THE POTENTIAL ROLE OF HEAT SHOCK PROTEINS IN CARDIOVASCULAR DISEASE: EVIDENCE FROM *IN VITRO* AND *IN VIVO* STUDIES

M. Ghayour-Mobarhan,[*,†] A.A. Rahsepar,[*,†] S. Tavallaie,[†] S. Rahsepar,[*,†] and G.A.A. Ferns[‡,1]

[*]Cardiovascular Research Center, Avicenna Research Institute, Mashhad University of Medical Science (MUMS), Mashhad 91376-73119, Iran
[†]Department of Nutrition and Biochemistry, Faculty of Medicine, MUMS, Mashhad 91376-73119, Iran
[‡]Postgraduate Medical School, University of Surrey, Guildford, Surrey GU2 7WG, UK

[1]Corresponding author: GAA Ferns, e-mail: g.ferns@surrey.ac.uk

0065-2423/09 $35.00
DOI: 10.1016/S0065-2423(09)48002-8

1. Abstract

The heat shock proteins (HSPs) are highly conserved families of proteins expressed by a number of cell types following exposure to stressful environmental conditions. These conditions include several known risk factors for cardiovascular disease. A number of the HSPs have been shown to be molecular chaperones that are involved in the refolding of other damaged protein molecules. Over the past two decades there has been an increasing interest in the relationship between HSPs and cardiovascular disease, and particularly whether an autoimmune response may be implicated. The fact that microorganisms also produce HSPs, and that these are homologous to human HSPs has given rise to concept of molecular mimicry. While most of the past studies have focused on HSP 65 and 70, there has been recent interest and investigations of the possible role of the smaller HSPs, such as HSP27, in atherogenesis. Furthermore, the possibility that autoimmunity may be mediating the deleterious effects of HSPs has led some investigators to explore tolerization as a potential therapeutic approach.

2. Introduction

2.1. Discovery of the HSPs, Their Classification and Their Functions

Approximately four decades ago, Ritossa and colleagues [1] observed that exposing larval salivary glands from Drosophila to heat induced specific genes in the giant chromosomes of the gland cells; it is now known that these genes encode proteins called HSPs. The HSPs are highly conserved families of proteins found in the cells of all organisms and several of them are known to function as molecular chaperones. The HSPs may be divided into seven major families according to their molecular weights: HSP10, small HSPs (15–30 kDa), HSP40, HSP60, HSP70, HSP90, and HSP100 (Table 1). HSP expression is increased in response to several environmental stresses in addition to heat stress; these include: certain forms of nutritional deficiency, oxidative stress, and ultraviolet radiation. This is mediated by the release of heat shock factor 1 and its binding to heat shock elements in the flanking regions of the HSP genes [2] (Fig. 1). Moreover, in addition to their role as chaperones, HSP have other putative roles [3–6]. Table 1 shows a summary of their functions.

2.2. Atherosclerosis

Atherosclerosis is a chronic multifactorial disease that underlies the pathophysiology of cardiovascular disease (CVD), stroke and peripheral vascular disease (PVD), and is the major cause of mortality worldwide [7, 8]. It is

TABLE 1
Summary of the Nomenclature, Location, and Function of the Major Heat Shock Protein Families

Family	Organism	HSP-related proteins	Location	Functions
Small HSPs	*E. coli*	Lbp A and B	Cytosol	Suppresses aggregation and heat inactivation of proteins *in vitro*; confers thermotolerance through stabilization of microfilaments; antiapoptotic activity
	S. cerevisiae	HSP27	Cytosol	
		A and B crystallin	Cytosol	
		HSP27	Cytosol	
Hsp40	*E. coli*	DnaJ	Cytosol	Essential cochaperone activity with Hsp70 proteins to enhance rate of adenosine triphosphatease activity and substrate release
	S. cerevisiae	Ydj 1	Cytosol/nucleus	
	Mammals	Hdj 1 and Hdj 2		
Hsp60	*E. coli*	GroEL	Cytosol mitochondria	Refolds and prevents aggregation of denatured proteins *in vitro*; may facilitate protein degradation by acting as a cofactor in proteolytic system; role in the assembly of bacteriophages and Rubisco (an abundant protein in the chloroplast)
	S. cerevisiae	HSP60	Chloroplasts mitochondria	
	Plants	Cpn60		
	Mammals	HSP60		
Hsp70	*E. coli*	DnaK	Cytosol	Roles in lambda phage replication; autoregulation of the heat shock response; interaction with nascent chain polypeptides; functions in interorganellar transport; roles in signal transduction; refolds and maintains denatured proteins *in vitro*; role in cell cycle and proliferation; antiapoptotic activity; potential antigen-presenting molecule in tumor cells
	S. cerevisiae	Ssa 1–4	Cytosol	
		Ssb 1,2	Cytosol	
		Kar2	ER mitochondria	
		Ssc1		
	Mammals	HSC70	Cytosol/nucleus	
		HSP70	Cytosol/nucleus	
		BIP	ER mitochondria	
		MHSP70		
Hsp90	*E. coli*	HtpG	Cytosol	Role in signal transduction (e.g., interaction with steroid hormone receptors, tyrosine kinases, serine/threonine kinases); refolds and maintains proteins *in vitro*; autoregulation of the heat shock response; role in cell cycle and proliferation
	S. cerevisiae	HSP83	Cytosol	
	Mammals	HSP90	Cytosol	
		GRP94	ER	
Hsp100	*E. coli*		Cytosol	Role in stress tolerance; helps the solubilization of heat-inactivated proteins from insoluble aggregates
	S. cerevisiae		Cytosol	

HSP, heat shock protein; *E. coli*, *Escherichia coli*; *S. cerevisiae*, *Saccharomyces cerevisiae*; ER, endoplasmic reticulum.
Modified from Lamb *et al*. [2]. Publisher and year of copyright: Elsevier, 2002.

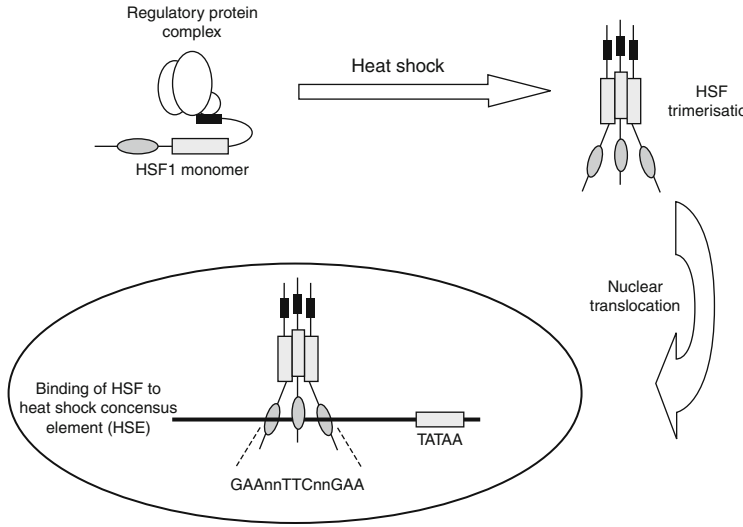

FIG. 1. Schematic representation of the regulation of mammalian heat shock protein expression. HSF, heat shock transcription factors; HSE, heat shock consensus element; TATAA, DNA sequence containing TATAA repeats. Reference: [2]. Publisher and year of Copyright: Elsevier, 2002. Permission for reproduction/adaptation was granted by the copyright holder.

characterized by the accumulation of lipids and extracellular matrix in the intima of large and medium sized arteries. It is associated with mononuclear cell infiltration, and smooth muscle proliferation [9]. Risk factors for CVD include: age, male sex, family history of CVD, hypertension, hypercholesterolemia, smoking, diabetes mellitus, socioeconomic status, and obesity [9]. There are several emerging risk factors for CVD including markers of oxidative stress, inflammation, and autoimmunity [10].

2.2.1. *Atherosclerosis and the Role of Inflammation*

The inflammatory nature of atherosclerosis was first described in the 1850s [11], however, more recent interest has developed because immunocytochemical studies have allowed the cellular composition of atherosclerotic plaques to be determined and related to the onset of clinical events, such as plaque rupture [12]. Furthermore, inflammatory processes also appear to be involved in atherogenesis [13]. The earliest lesions in atherogenesis, are fatty streaks, and these are commonly found in infants and young children [14]. They are characterized by a relative paucity of lipid accumulation and comparative abundance of intimal inflammatory cells that include activated T lymphocytes (helper, suppressor, and regulator), mast cells, macrophages, dendritic cells [15], and less commonly granulocytes and NK cells [16–18].

Epidemiological studies have supported the role of inflammation in CVD. Serum C-reactive protein (CRP) concentrations have been reported to be a stronger independent predictor of coronary events than low density lipoprotein (LDL) cholesterol levels [19–22]. It has also been reported that elevated levels of soluble intercellular adhesion molecule (ICAM)-I, a marker of endothelial cell activation, are associated with increased coronary risk [23] and its expression is increased in human atherosclerotic lesions [24]. Complement activation [25–27] may play a role in endothelial injury during atherogenesis and may be a consequence of autoimmune responses to modified LDL [28] or denatured HSPs [29]. The expression of human lymphocytic antigen (HLA) class II antigen and secretion of several cytokines, within atherosclerotic lesions supports the involvement of inflammation in atherosclerosis [30]. Advanced atheromatous lesions also contain large numbers of T lymphocytes [30], most of which are T helper (h) type 1 cells bearing alpha/beta receptor [17]. Furthermore, activated T cells bearing gamma/delta receptors are abundant at the earliest stages of atherogenesis [31] and atherosclerosis can be inhibited by depletion of T lymphocytes [32].

Xu *et al.* [33] have suggested that CD4+ cells predominate within the T cell population in early lesions, while Van Der Wal *et al.* [18] have reported an increased CD8/CD4 ratio in both early and late lesions. There is a preponderance of pro-inflammatory Th1 cells expressing IFN-γ and IL-2 compared to Th2 cells producing interleukin (IL)-4, IL-5, and IL-10 [34, 35]. In apolipoprotein E deficient mice it has been reported that Th1-inhibition is associated with a 60% reduction in atherosclerotic lesion area [36]. Regulatory T cells (Treg) are a subpopulation of T cells which exert important regulatory effects on immune function [37–39], Type 1 Treg cells can inhibit immune responses by secreting TGF-β and IL-10 [40, 41], while Th2 cells suppress inflammation and dampen macrophage activity via a broader spectrum of anti-inflammatory cytokines, and may have protective effects against atherogenesis [35, 42–44]. Switching the balance of activity from Th1 to Th2 may therefore be protective in atherogenesis [45]. Depletion of CD4+ and CD8+ T cells has been reported to reduce the formation of fatty streaks in C57BL/6J mice [46], which supports the importance of T cells in atherogenesis. However, there remains controversy about the precise role of cellular immunity in atherogenesis as some studies have shown that immune-suppression may result in enhanced atherogenesis in experimental models [47, 48].

2.2.2. *Atherosclerosis and the Role of Infection*

Several studies have shown a positive association between the degree of atherosclerosis burden and presence of chronic infectious microorganisms [19, 49], these include: the Herpes group of viruses, notably *Cytomegalovirus* (CMV) and *herpes simplex virus type 1* (HSV-1) [50], *Helicobacter (H) pylori*

[51], *Chlamydia (C) pneumonia* [52], *Hepatitis A virus* (HAV) [53], and infectious organisms that give rise to gingivitis [54]. These infective processes may exert a pro-atherogenic effect in early life. Pesonen and coworkers [55] have shown that in young children, the presence of antibodies to several microorganisms was positively associated with carotid intimal thickening, a marker of atherosclerosis. It has been proposed that infection acquired during childhood may lead to atherosclerosis in later life [56]; and it has been reported that there is a positive association between the number of infectious organisms a person has been exposed to and the extent of CVD [57] (Fig. 2). Splenectomy is associated with an increased susceptibility to both infection by organisms such as *C. pneumonia* and more severe atherosclerosis [58–60]. Individuals with chronic infections have high serum levels of HSP60, which are also associated with severity of atherosclerosis [61]. The potential mechanisms by which infections may induce atherosclerosis and their interaction with other pro-inflammatory processes is shown in Figs. 3 and 4.

