPEUTIC INTERVENTIONS ON HSP

There have been a few reports on the effects of drugs, herbal medications, and acupuncture on HSP and anti-HSP.

6.1. Herbal medicines

Curcumin extracted from dried rhizomes of the herb *Curcuma longa* (turmeric) and administered (1 g/day) for a period of 30 days had no significant effect on serum anti-HSP27 [243]. Subjects with metabolic syndrome were treated with saffron (100 mg/day) or placebo for 12 weeks and anti-HSP27, 60, 65, and 70 were determined. Saffron was found to decrease anti-HSP27 and 70.

6.2. Statin therapy

Moohebati *et al.* evaluated the effects of statin therapy on serum HSP antibodies in dyslipidemic patients [86]. In this study, 102 subjects were treated with simvastatin (40 mg/day) in a placebo controlled cross-over trial. Simvastatin decreased anti-HSP60, 65, and 70.

7. CONCLUSION

There is good evidence that HSPs are involved in atherogenesis in man and animal models. Although there are a number of options to modulate HSP, it is unclear whether this approach will be beneficial. Tolerization may reduce the immune response to HSP and could be an important factor in mitigating atherosclerotic progression. Several drugs and herbal medicines influence anti-HSP, but their effect is modest and unlikely to provide a viable therapeutic option. While promising, these preliminary studies clearly warrant additional and more comprehensive well-controlled prospective studies to clarify the role of HSP and their antibodies in CVD.

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