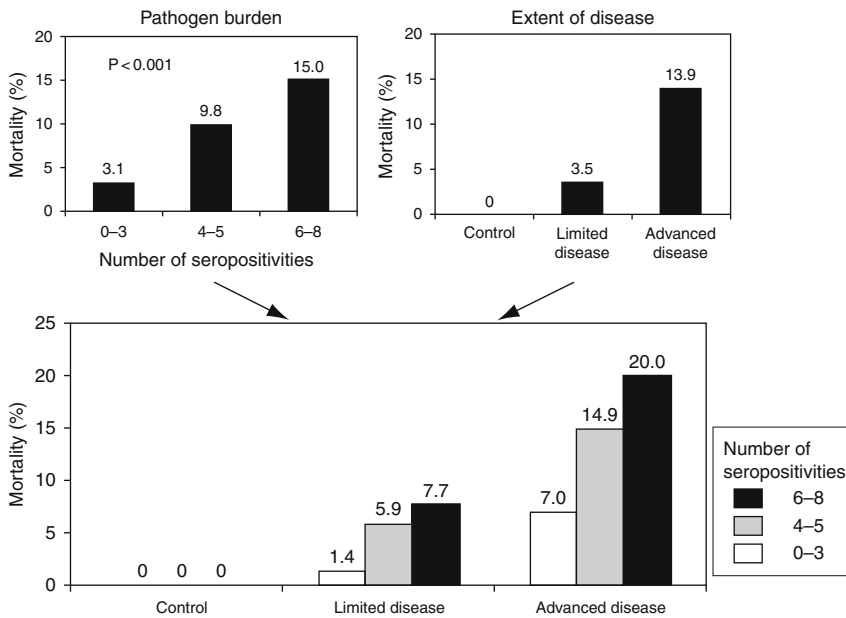

FIG. 2. Cardiovascular mortality rate according to pathogen burden and extent of atherosclerosis. Reference: [57]. Publisher and year of Copyright: American Heart Association, 2002. Permission for reproduction/adaptation was granted by the copyright holder.

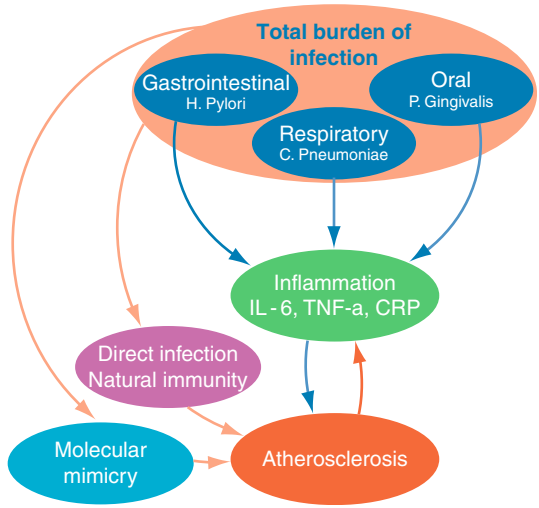

FIG. 3. Possible mechanisms of infection-induced atherosclerosis. Reference: [234]. Publisher and year of copyright: Faculty of Dental Practitioners, 2007. Permission for reproduction/adaptation was granted by the copyright holder.

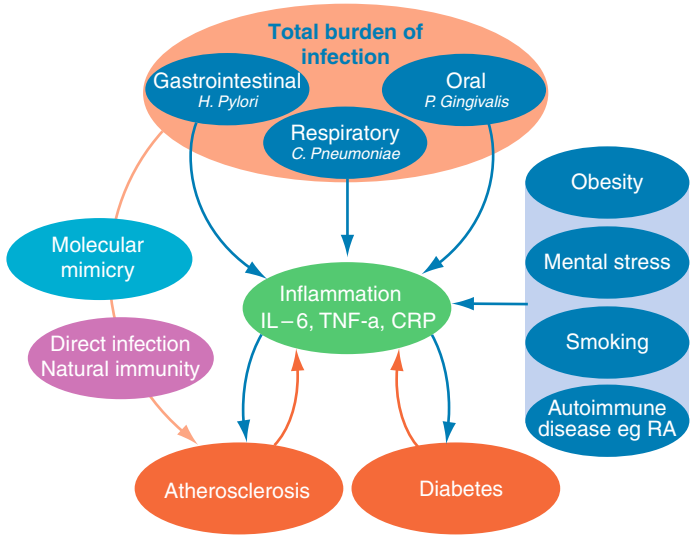

FIG. 4. Chronic inflammatory conditions such as smoking, stress, obesity, and rheumatoid arthritis may contribute to the total burden of inflammation and hence to atherosclerosis. Reference: [234]. Publisher and year of copyright: Faculty of Dental Practitioners, 2007. Permission for reproduction/adaptation was granted by the copyright holder.

2.2.3. *Autoimmunity in Atherogenesis*

Antigen presenting cells (APCs) such as macrophages and dendritic cells have been identified within atherosclerotic lesions, and autoantibodies are present in the serum of individuals with atherosclerosis, and hence it has been proposed that an autoimmune reaction may be initiated within atherosclerotic plaques [62]. Wick and colleagues [63] 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. Several other potential autoantigens have now been identified including modified LDL (oxidized LDL and malondialdehyde modified LDL) and beta-2-Glycoprotein-I [64].

3. HSPs and Atherogenesis

The potential relationship between serum HSPs, HSP antibody concentrations, and CVD was initially explored in the early 1990s [17, 65, 66]. Since then most interest has focused on HSPs-60 and-70 and there have been a number of cross-sectional and cohort studies investigating the relationship between antigen and antibody concentrations in coronary and PVD [67–69]. The expression of HSP-60 and-70 in atherosclerotic lesions was first reported by Kleindienst *et al.* [17] and Berberian *et al.* [65]. HSP60 expression was found to be highest in the shoulder regions and around the necrotic core of atherosclerotic plaques [70] (Fig. 5). Pockley and colleagues [66] demonstrated that HSP60 and HSP 60 antibodies were present in the circulation of normal individuals and later studies have shown a positive relationship between serum HSP-60 and atherosclerosis burden [71–73], particularly in the early stages of disease [74].

Expression of HSP70 was shown to be most concentrated in the center of thickened atheromatous plaques; the intensity of HSP70 staining was reported to correlate with the thickness of the atherosclerotic plaque. HSP70 appears to have an athero-protective role, as indicated by several cross-sectional studies [75, 76]; this may be mediated by its effect on the survival of smooth muscle cells (SMCs). It was subsequently shown that the localization of HSP70 expression changed during plaque evolution and was positively associated with severity of atherosclerosis and the altered patterns of HSP70 staining [77]. In advanced atherosclerotic lesions, HSP70 was found to be expressed by several cell types including SMCs, dendritic cells, and monocyte/macrophages, while in early atherosclerotic lesions only dendritic cells expressed it [78].

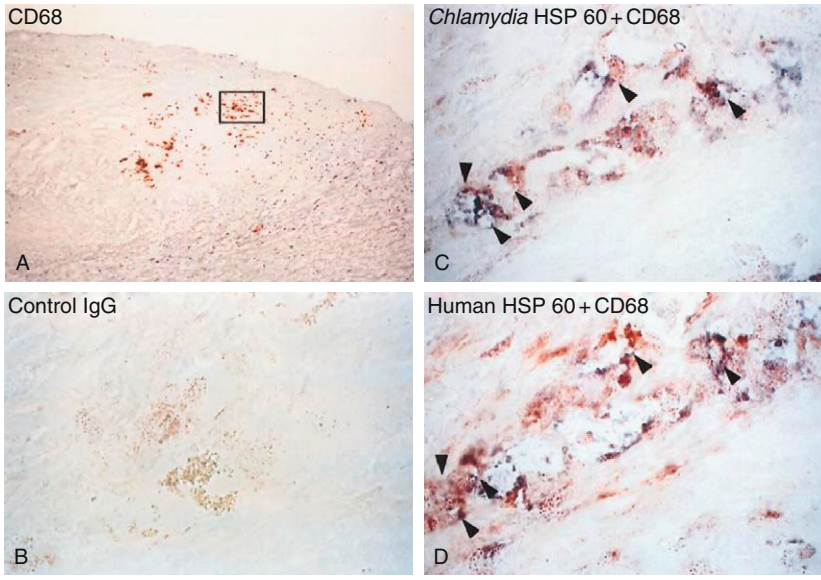

FIG. 5. Co-localization of Chlamydial and human HSP 60 and macrophages in human atherosclerotic lesions. Reference: [70]. Publisher and year of copyright: American Heart Association, 1998. Permission for reproduction/adaptation was granted by the copyright holder.

An increased serum HSP70 concentration is reported to be associated with a lower risk of CVD [79], which was found to be independent of CVD risk factors, although it is known that some of these risk factors can induce HSP70 expression by ECs and SMCs [80]. It has also been reported that the severity of coronary disease (number of diseased vessels) is inversely related to serum HSP70 concentrations [79], although elevated levels of HSP70 have been found in patients with chronic heart failure [81]. Studies have also reported a cellular and humoral response to HSP65 in humans with carotid and coronary atherosclerosis [82, 83].

Cellular immunity directed against HSP60 was found to be related to intima: media thickness in young male individuals but not in the elderly, suggesting a possible role of specific cellular immunity to HSP60 in the early stages of atherosclerosis [84]. However, these results are not in accord with the Bruneck study [85, 86], which showed no relationship between circulating HSP60-specific T cells and late stages of atherosclerosis. However, Ramage *et al.* [87] have reported that the proliferative response of human T lymphocytes to highly purified hHSP60 is confined to the adult $CD45RA^{-}$ RO^{+} naïve subset, whereas both memory and naïve T cell populations proliferated to bacterial HSP60. Increased T cell responses to microbial HSP65 (mHSP65) as well as

raised levels of circulating anti-mHSP65 and HSP60 antibodies have been found in patients with different autoimmune conditions, and in patients with established atherosclerosis [88]. The possible mechanisms by which HSPs may be involved in atherosclerosis are summarized in Fig. 6.

3.1. HSPs and Animal Models of Atherogenesis

The effects of HSPs have predominantly been studied in LDL-receptor-deficient and apolipoprotein E knockout mice and cholesterol-fed rabbits. The LDL-receptor deficient (LDL-RD) mouse develops significant atherosclerosis when fed a high fat diet [89], while apolipoprotein E knockout mice spontaneously develop hypercholesterolemia with concomitant atherosclerosis [90, 91], although they are also often fed an atherogenic, high fat diet. Arterial injury models have been used in mouse, rat, and rabbit and are associated with the rapid development of intimal lesions that are SMC rich.

3.1.1. *Mouse and Rat*

A potential protective role of HSP70 is indicated by the ability of HP70 administration to limit infarct size following the exposure of the heart to ischemia–reperfusion injury in the rat [92] and rabbit [93]. In the LDL-R knockout mouse fed a normal diet, immunization with HSP65 or with heat-killed Mycobacterium tuberculosis develop atherosclerosis more rapidly than control animals [94]. Moreover, lesion formation was also enhanced in wild-type C57BL/6J mice similarly immunized with HSP65 or mycobacterial HSP65 [95], and in the rat model of arterial injury [96], neointimal thickening has been reported to be increased following immunization with mHSP65 [97].

Antibodies directed against, and lymphocytes reactive to HSP65 have been shown to promote fatty-streak formation in LDL-RD mice, providing further evidence for the pro-atherogenic potential of cellular and humeral immunity to HSP65 [98]. In a murine model that combines hyperglycemia with diet-induced hyperlipidemia, the accelerated atherosclerotic process has been reported to be associated with a significant immune response to HSP65 and elevated levels of anti-HSP65 [99].

Further evidence supporting the role of an autoimmune response to HSP in atherogenesis comes from experiments in which HSP60 autoreactive T lymphocytes were transferred to LDL-RD mice and which led to enhanced atherosclerotic changes [98].

3.1.2. *Rabbit*

Immunization of normocholesterolemic rabbits with HSP65 promotes atherosclerotic lesion formation [100], although these lesions regress in the absence of additional risk factors indicating that the inflammatory response

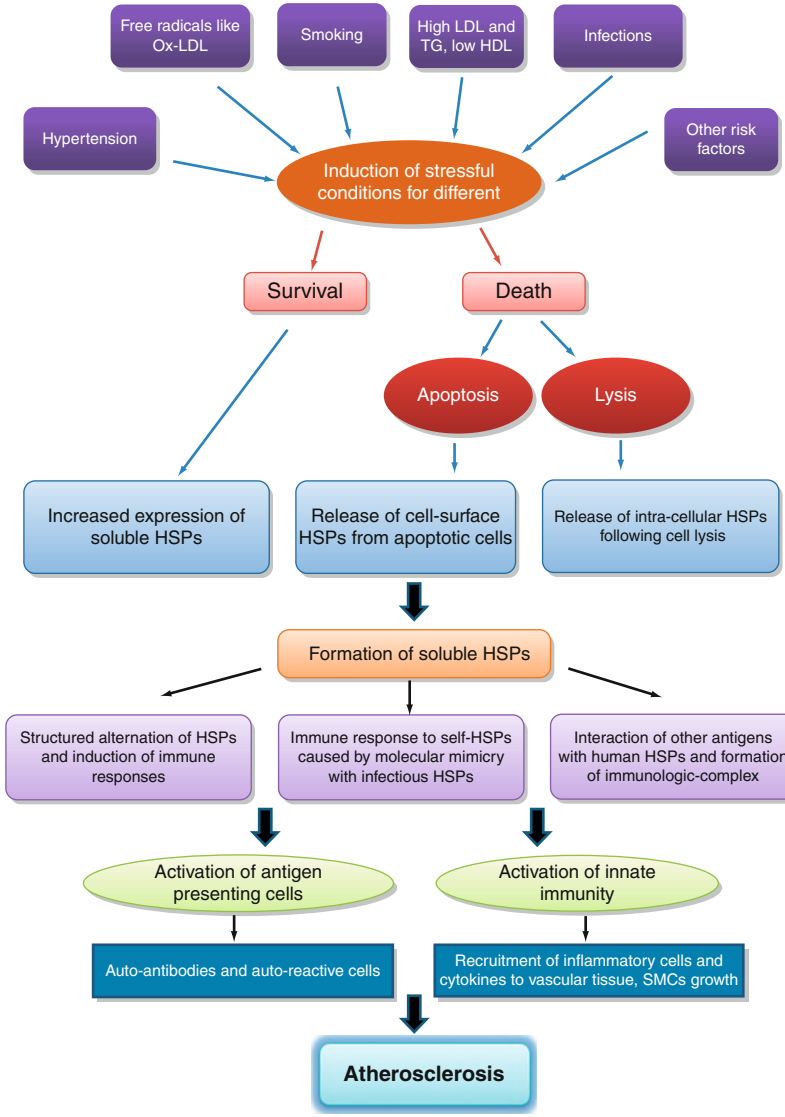

FIG. 6. Possible mechanisms of involvement of HSPs in atherosclerosis. Modified from Reference [133]. Publisher and year of copyright: Elsevier, 2004. Permission for reproduction/adaptation was granted by the copyright holder.

on its own is insufficient to drive atherogenesis over prolonged periods of time [101]. The initial phase of lesion formation in this latter model lacks foam cells and appears to be partially reversible, however in the presence of hypercholesterolemia the lesions develop further [101]. T cells isolated from these lesions were found to respond specifically to HSP 65 *in vitro* [32, 102]. Immunization with the Bacillus Calmette Guerin (BCG) vaccine, which contains large quantities of HSPs, has also been reported to enhance atherogenesis in the cholesterol-fed rabbit [103] and the mechanisms that may account for this are shown in Fig. 7.

Plasma levels of anti-HSP70 increased in both BCG-immunized and control rabbits following the initiation of a high cholesterol-fed diet [104]. We [105] have also demonstrated that a high-cholesterol diet can induce the expression of anti-HSP60, 65 and 70 in rabbits, and this was associated with increasing concentrations of von Willbrand factor (vWF), a marker of endothelial injury (Fig. 8).

It has been reported that depletion of peripheral blood T lymphocytes results in less atherosclerosis in rabbits immunized with HSP60 [32]. Xu *et al.* [102] have found that a population of T lymphocytes isolated from the atherosclerotic lesions of rabbits responded specifically to HSP65; IL-2 expanded T cell lines derived from atherosclerotic lesions, showed a significantly higher HSP-65 reactivity than those from the peripheral blood of the same animal. This finding supports the proposal that HSPs are an important autoantigen recognized in atherosclerotic lesions. Furthermore, T cell lines derived from the lesions of rabbits that were not immunized but only fed cholesterol rich diet, showed hyper-reactivity to HSP65 as compared to T cells from the peripheral blood of the same animals [102]. T cells derived from rabbit atherosclerotic lesions were also found to undergo a strong proliferative response to HSP65 *in vitro* [102]. Table 2 summarizes the animal studies that have investigated the relationship between HSPs and HSP antibodies and atherosclerosis.

3.2. Modulation of HSP Expression in Cells Involved in Atherogenesis *In Vitro*

Several of the cell types involved in atherosclerosis express HSPs, although the factors stimulating their expression vary; for example, HSP60 overexpression by endothelial cells may be modulated by hemodynamic factors, whereas the expression by SMCs and mononuclear cells appears to be driven by the inflammatory process [17]. There is also evidence that HSP60 and HSP70 are expressed on all major cell types in lesion-prone sites during atherogenesis [106].

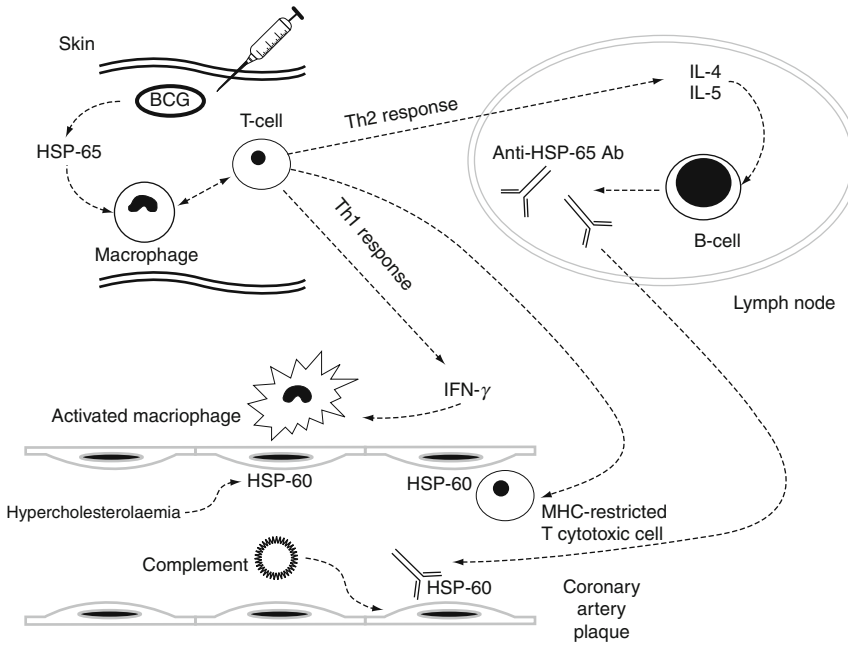

FIG. 7. HSP-65 from BCG is taken up by tissue macrophages within the dermis which present HSP-65 derived peptides with class II MHC molecules to either type 1 (Th1) or type 2 (Th2) T helper cells or T cytotoxic cells. HSP-65 that drains to the lymph nodes is also endocytosed and processed by B-cells who express derivatized peptides in association with MHC class II molecules. Sensitized Th2 cells recognize this complex and secrete interleukin-4 (IL-4) and interleukin-5 (IL-5). These cause the B-cell to differentiate and express HSP-65 specific immunoglobulin. These antibodies cross-react with HSP-60 expressed by endothelium as a result of hypercholesterolemia or other stresses, allowing complement to bind and mediating the lysis of the cell. Activated Th1 cells secrete interleukin-2 (IL-2) and interferon-γ (IFN-γ) which activate macrophages and may increase the activation state of macrophages within plaques. Activated T cytotoxic cells restricted to MHC class I antigen recognition may recognize endothelial HSP-60 where expressed with MHC class I molecules and mediate cell endothelial cell death. Reference: [2]. Publisher and year of copyright: Elsevier, 2002. Permission for reproduction/adaptation was granted by the copyright holder.

3.2.1. *Endothelial Cells*

Endothelial cells are directly exposed to stressors and cardiovascular risk factors present in blood that can lead to endothelial injury. While the subsequent increase in expression of HSPs has potential protective effects it may also have adverse effects. HSPs are expressed on the cell surface [107] and in the presence of cross-reacting anti-mHSP65/-hHSP60 IgG or IgM

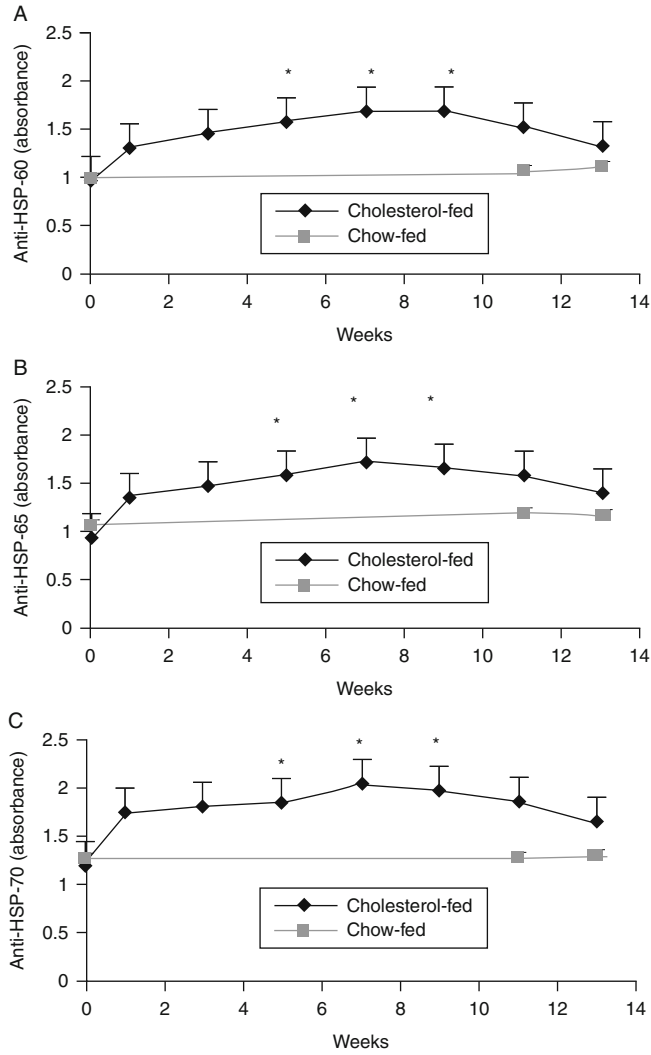

FIG. 8. Time course for changes in plasma anti-Hsp-60,-65, and-70 titers in normal chow and cholesterol-fed rabbits. (A) Antibody titers to Hsp 60 were significant higher for cholesterol compared to normal chow-fed animals during the experimental period ($p = 0.0013$, by ANOVA), also being significantly higher at weeks 5 ($p < 0.05$), 7, and 9 ($p < 0.01$) compared with baseline. (B) Antibody titers to Hsp 65 were significant higher for cholesterol compared to normal chowfed animals during the experimental period ($p = 0.001$, by ANOVA), also being significantly higher at weeks 5 ($p < 0.05$), 7, and 9 ($p < 0.01$) compared with baseline. (C) Antibody titers to Hsp 70 were significantly higher for cholesterol compared with normal chow-fed animals during the experimental period ($p = 0.0016$, by ANOVA), also being significantly higher at weeks 5 ($p < 0.05$), 7, and 9 ($p < 0.01$) compared with baseline. Reference: [105]. Publisher and year of copyright: Blackwell Publishing, 2007. Permission for reproduction/adaptation was granted by the copyright holder.

TABLE 2

ANIMAL STUDIES INVESTIGATING THE RELATIONSHIP BETWEEN HSPS AND HSP ANTIBODIES AND ATHEROSCLEROSIS

Animal model	Aim of the study	Outcome	References
Mouse	Investigation about myocardial protection and changes in gene expression following by whole body heat stress	Increased myocardial HSP70 expression results in protection of the heart against ischemic injury	[92]
	Investigation whether the expressions of HSP60 and HSP70 are correlated with the development of atherosclerotic lesions	HSP60 and HSP70 are temporally expressed on all major cell types in lesion-prone sites during atherogenesis	[106]
	To examine the individual contribution of specific HSPs in primary rat cardiomyocytes to any protection observed following lethal heat stress or simulated lethal ischemia	Transfection of the inducible heat stress protein 70 was found to increase survival following a lethal heat stress and against lethal ischemia	[114]
Rat	To test the hypothesis that the degree of protection from ischemic injury in heat-shocked rats correlates with the degree of prior HSP72 induction	The improved salvage after heat-shock pretreatment may be related to the amount of HSP72 induced before prolonged ischemia and reperfusion	[118]
	To examine whether the overexpression of HSP27 and alphaB-crystallin in rat cardiomyocytes would protect against ischemic injury	The increased expression of HSP27 and alphaB-crystallin protects against ischemic injury in adult cardiomyocytes	[124]
	To test whether phosphorylation of HSP27 is required for the protective role this protein plays in the cell	Phosphorylation of HSP27 seems not to play a role in its ability to protect adult rat cardiomyocytes against ischemic damage	[125]
	Investigation about the possible autoantigens involved in atherosclerosis	Induction of arteriosclerosis in normocholesterolemic rabbits by immunization with HSP65	[100]
	Investigation a possible relationship between HSP60 expression and the antigenic specificities of infiltrating T cells in the lesion	Increased expression of HSP65 coincides with a population of infiltrating T lymphocytes in atherosclerotic lesions of rabbits specifically responding to HSP 65	[102]
	Investigation about the immune mechanisms in atherosclerosis	Regression of arteriosclerotic lesions induced by immunization with HSP65 containing material in normocholesterolemic, but not hypercholesterolemic rabbits	[101]

(*continues*)

TABLE 2 (*Continued*)

Animal model	Aim of the study	Outcome	References
Rabbit	Investigation about the immune mechanisms in atherosclerosis	Inhibition of arteriosclerosis by T cell depletion in normocholesterolemic rabbits immunized with HSP65	[32]
	Investigation about the immune mechanisms in atherosclerosis	Immunization with BCG vaccine increases aortic atherosclerosis in the cholesterol-fed rabbit	[103]
	Investigation about the relationship between the immune responses to HSP and subsequent atherosclerosis	Immune responses to HSP may be implicated in the relationship between specific infections and CVD	[104]
	Investigation the time course of appearance of Hsp-60,-65, and-70 antibodies in the cholesterol-fed rabbit and to relate antibody titers to serum concentrations of von Willbrand factor	In cholesterol-fed rabbits, antibody titers to Hsp-60,-65, and-70 appear to rise in association with a marker of endothelial injury, peaking at approximately the same time after starting a high cholesterol diet	[105]
	Induction of stress proteins, such as heat-shock protein 71 (HSP71), is associated with cardioprotection in isolated ischemic myocardium	Heat shock-induced cardioprotection is transient and delays the onset of irreversible myocardial injury caused by ischemia	[113]

HSP, heat shock protein; CVD, cardiovascular disease.

antibodies may be susceptible to complement-mediated cell lysis [108], or endothelial cell apoptosis [109].

3.2.2. *Smooth Muscle Cells*

SMCs are important players in atherogenesis and can be induced to express HSPs as part of a survival mechanism following exposure to a variety of stressors; for example, exposure to high blood pressure. Berberian *et al.* [65] have reported increased levels of HSP70 expression in human atherosclerotic lesions and showed a protective role for HSP70 in the survival of SMCs, which has been confirmed by others [110]. However, the reported expression of HSPs by SMCs in complex lesions appears to be inconsistent [77]. Furthermore, mechanical stresses evoke rapid activation of HSP70 expression in SMCs [111] and Kleindienst *et al.* [17] showed the presence of HSP60 on SMCs in aortic and carotid specimens.

3.2.3. *Cardiac Myocytes*

HSPs have an important role in protecting myocardial cells from a number of environmental stressors. This has been investigated using *in vitro* and *in vivo* approaches in animal models [112, 113]. For example, it has been shown that overexpression of HSP70 in cultured primary cardiac cells protect these cells against ischemic or thermal stress, while overexpression of HSP60 did not have a protective effect [114–116]. To show the cardioprotective role of HSP70, transgenic mice overexpressing HSP70 were generated and these animals were found to be more resistant to ischemic injury [92, 117]. In rats treated with whole body hyperthermia there was an induction of HSP72 and a reduction of myocardial infarction (MI) size following experimental ischemia [118]. Experimental coronary artery occlusion induces myocardial ischemia and elevation of HSP70 in heart tissues [119], and specifically in myocardial cells [120, 121]. Moreover, in other studies HSP70 has been shown to increase arterial and myocardial cell survival [92, 117, 122] and it has been proposed that HSP70 is associated with protective mechanisms in normal and diseased arteries [123]. Other HSPs also appear to protect cardiac myocytes from ischemic injury. Martin *et al.* [124, 125] reported that both HSP27 and HSP70 were able to protect cardiac myocytes from the effect of ischemia and that decreasing the level of endogenous HSP27 resulted in an enhancement of the damaging effects of a subsequent ischemic stimulus. These findings suggest that HSP27 may also be protective in myocardial cells. The authors propose that plasma HSP27 concentrations could be a potential marker of atherosclerosis, although further validation in larger patient cohorts is required. It has also been suggested that increased expression of HSP27, could be important for cardiac self-protection in cardiac allograft rejection, [126].

3.2.4. *Monocyte/Macrophages*

Monocyte/macrophages are APCs which are able to process and present HSP related peptides to lymphocytes and may be involved in the generation of an autoimmune response associated with atherosclerosis. Lymphocyte activation (particularly Th1 cell stimulation) may enhance the inflammatory process [34, 35]. Immunocytochemical studies of both early and late atherosclerotic lesions have revealed a high level of expression of both HSP60 [17] and HSP70 [65] by macrophages, particularly those adjacent to the necrotic core of advanced lesions. Furthermore, *in vitro* studies have shown that HSPs and anti-HSP antibodies induce the production of pro-inflammatory cytokines by macrophages that may stimulate adhesion molecule expression and thereby further enhance the inflammatory process [71, 127].

3.2.5. *Lymphocytes*

It has been established that atherosclerotic lesions contain large numbers of T lymphocytes [30], and that several types of T cells are involved in modulating the inflammatory response that include helper, suppressor, and regulator cells. It is also hypothesized [102] that HSP65 is an autoantigen which is recognized by these cells, and T cells, isolated from atheromatous plaque appear to be stimulated by HSP65 *in vitro*.

3.2.6. *HSPs and Apoptosis*

HSP27 has been shown to bind to cytochrome C and prevent its interaction with Apaf-1 [128, 129] causing an inhibition of cell apoptosis, HSP70 can also inhibit apoptosis in a caspase independent manner [5] and further that overexpression of HSPs in cardiac myocytes has been shown to inhibit apoptosis [130]. HSP27 induces human monocytes to produce large amounts of the anti-inflammatory cytokines [131] and inhibits toll like receptor-4 (TLR-4) expression on monocytes and their differentiation into dendritic cells [132].

3.3. Soluble or Circulating HSPs

There are a number of possible sources of soluble HSPs in blood, as previously discussed [133]: (1) increased synthesis of HSP by host cells as an immune defense in infectious organisms within the host [134]; (2) release of intracellular HSPs following cell lysis [135]; (3) increased expression of soluble HSPs because of general inflammatory processes, for example, during atherogenesis; (4) release of soluble HSP from necrotic cells within the plaque; (5) and finally the release of cell-surface HSPs from apoptotic cells via the formation of microparticles [136–139]. It is reported that the TLR

4/CD14 complex is a receptor for soluble HSP [140], and hence cell surface bound HSP may also originate from exogenous sources.

Some soluble HSPs may have pro-inflammatory effects; for example, *Chlamydial* and human HSP60 can induce the expression of tumor necrosis factor (TNF)-α and matrix metalloproteinase (MMP)-9 [70], IL-6 [141], IL-12, IL-15 [127]; and exogenous HSP-70 has been reported to upregulate IL-1, IL-6, TNF-α expression in human monocytes [142]. Binding of soluble HSPs to the TLR 4/CD14, stimulates an innate immune response that includes the production of pro-inflammatory cytokines by macrophages and adhesion molecules in endothelial cells via NF-κB activation [140]. These data suggest that soluble HSPs may serve as a danger signal for the innate immune system [143, 144].

4. HSPs and Autoimmunity in Atherogenesis

4.1. General Consideration

The presence of professional APCs such as macrophages and dendritic cells within atherosclerotic plaques provides an opportunity for some plaque-related antigens to become autoantigens. Wick *et al.* [145] have proposed that the accumulation of mononuclear cells within the arterial intima, could be termed the vascular-associated lymphoid tissue (VALT), and may be viewed as being analogous to the mucous associated lymphoid tissue (MALT). They have proposed that the VALT has a similar role as the MALT, which may include monitoring for potentially harmful autologous and exogenous antigenic material contained in the blood. In an attempt to identify which antigens may be implicated in early atherogenesis, Xu *et al.* [100] immunized normocholesterolemic rabbits with a variety of mixed antigens in complete Freund's adjuvant. Surprisingly, all immunized animals were found to develop atherosclerotic lesions at the known predilection sites [100]. The authors proposed that this was due to the mycobacteria-derived HSP it contained. They subsequently immunized rabbits with recombinant purified mycobacterial HSP65. Animals initially developed vascular lesions that did not progress unless they were also fed a cholesterol rich diet [146].

Wick *et al.* [63] hypothesized that autoimmune responses to HSPs could be crucial in the initiation of atherosclerosis [95, 100] and this is supported by human studies [19, 49, 67, 82, 147]. While immune responses have a potentially important role in atherogenesis, immunosuppressed rabbits [32] or immunologically compromised mice [148–151] do nevertheless develop atherosclerosis. It is likely that both humeral and cellular responses to HSP65 are implicated, with a predominant role of Th1 cells [36, 98, 152], and it is

hypothesized that T cells are involved in the initiation of disease and the humoral response plays a facilitating role [153]. It is possible that HSP27 and-90 are putative autoantigens involved during atherogenesis [154, 155]. Furthermore, autoimmunity to HSPs may lead to a systemic inflammatory response associated with elevated CRP which may also promote atherogenesis.

However, autoantibodies to HSPs can be found in normal subjects. Perschinka *et al.* [156] found that antibodies to mHSP60/65 recognize epitopes on human HSP60; these cross-reactive epitopes were shown to serve as autoantigenic targets in incipient atherosclerosis and also HSP60 could be targeted by a proportion of anti-EC antibodies including anti-HSP antibodies. These are able to trigger apoptosis of ECs [109], which is dependent on HSP60 epitope specificity. It is reported that high antibody titers against mHSP65 are associated with increased cardiovascular morbidity and mortality [67]; there were similar findings for anti-HSP60 [157], -70 [158, 159], and -27 [160] antibodies. One possible mechanism accounting for this is via the induction of pro-inflammatory cytokines by macrophages [71, 127, 133], leading to plaque instability. However, one study has reported no significant relationship between inflammatory factors and anti-HSPs antibody titers [161]. The anti-HSP antibodies could also lead to endothelial injury by antibody-dependent, complement mediated cellular cytotoxicity. We [105] have demonstrated that there is a relationship between vWF concentrations, a marker of endothelial injury or dysfunction [69], extent of atherosclerosis and antibody titers to HSP60, -65, -70 in the cholesterol-fed rabbit.

Titers of these anti-HSP antibodies may be induced and maintained by several mechanisms [133]; (1) an immune response to HSP60 derived from microorganisms, but homologous to human HSP65, because of the phenomenon of molecular mimicry [162]; (2) HSP may be rendered immunogenic because of structural alteration or posttranslational modification resulting from oxidation or metabolic alteration [163]; (3) Other foreign or self-antigens could interact with HSP60 to form immunogenic complexes, and thereafter be recognized as foreign by B or T cells [164]; (4) the recognition of soluble HSPs [71] by a population of T and B cells as a non-self-antigen; and (5) genetic susceptibility (supported by the strong association between a genetic polymorphism within the IL-6 promoter and anti-HSP60 antibody levels) [165].

Binding of anti-HSP antibodies to epitopes on stressed endothelial cells is followed by complement activation [147, 166] and endothelial injury. Hence, endothelial cells that are exposed to high temperature or inflammatory cytokines (e.g., TNF-α) are particularly susceptible to complement-dependent lysis by HSP60-specific antibody [167] in the presence of high concentrations of these antibodies [166]. There are marked differences in the ability to activate complement for different anti-HSP65 and HSP60

antibodies present in patients with coronary heart disease (CHD), and this may be an issue of epitope specificity. Only HSP60–antiHSP60 immune complexes have been shown to be strong activators of complement [166], and a strong positive correlation has been reported between the degree of complement activation and the concentrations of anti-HSP60 but not anti-mHSP65 IgG antibodies [166]. Furthermore, it has been reported that high levels of complement-activating autoantibodies against HSP60 may be an independent cardiovascular risk factor of CHD [168].

4.2. Molecular Mimicry and Relation to Infection

While anti-HSP antibodies are probably produced for the primary purpose of eliminating infectious organisms they may lead to endothelial injury; Mayr *et al.* [147] have reported that serum anti-HSP-antibodies to *E. coli and C. pneumonia* can mediate endothelial cell lysis of stressed, but not unstressed endothelial cells [108, 147]. Moreover, serum antibodies to HSP60/65 from subjects with atherosclerosis, appear to cross-react with hHSP60, GroEL, and *Chlamydial* HSP60 [147].

4.3. Antibodies to HSPs and Infections

Mayr *et al.* [49] have shown that antibody titers to *Mycobacterial* HSP65 correlated strongly with human IgA to *C. pneumonia* and with IgG to *H.* pylori, suggesting a role for infection in inducing the production of mHSP65 antibodies. Eradication of *H. pylori* in patients with confirmed *H. pylori* infection led to a significant fall in anti-mHSP65 titers, suggesting that *H. pylori* infection may be a determinant of anti-mHSP65 titers [169].

High levels of anti-HSP60 and *C. pneumonia* antibodies were found to be independent risk factors for coronary atherosclerosis [170] and their concurrent presence substantially increased the risk of CVD [171]. It was also shown that serum levels of antihuman (h)HSP60 IgG antibody and anti-*Chlamydial* IgM, but not IgG or IgA antibody were significantly higher in patients with acute coronary syndrome than in patients with stable ischemic heart disease [172]. A persistent elevation in antibodies to both hHSP60 and *C. pneumonia* was a better predictor of coronary events than transient or individual elevations in these antibodies [173]. Mayr *et al.* [49] also found that IgA antibodies to *C. pneumonia* were correlated with the extent of carotid and femoral atherosclerosis and were associated with antibodies to mHSP65.

However, these findings are not consistent with those of Hoymons *et al.* [174] who have reported that the antibody response to human and *Chlamydial* HSP60 are not associated with endothelial dysfunction, nor the presence or severity of CVD, arguing against the proposition that infection contributes to

disease progression. Furthermore, Jantos *et al.* [175] were unable to demonstrate the value of HSP60 antibody titers to *C. pneumonia* in discriminating between patients with and without CVD.

Deshpande *et al.* [176] have reported that a primary periodontopathic pathogen can invade ECs and there is evidence that antibodies to *P. gingivalis* GroEL in sera can cross-react with human HSP60 [177], which indicates that these antibodies could mediate endothelial cytotoxicity. It has been reported that in patients with severe periodontitis, elevated IgG antibody titers to both *P. gingivalis* HSP60 and human HSP60 are observed [178]. Increased levels of salivary anti-HSP65 IgA antibodies have also been reported in patients with gingivitis [179], although this has not been a consistent finding [158]; furthermore, anti-HSP-60/-65 IgA titers were found to be lower in smokers and this may be related to an impaired ability to mount a humeral responses to HSP60/65. Other possible confounding factors include the stage of disease.

4.4. Antibodies to HSPs and Cardiovascular Risk Factors

The association of antibodies directed against HSPs has been reviewed below for animal models and human studies, respectively.

4.4.1. *Animal Models*

Cardiovascular risk factors may be divided into those that are modifiable and those that are nonmodifiable. It has been reported that the response to heat treatment was attenuated with increasing age in an animal study [180].

Although acute changes in blood pressure can lead to an upregulation of HSP70 in the rat aorta [181], similar changes were not seen in spontaneously hypertensive rats, even though comparable basal blood pressures were sufficient to induce HSPs levels in normotensive Wistar–Kyoto rats [181].

Nonobese diabetic mice have been reported to develop high titers of anti-HSP60 [182]. Similarly, LDL-RD mice with an induced hyperglycemia, also developed higher antibody titers to HSP65, and accelerated atherosclerosis [99].

A high cholesterol diet in the rat may lead to a significant attenuation of the protective effects of ischemic preconditioning of their heart [183], which may be explained by the finding that hyperlipidemia can inhibit the heat shock response [184].

4.4.2. *Human Studies*

No significant relationship has been found between gender, positive family history of CVD and age and concentrations of soluble HSPs or anti-HSP-antibody titers [67, 71, 83, 160, 185]; and in a prospective study Xu *et al.* [83] were unable to find an association between any traditional risk factors and

HSP65 antibody titers. Furthermore, Frostegård *et al.* [186] found no significant correlation between serum anti-HSP 65 concentrations and several metabolic and anthropometric variables (i.e., lipoproteins, insulin, body mass index, and waist–hip ratio) and blood pressure. However, reports have been inconsistent; for example, Rea *et al.* [187] found that in healthy individuals aged from 20 to 96 years, there was a progressive decline in serum HSP60 and HSP70 antigen levels and a trend for an increase in serum HSP70 antibody levels with age. Similar results were found for IgG anti-HSP-27 concentrations which were strongly associated with age, gender, hypertension, and weakly with diabetes in patients with acute coronary syndrome [188]; however, other cardiovascular risk factors were not associated with anti-Hsp-27 IgG antibody concentrations. Furthermore, it was reported that anti-HSP27 antibody titers are inversely related to age but unrelated to several other established cardiovascular risk factors [189]. We were unable to demonstrate an association between anti-HSP27 antibody levels and several coronary risk factors in an Iranian cohort [160].

Blood vessels subjected to increased mechanical and shear stress express HSPs and are also more prone to the development of atherosclerosis [17, 108]. Frostegård *et al.* [186] have demonstrated that serum anti-HSP antibodies correlate positively with hypertension, supporting the effects of altered hemodynamic stress on HSP. Elevated levels of plasma HSP60 have also been reported in patients with borderline hypertension and were associated with increased intima-media thickness [74]. While other studies have reported that anti-hHSP60 titers were significantly lower in individuals with borderline hypertension [190], and that although anti-HSP65 titers were associated with diastolic blood pressure, they were not related to systolic blood pressure. Other investigators have reported a trend for circulating HSP60 antigen and anti-HSP65 levels to be higher and anti-HSP60 levels to be lower in patients with borderline hypertension [74]. Pockley *et al.* [75] found that in patients with established hypertension whose intima-media thickness was measured over a 4-year period the smallest changes were found in subjects with high HSP70 levels, and anti-HSP70 and anti-mHsp65 antibodies were significantly and independently elevated in patients with established hypertension compared to normotensive controls [190, 191]. The latter result is in contrast to the findings in patients with borderline hypertension, in whom anti-HSP70 concentrations were not elevated relative to controls, and elevation in anti-mHSP65 levels was consistent between patients with borderline and established hypertension [74, 186] so the reported relationship between HSP antigen and antibody levels and blood pressure has been inconsistent in clinical studies.

HSP70 antibodies appear to have a protective effect in hypertensive subjects by modifying the progression of atherosclerosis [75], and increased levels of

circulating HSP70 have been related to a lower risk of CVD and decreased intima-media thickness in hypertensive patients [75, 79]. Moreover, it has been found that peripheral blood lymphocytes from hypertensive subjects contain more HSP70 mRNA compared with normotensive individuals [192], and another study has reported that anti-HSP65 and 70 antibody levels were both associated with hypertension, independent of age, smoking habit, and blood lipids [190]. With respect to HSP27, Shams *et al.* [188] found an inverse relationship between hypertension and Hsp-27 IgG antibody concentrations in patients with chest pain. We have previously reported similar results for HSP-65 antibodies [193]. The combination of hypertension and presence of high anti-HSP60 titers was associated with a >4-fold higher risk of CVD compared to normotensive subjects with low concentrations anti-HSP60 [194]. A similar additive effect on CVD risk was observed with the combination of diabetes and high concentrations of anti-HSP60 [194].

Smoking induces a necrotic and, hence, pro-inflammatory type of cell death in endothelial cells, that may lead to the release HSP60 [195–197]. Individuals who had never smoked or who were not current smokers were found to have higher serum HSP60 concentrations than individuals who were smokers [185]. Frostegård *et al.* [186] found that smokers with atherosclerotic lesions and borderline hypertension had significantly decreased antibody titers to HSP65 compared with age-matched normotensive smokers, but not nonsmokers. Although smoking may be expected to cause an induction of HSP expression, it may also lead to increased cell necrosis with the clearance of HSP65 via the formation of immune complexes with HSP65 antibodies which would lead to a subsequent decrease of antibody titers [186]. It has also been proposed that high concentrations of plasma HSP60 may lead to a suppression of the anti-HSP65 immune response [198]. Another possible reason for low HSP60 titers is that smoking stimulates HSP60 clearance from the plasma by increased catabolism or cellular uptake [185]. Kervinen *et al.* [199] have shown that while a high anti-HSP60-antibody level in hypertensive patients increased coronary risk by approximately 50%, smoking more than doubled the risk, indicating the important role of smoking in the promotion of atherosclerosis.

In diabetic patients anti-HSP60 plasma concentrations were reported to be higher than for nondiabetics [194]. IgA anti-HSP70 antibody concentrations were also shown to be significantly higher in type I and II diabetics than in nondiabetics [200]. Similarly, anti-HSP70 and 90 have also been reported to be higher in diabetic patients [201], and a significantly higher proportion of diabetic patients with CVD had measurable levels of plasma HSP60 compared with those with no evidence of CVD [185]. Anti-HSP60 antibody

concentrations were found to be independently associated with CVD risk, and the combination of diabetes and high concentrations of anti-HSP60 was associated with a substantially increased CVD risk [194].

A positive association between soluble HSP60 and total serum cholesterol [202] and LDL cholesterol [71] has been reported. Plasma HSP27 concentrations have also been reported to be correlated with total serum cholesterol concentrations in patients with acute coronary syndrome [203]. In contrast, a negative association was reported between anti-HSP60 antibody titers and serum HDL cholesterol [199]. Among dyslipidemic patients, serum high sensitivity (hs-) CRP concentrations and anti-HSP60, 65, 70 titers were significantly higher than for controls, but there was no significant association between HSP antibody titers and serum hs-CRP concentrations [204]. Huittinen *et al.* [205] were unable to find a significant relationship between plasma HSP60 antibody titers and coronary risk amongst dyslipidemic middle-aged males. Moreover, patients with hyperlipidemia and hypertension had lower levels of anti-HSP27 antibody than those with neither [188]. The presence of both dyslipidemia and high anti-HSP60 antibody titers was associated with a high risk of coronary atherothrombotic events [199].

We have recently reported a significant relationship between HSP-60, -65 and -70 antibody titers with specific dietary constituent [105, 204]; in subjects with dyslipidemia, plasma antibody titers to HSP60, -65, -70 were associated with dietary antioxidant vitamins and saturated fat [204]. We and others have also found a significant relationship between antibody titers to HSP60 versus HSP65, HSP60 versus HSP70, and HSP65 versus HSP70 [206, 207], although these findings do not accord with those of Kocsis *et al.* [208].

Statins are used to lower LDL cholesterol concentrations and have been shown to reduce the risk of CVD. They also appear to be associated with a reduction in anti-HSP-antibody titers [206, 207]. In addition, cardiac rehabilitation therapy was also found to be associated with a significant reduction in the antibody titers to HSP60 and 70 [207]. Statins are also inhibitors of MHC class II mediated T cell activation [209], and it is therefore possible that some beneficial effects of compounds such as these may be due to their immunemodulatory effects rather than their action on cholesterol metabolism.

Psychological factors, such as stress are known to contribute to CVD risk [210]. Lewthwaite *et al.* [211] have reported an inverse association between serum HSP60 concentrations, social isolation, low socioeconomic status, and psychological distress, a finding that was confirmed in a larger cohort [202].

Table 3 provides a summary of the studies that have investigated the relationship between HSPs and anti-HSP antibodies with known coronary risk factors.

TABLE 3
STUDIES INVESTIGATING THE CORRELATION BETWEEN HSPS AND ANTI-HSP ANTIBODIES WITH CORONARY RISK FACTORS

Risk factor	Subjects	Outcome	References
Nonmodifiable and modifiable risk factors	750 subjects from general population	Negative association with anti-HSP65 antibody titers between these risk factors in these subjects ($p > 0.05$)	[67]
Nonmodifiable and modifiable risk factors	826 subjects from general population	Negative association with soluble HSP60 between these risk factors in these subjects ($p > 0.05$)	[71]
Nonmodifiable and modifiable risk factors	94 patients with CVD	Negative association with anti-HSP27 antibody titers between these risk factors in these subjects ($p > 0.05$)	[160]
Age and Hypertension	60 patients with acute cardiac events	Positive association between anti-HSP27 antibody titers and age and hypertension($p < 0.001$)	[188]
Age	255 initially healthy participants from a cohort study	Inverse association between anti-HSP27 antibody titers and age ($p < 0.001$)	[189]
Hypertension	72 men with borderline hypertension	Positive association between circulating HSP60 ($p = 0.001$) and anti-HSP65 antibody levels ($p < 0.001$), Negative association with HSP70 and anti-HSP70 antibody levels ($p > 0.05$)	[74]

Hypertension	66 men with borderline hypertension	Positive association between anti-HSP65 antibody titers and hypertension ($p < 0.05$)	[186]
Hypertension	111 men with established hypertension	Positive association between anti-HSP65 and 70 antibody titers and hypertension ($p < 0.001$)	[190]
Smoking	855 patients (17.2% type I and 82.8% type II)	Positive association between HSP60 concentrations in nonsmoker group ($p = 0.01$)	[185]
Diabetes mellitus	67 patients (27 type I and 40 type II)	Positive association between IgA antibody to HSP70 and diabetes mellitus type II ($p < 0.05$)	[200]
Total cholesterol	27 patients with acute coronary syndrome	Positive association between HSP27 titers and total cholesterol ($p < 0.05$)	[203]
HDL-cholesterol	233 middle-aged men from a cohort study	Negative association between anti-HSP60 and HDL ($p > 0.05$)	[199]
High levels of hs-CRP	238 dyslipidemic patient	Negative association between anti-HSP60, 65, 70, and hs-CRP ($p > 0.05$)	[204]
Psychological factors	126 men and 103 women	Inverse association between soluble HSP60 levels and Psychological factors in women ($p < 0.05$)	[211]
Psychological factors	541 men and 319 women	Positive association between soluble HSP60 levels and Psychological factors in women and men ($p < 0.05$)	[202]

HSP, heat shock protein; CVD, cardiovascular disease; Ig, Immunoglobulin; hs-CRP, high-sensitive C-reactive protein.

4.5. Antibody Titers to HSPs and Their Relationship to CVD Burden

There have been a number of observational and prospective studies that have shown associations between antibody titers to several HSPs and atherosclerosis.

4.5.1. *Observational Studies*

Anti-HSP60 antibodies were found to be present at particularly high levels in subjects with unstable angina or following myocardial infarction (MI) [161]. Elevated levels of serum anti-HSP60 titers have also been reported in patients with ECG abnormalities, including sinus arrhythmia, chronic myocardial ischemia, and ectopic rhythm [212]. Moreover, high antibody titers to mHSP65 but not hHSP60 were found to be associated with coronary calcification [213]. There are reports that some HSP antibody titers fall after MI or angioplasty [82, 213, 214], and this may be explained by the formation of immune complexes between the circulating antibodies with HSPs released as a consequence of tissue necrosis; these being rapidly cleared by the liver [198]. Acute cardiovascular events may therefore be associated with acute changes in anti-HSP60 antibody titers, as has been reported [82].

Serum mHSP65 antibodies have been shown to cross-react with recombinant human HSP60, homogenates from atherosclerotic plaque and HSP60 present in the endothelial cells within atheromatous lesions [215]. Several studies have also reported that anti-HSP60/65 is cross-reactive [88, 182], and this has been confirmed by Zhu [157] and Xu [67] in subjects with carotid atherosclerosis. However, it has been suggested that anti-HSP60 and anti-HSP65 antibodies from CHD patients are only partially cross-reactive [166, 190], and it appears that the recognition and production of antibodies to different HSP60 epitopes expressed on ECs can result in diverse consequences. For instance, the anti-hHSP60 monoclonal antibody II-13 was cytotoxic for stressed ECs, while another monoclonal antibody, ML-30, which recognizes a different epitope, was not [167]. So it appears that distinct epitopes are accessible to different antibodies, indicating that surface orientation of HSP60 is important, or that discrete domains of hHSP60 are present on the outer surface of the cells.

There have been inconsistent reports of the importance of HSP70 antibody titers in CVD [159, 208]. Zhu *et al.* [79] found no association between anti-HSP70 IgG sero-positivity and the prevalence or severity of CVD whereas there have been reports in which patients with coronary atherosclerosis or stable/unstable angina were found to have lower levels of anti-HSP70 antibody [216]. Herz *et al.* [216] found higher titers of anti-HSP70 in patients with unstable angina compared to those with stable chronic angina, and Vogt [217] reported that higher titers of anti-HSP70 were associated with cardiac

output and pulmonary capillary wedge pressure in those patients who were positive for the anti-HSP70 antibody undergoing heart surgery. It was proposed that patients whose preoperative stress levels, reached the threshold for anti-HSP70 antibody production, were protected from the subsequent CABG procedure.

Kramer *et al.* [218] found that there was no difference in serum titers of either anti-HSP60 or anti-HSP65 antibodies between patients with cerebrovascular disease and age-matched healthy subjects. The authors proposed that stimuli that enhance HSP expression in coronary arteries may not have a similar effect in carotid arteries, which may be more resistant to pro-atherogenic factors.

A significant correlation between anti-HSP70 antibody levels and vascular disease severity has been reported in patients with lower limb claudication, or lower limb critical ischemia [159]. In another study, although levels of serum HSP70 were significantly elevated in 20 patients with PVD, as were serum anti-HSP60 and anti-HSP70 titers, HSP60 were not significantly related to the extent of disease [68].

4.5.2. *Prospective Studies*

Several studies have shown that human anti-HSP60 antibodies are positively associated with the development of atherosclerosis [83, 157, 205] and higher titers of anti-HSP antibodies were strongly associated with CVD [82, 169], particularly anti-HSP60 titers [157]. In one of the first prospective cohort studies of HSP antibody titers and vascular disease, Xu *et al.* [83] found that elevated levels of anti-mHSP65 antibodies were an independent prognostic marker of the incidence, severity, progression, and mortality associated with carotid atherosclerosis in a population that was initially clinically healthy [67, 83, 169, 186]. Serum soluble HSP60 concentrations and antibody titers to HSP65 were also found to predict carotid disease [19] and mortality [67], respectively.

Antibody titers to HSP60 have been reported to be associated with both the presence and severity of clinically significant CVD, independent of traditional coronary risk factors [157, 171], indicating that a high anti-hHSP60 titers may be an important risk factor for coronary atherosclerosis [208, 219].

However, it appears that subtype specificity may be important; for example, it has been reported that anti-HSP60 IgA titers, but not IgG or *C. pneumonia* HSP60 antibodies were a significant risk factor for coronary events. An association between the hHSP60 IgA antibody titers and serum CRP concentration has also been reported [199, 205]. However, elevated concentrations of anti-HSP60 IgA antibody were not found to be a risk factor for CVD unless CRP levels were also elevated, when the presence of an elevated IgA antibodies against hHSP60 was predictive of coronary death

and MI. However, antibodies titers against human and *Chlamydial* HSP60 do not appear to be consistent markers for coronary atherosclerosis or arterial dysfunction [174].

Some studies have shown that anti-mHSP65 antibody levels are predictive of cardiovascular events [67, 220], however, these findings are in contrast to the report of Pockley *et al.* [75] who found no relationship to intima-media thickness, and no association between HSPs or anti-HSP antibodies and intima-media thickness in subjects with established hypertension [190]. The explanation for these discordant findings may be patients selection, or that high HSP antibody titers are a marker of plaque instability and the risk of an acute event, rather than stable plaque formation.

Anti-HSP65 titers have also been reported to be a predictive marker of outcome following coronary angioplasty; patients in whom a fall in antibody levels immediately after PTCA was observed, did not develop restenosis, while in patients who developed subsequent restenosis this decrease was not observed [213, 214]. Anti-HSP65 and anti-HSP70 titers were found to be elevated 48 h after an ischemic stroke, and elevated levels of these antibodies were found to be independent risk factors for stroke [221]. HSP70 antigen is likely to be neuro-protective during the early phases of ischemic stroke; lymphocyte-associated HSP70 is elevated in patients with cerebral infarction, and its level decreased during the period of recovery [222]. It has also been reported that detectable IgG titers against HSP60/65 is associated with an increased risk of stroke [223], although anti-mHSP65 titers appear to have a poor predictive value for atherosclerosis [169, 224].

Hence, antibody titers to HSP60, -65, and -70 have been reported to be associated with increased risk of CVD [141], the severity of cardiovascular [164], and vascular endpoints in patients with established disease [167, 225] while there are relatively few consistent data for other HSPs, including small HSPs [193].

Table 4 summarizes the clinical studies investigating the relationship between HSPs and HSP antibodies and atherosclerosis.

4.6. Changes in Titers of HSP Antibodies During Acute Coronary Syndromes

There have been a few studies that report changes in antibody titers to HSPs in patients with acute coronary syndrome [67, 169, 226]. There have been several reports of reductions in antibody titers after MI or angioplasty [82, 214]; the latter may be due to the formation of immune complexes with HSPs released as a consequence of tissue necrosis [198]. Furthermore, it has been reported that there is a significant increase in serum HSP27 antigen levels in patients with acute coronary syndrome [203]. Shams *et al.* [188]

TABLE 4

CLINICAL STUDIES INVESTIGATING THE RELATIONSHIP BETWEEN HSPS AND HSP ANTIBODY TITERS AND ATHEROSCLEROSIS

Design	Subjects	Outcome	References
	$N = 219$ CVD patients	Positive relationship with anti-HSP60 antibody titers ($p < 0.05$)	[161]
	$N = 396$ autoworkers exposed to noise	Positive relationship with anti-HSP 60 ($p < 0.01$) and -70 ($p < 0.05$) antibody titers	[212]
	$N = 391$ CVD patients	Positive relationship with anti-HSP60 antibody titers ($p < 0.01$)	[157]
Observational, Cross-sectional	$N = 99$ CVD patients	Negative relationship with anti-HSP 70 antibody titers ($p > 0.05$)	[208]
		Positive relationship with anti-HSP60 and -65 antibody titers ($p < 0.001$)	
	$N = 61$ vascular patients	Positive relationship with anti-HSP70 antibody titers ($p < 0.05$)	[159]
	$N = 421$ CVD patients	Positive relationship between low risk of CVD and HSP70 titers ($p < 0.001$)	[79]
	$N = 131$ CVD patients	Positive relationship between lower levels of anti-HSp70 antibody titers and CVD ($p < 0.001$)	[216]
	$N = 292$ cerebrovascular patients	Negative relationship with anti-HSP60 and -65 antibody titers ($p > 0.05$)	[218]
Prospective cohort, coronary disease	$N = 203$ MI and CVD patients	Positive relationship with anti-HSP-65 antibody titers ($p < 0.05$)	[82]
	$N = 357$ CVD patients	Positive relationship with anti-HSP60 antibody titers ($p < 0.001$)	[219]
	$N = 136$ CVD patients	Positive relationship with anti-HSP65 antibody titers ($p < 0.05$)	[169]
Carotid atherosclerosis	$N = 867$ normal subjects	Positive relationship with anti-HSP65 antibody titers ($p < 0.05$)	[83]
	$N = 750$ subjects	Positive relationship with HSP65 antibody titers ($p < 0.05$)	[67]
	$N = 66$ patients with borderline hypertension	Positive relationship with anti-HSP65 antibody titers ($p < 0.05$)	[186]
	$N = 239$ CVD patients	Positive relationship with human IgA antibodies to HSP60 ($p < 0.05$)	[205]
Stroke	$N = 180$ stroke patients	Positive relationship with HSP65 and 70 antibody titers ($p < 0.0001$)	[221]
	$N = 65$ stroke patients	Positive relationship with HSP70 ($p < 0.05$)	[222]
	$N = 93$ stroke patients	Positive relationship with anti-HSP60 and -65 antibody titers ($p < 0.01$)	[223]
Peripheral vascular disease	$N = 20$ PVD patients	Positive relationship with HSP70 ($p < 0.01$) and negative relationship with HSP60 ($p > 0.05$)	[68]

HSP, heat shock protein; CVD, cardiovascular disease; PVD, peripheral vascular disease; Ig, Immunoglobulin; MI, myocardial infarction.

found higher antibody concentrations to HSP27 in patients with chest pain compared to healthy controls. We [160] have also reported that in patients with acute coronary syndrome HSP27 antibody titers are high during the first 12 h following the event, then fall to near normal levels after about 12 h.

5. Therapeutic Implications

It appears that autoimmune responses may be generated against antigens present within the atherosclerotic plaque, and this leads to a cycle of ongoing vascular injury. It has been proposed that inducing a state of tolerance to these atherosclerosis associated antigens may inhibit atherogenesis and hence it may be a feasible therapeutic approach. It has been shown that immune tolerance can be induced by mucosal administration of these antigens. The efficiency of oral tolerization is dependent on the dose of antigen administered; at high doses mucosal administration leads to clonal deletion/anergy; whereas low doses induce T regulatory cells, capable of altering cytokine production [155]. Harats and colleagues have shown that tolerization to HSP65 led to a reduction of plaque formation in a murine model of atherosclerosis [227]. Tolerization was also associated with reduced macrophage and T cell infiltration and increased expression of the anti-inflammatory cytokine, IL-10 expression [227, 228]. However, it should be noted that the effects of immunization may vary with the epitope of HSP65 used for immunization; some appear to enhance while others inhibit atherosclerosis [229]. It is also interesting that some HSPs may be used as effective carriers for delivering B cell epitopes to the immune system in the absence of adjuvant [230, 231].

'Whole pathogen' vaccines such as the BCG contain potentially immunogenic HSP, and while these vaccines reduce morbidity and mortality associated with infection, they may simultaneously stimulate proatherogenic mechanisms [2]. BCG is a live, whole organism vaccine attenuated from the bovine tubercle bacillus. It elicits a strong cell mediated immune response to several Mycobacterial antigens [232], principally a secreted form of HSP-65 [233]. Immunization with BCG vaccine, which contains HSPs, increases the extent of atherosclerosis in the cholesterol-fed rabbit, and the anti-HSP60 titers in BCG immunized rabbits were found to be correlated with the atherosclerotic plaque formation suggesting that the specific immune response to BCG-associated HSP might be proatherogenic [103, 104]. However, atherosclerotic lesions induced by BCG immunization alone in the absence of traditional risk factors such as hypercholesterolemia were found to regress with time suggesting that in the absence of other CVD risk factors, the inflammatory response to HSP60 is not enough to drive atherogenesis over prolonged period of time [101].

6. Conclusions

Hence, cells involved in atherogenesis express large quantities of HSPs in response to exposure to several stressors that may also promote atherosclerosis. Measures of HSP expression, including serum antigen, or antibody concentrations may be useful as markers of disease susceptibility, although the reported data are inconsistent. This may be due to complex interactions between HSP production, release, clearance, and autoimmune responses. Studies using experimental animal models indicate that the role of HSPs in atherogenesis may not be straightforward with different HSPs potentially having pro- or antiatherogenic roles. In addition to a potential role in the initiation of atherosclerosis, they may also be involved in the later stages of disease by inducing a pro-inflammatory autoimmune response [82, 83], and the recruitment of a HSP-specific inflammatory lymphocyte population [17].

The immune response to Ox-LDL appears to be antiatherogenic while that directed against HSP65 or β2-GPI is possibly proatherogenic. The relationship between other HSP antibodies (e.g., HSP70) is less clear and studies have reported either protective or deleterious effects [159, 208]. Despite these previous data it is unclear whether the HSPs have a direct role in atherogenesis. Longer term prospective studies in different populations with a more careful assessment of the time course of appearance of the HSPs and antibodies relative to the development of clinical events will be required. Further work on the effects of tolerization may also be useful to elucidate the importance of the HSP immune response in atherogenesis.

References

[1] F. Ritossa, A new puffing pattern induced and temperature shock and DNP in Drosophilia, Experimentia 18 (1962) 571–573.
[2] D.J. Lamb, W. El-Sankary, G.A. Ferns, Molecular mimicry in atherosclerosis: a role for heat shock proteins in immunisation, Atherosclerosis 167 (2) (2002) 177–185.
[3] K.A. Buzzard, A.J. Giaccia, M. Killender, R.L. Anderson, Heat shock protein 72 modulates pathways of stress-induced apoptosis, J. Biol. Chem. 273 (27) (1998) 17147–17153.
[4] C. Garrido, S. Gurbuxani, L. Ravagnan, G. Kroemer, Heat shock proteins: endogenous modulators of apoptotic cell death, Biochem. Biophys. Res. Commun. 286 (3) (2001) 433–442.
[5] M. Jaattela, D. Wissing, K. Kokholm, T. Kallunki, M. Egeblad, Hsp70 exerts its anti-apoptotic function downstream of caspase-3-like proteases, EMBO J. 17 (21) (1998) 6124–6134.
[6] S.R. Kirchhoff, S. Gupta, A.A. Knowlton, Cytosolic heat shock protein 60, apoptosis, and myocardial injury, Circulation 105 (24) (2002) 2899–2904.
[7] E. Braunwald, Shattuck lecture—Cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities, N. Engl. J. Med. 337 (19) (1997) 1360–1369.

[8] J.L. Breslow, Cardiovascular disease burden increases, NIH funding decreases, Nat. Med. 3 (6) (1997) 600–601.
[9] R. Ross, The pathogenesis of atherosclerosis: a perspective for the 1990s, Nature 362 (6423) (1993) 801–809.
[10] G.A. Ferns, New and emerging risk factors for CVD, Proc. Nutr. Soc. 67 (2) (2008) 223–231.
[11] R. Virchow, Der Ateromatose Prozess der Arterien, Wien. Med. Wochenschr. 6 (1856) 825–827.
[12] Q.B. Xu, G. Oberhuber, M. Gruschwitz, G. Wick, Immunology of atherosclerosis: cellular composition and major histocompatibility complex class II antigen expression in aortic intima, fatty streaks, and atherosclerotic plaques in young and aged human specimens, Clin. Immunol. Immunopathol. 56 (3) (1990) 344–359.
[13] P. Libby, G.K. Hansson, Involvement of the immune system in human atherogenesis: current knowledge and unanswered questions, Lab. Invest. 64 (1) (1991) 5–15.
[14] C. Napoli, F.P. D'Armiento, F.P. Mancini, et al., Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions, J. Clin. Invest. 100 (11) (1997) 2680–2690.
[15] G. Millonig, H. Niederegger, W. Rabl, et al., Network of vascular-associated dendritic cells in intima of healthy young individuals, Arterioscler. Thromb. Vasc. Biol. 21 (4) (2001) 503–508.
[16] C. Buono, C.E. Come, G. Stavrakis, G.F. Maguire, P.W. Connelly, A.H. Lichtman, Influence of interferon-gamma on the extent and phenotype of diet-induced atherosclerosis in the LDLR-deficient mouse, Arterioscler. Thromb. Vasc. Biol. 23 (3) (2003) 454–460.
[17] R. Kleindienst, Q. Xu, J. Willeit, F.R. Waldenberger, S. Weimann, G. Wick, Immunology of atherosclerosis. Demonstration of heat shock protein 60 expression and T lymphocytes bearing alpha/beta or gamma/delta receptor in human atherosclerotic lesions, Am. J. Pathol. 142 (6) (1993) 1927–1937.
[18] A.C. Van der Wal, P.K. Das, B.D. van de Berg, C.M. Van der Loos, A.E. Becker, Atherosclerotic lesions in humans. In situ immunophenotypic analysis suggesting an immune mediated response, Lab. Invest. 61 (2) (1989) 166–170.
[19] S. Kiechl, G. Egger, M. Mayr, et al., Chronic infections and the risk of carotid atherosclerosis: prospective results from a large population study, Circulation 103 (8) (2001) 1064–1070.
[20] P.M. Ridker, M.J. Stampfer, N. Rifai, Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease, J. AM. Med. Assoc. 285 (19) (2001) 2481–2485.
[21] P.M. Ridker, N. Rifai, L. Rose, J.E. Buring, N.R. Cook, Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events, N. Engl. J. Med. 347 (20) (2002) 1557–1565.
[22] N. Rifai, P.M. Ridker, High-sensitivity C-reactive protein: a novel and promising marker of coronary heart disease, Clin. Chem. 47 (3) (2001) 403–411.
[23] H. Yu, N. Rifai, High-sensitivity C-reactive protein and atherosclerosis: from theory to therapy, Clin. Biochem. 33 (8) (2000) 601–610.
[24] R.N. Poston, D.O. Haskard, J.R. Coucher, N.P. Gall, R.R. Johnson-Tidey, Expression of intercellular adhesion molecule-1 in atherosclerotic plaques, Am. J. Pathol. 140 (3) (1992) 665–673.
[25] J. Torzewski, R. Oldroyd, P. Lachmann, C. Fitzsimmons, D. Proudfoot, D. Bowyer, Complement-induced release of monocyte chemotactic protein-1 from human smooth muscle cells. A possible initiating event in atherosclerotic lesion formation, Arterioscler. Thromb. Vasc. Biol. 16 (5) (1996) 673–677.

[26] J. Torzewski, D.E. Bowyer, J. Waltenberger, C. Fitzsimmons, Processes in atherogenesis: complement activation, Atherosclerosis 132 (2) (1997) 131–138.
[27] M. Torzewski, J. Torzewski, D.E. Bowyer, et al., Immunohistochemical colocalization of the terminal complex of human complement and smooth muscle cell alpha-actin in early atherosclerotic lesions, Arterioscler. Thromb. Vasc. Biol. 17 (11) (1997) 2448–2452.
[28] E. Wieland, B. Dorweiler, U. Bonitz, S. Lieser, I. Walev, S. Bhakdi, Complement activation by oxidatively modified low-density lipoproteins, Eur. J. Clin. Invest. 29 (10) (1999) 835–841.
[29] Z. Prohaszka, J. Duba, G. Lakos, et al., Antibodies against human heat-shock protein (hsp) 60 and mycobacterial hsp65 differ in their antigen specificity and complement-activating ability, Int. Immunol. 11 (9) (1999) 1363–1370.
[30] G.K. Hansson, Immune mechanisms in atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 21 (12) (2001) 1876–1890.
[31] G. Millonig, G.T. Malcom, G. Wick, Early inflammatory-immunological lesions in juvenile atherosclerosis from the Pathobiological Determinants of Atherosclerosis in Youth (PDAY)-study, Atherosclerosis 160 (2) (2002) 441–448.
[32] B. Metzler, M. Mayr, H. Dietrich, et al., Inhibition of arteriosclerosis by T-cell depletion in normocholesterolemic rabbits immunized with heat shock protein 65, Arterioscler. Thromb. Vasc. Biol. 19 (8) (1999) 1905–1911.
[33] Q.B. Xu, G. Oberhuber, M. Gruschwitz, G. Wick, Immunology of atherosclerosis: cellular composition and major histocompatibility complex class II antigen expression in aortic intima, fatty streaks, and atherosclerotic plaques in young and aged human specimens, Clin. Immunol. Immunopathol. 56 (3) (1990) 344–359.
[34] S.A. Huber, P. Sakkinen, C. David, M.K. Newell, R.P. Tracy, T helper-cell phenotype regulates atherosclerosis in mice under conditions of mild hypercholesterolemia, Circulation 103 (21) (2001) 2610–2616.
[35] L.J. Pinderski, M.P. Fischbein, G. Subbanagounder, et al., Overexpression of interleukin-10 by activated T lymphocytes inhibits atherosclerosis in LDL receptor-deficient Mice by altering lymphocyte and macrophage phenotypes, Circ. Res. 90 (10) (2002) 1064–1071.
[36] E. Laurat, B. Poirier, E. Tupin, et al., *In vivo* downregulation of T helper cell 1 immune responses reduces atherogenesis in apolipoprotein E-knockout mice, Circulation 104 (2) (2001) 197–202.
[37] H. Groux, An overview of regulatory T cells, Microbes. Infect. 3 (11) (2001) 883–889.
[38] P. McGuirk, C. McCann, K.H. Mills, Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by Bordetella pertussis, J. Exp. Med. 195 (2) (2002) 221–231.
[39] M.G. Roncarolo, R. Bacchetta, C. Bordignon, S. Narula, M.K. Levings, Type 1 T regulatory cells, Immunol. Rev. 182 (2001) 68–79.
[40] A. Doetze, J. Satoguina, G. Burchard, et al., Antigen-specific cellular hyporesponsiveness in a chronic human helminth infection is mediated by T(h)3/T(r)1-type cytokines IL-10 and transforming growth factor-beta but not by a T(h)1 to T(h)2 shift, Int. Immunol. 12 (5) (2000) 623–630.
[41] H. Groux, A. O'Garra, M. Bigler, et al., A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis, Nature 389 (6652) (1997) 737–742.
[42] G. Caligiuri, M. Rudling, V. Ollivier, et al., Interleukin-10 deficiency increases atherosclerosis, thrombosis, and low-density lipoproteins in apolipoprotein E knockout mice, Mol. Med. 9 (1–2) (2003) 10–17.
[43] D.J. Grainger, C.M. Witchell, J.C. Metcalfe, Tamoxifen elevates transforming growth factor-beta and suppresses diet-induced formation of lipid lesions in mouse aorta, Nat. Med. 1 (10) (1995) 1067–1073.

[44] E. Lutgens, M. Gijbels, M. Smook, et al., Transforming growth factor-beta mediates balance between inflammation and fibrosis during plaque progression, Arterioscler. Thromb. Vasc. Biol. 22 (6) (2002) 975–982.
[45] C. Buono, C.J. Binder, G. Stavrakis, J.L. Witztum, L.H. Glimcher, A.H. Lichtman, T-bet deficiency reduces atherosclerosis and alters plaque antigen-specific immune responses, Proc. Natl. Acad. Sci. USA 102 (5) (2005) 1596–1601.
[46] E.E. Emeson, M.L. Shen, C.G. Bell, A. Qureshi, Inhibition of atherosclerosis in CD4 T-cell-ablated and nude (nu/nu) C57BL/6 hyperlipidemic mice, Am. J. Pathol. 149 (2) (1996) 675–685.
[47] E.E. Emeson, M.L. Shen, Accelerated atherosclerosis in hyperlipidemic C57BL/6 mice treated with cyclosporin A, Am. J. Pathol. 142 (6) (1993) 1906–1915.
[48] S.E. Roselaar, G. Schonfeld, A. Daugherty, Enhanced development of atherosclerosis in cholesterol-fed rabbits by suppression of cell-mediated immunity, J. Clin. Invest. 96 (3) (1995) 1389–1394.
[49] M. Mayr, S. Kiechl, J. Willeit, G. Wick, Q. Xu, Infections, immunity, and atherosclerosis: associations of antibodies to *Chlamydia pneumoniae, Helicobacter pylori,* and cytomegalovirus with immune reactions to heat-shock protein 60 and carotid or femoral atherosclerosis, Circulation 102 (8) (2000) 833–839.
[50] S.E. Epstein, Y.F. Zhou, J. Zhu, Infection and atherosclerosis: emerging mechanistic paradigms, Circulation 100 (4) (1999) e20–e28.
[51] A.R. Folsom, F.J. Nieto, P. Sorlie, L.E. Chambless, D.Y. Graham, Helicobacter pylori seropositivity and coronary heart disease incidence. Atherosclerosis Risk in Communities (ARIC) Study Investigators, Circulation 98 (9) (1998) 845–850.
[52] P. Saikku, M. Leinonen, K. Mattila, et al., Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction, Lancet 2 (8618) (1988) 983–986.
[53] J. Zhu, A.A. Quyyumi, J.E. Norman, et al., Effects of total pathogen burden on coronary artery disease risk and C-reactive protein levels, Am. J. Cardiol. 85 (2) (2000) 140–146.
[54] K.J. Mattila, M.S. Nieminen, V.V. Valtonen, et al., Association between dental health and acute myocardial infarction, Br. Med. J. 298 (6676) (1989) 779–781.
[55] E. Pesonen, I. Paakkari, J. Rapola, Infection-associated intimal thickening in the coronary arteries of children, Atherosclerosis 142 (2) (1999) 425–429.
[56] M. Chmiela, M. Kowalewicz-Kulbat, A. Miszczak, et al., A link between *Helicobacter pylori* and/or *Chlamydia* spp. infections and atherosclerosis, FEMS Immunol. Med. Microbiol. 36 (3) (2003) 187–192.
[57] C. Espinola-Klein, H.J. Rupprecht, S. Blankenberg, et al., Impact of infectious burden on extent and long-term prognosis of atherosclerosis, Circulation 105 (1) (2002) 15–21.
[58] G. Caligiuri, A. Nicoletti, B. Poirier, G.K. Hansson, Protective immunity against atherosclerosis carried by B cells of hypercholesterolemic mice, J. Clin. Invest. 109 (6) (2002) 745–753.
[59] A. Nicoletti, G. Caligiuri, G. Paulsson, G.K. Hansson, Functionality of specific immunity in atherosclerosis, Am. Heart J. 138 (5 Pt 2) (1999) S438–S443.
[60] J.L. Witztum, Splenic immunity and atherosclerosis: a glimpse into a novel paradigm? J. Clin. Invest. 109 (6) (2002) 721–724.
[61] Q. Xiao, K. Mandal, G. Schett, et al., Association of serum-soluble heat shock protein 60 with carotid atherosclerosis: clinical significance determined in a follow-up study, Stroke 36 (12) (2005) 2571–2576.
[62] R. Ohashi, H. Mu, Q. Yao, C. Chen, Atherosclerosis: immunopathogenesis and immunotherapy, Med. Sci. Monit. 10 (11) (2004) RA255–RA260.

[63] G. Wick, G. Schett, A. Amberger, R. Kleindienst, Q. Xu, Is atherosclerosis an immunologically mediated disease? Immunol. Today 16 (1) (1995) 27–33.
[64] K. Mandal, M. Jahangiri, Q. Xu, Autoimmune mechanisms of atherosclerosis, Handb. Exp. Pharmacol. 170 (2005) 723–743.
[65] P.A. Berberian, W. Myers, M. Tytell, V. Challa, M.G. Bond, Immunohistochemical localization of heat shock protein-70 in normal-appearing and atherosclerotic specimens of human arteries, Am. J. Pathol. 136 (1) (1990) 71–80.
[66] A.G. Pockley, J. Bulmer, B.M. Hanks, B.H. Wright, Identification of human heat shock protein 60 (Hsp60) and anti-Hsp60 antibodies in the peripheral circulation of normal individuals, Cell Stress Chaperones 4 (1) (1999) 29–35.
[67] Q. Xu, S. Kiechl, M. Mayr, et al., Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis: clinical significance determined in a follow-up study, Circulation 100 (11) (1999) 1169–1174.
[68] B.H. Wright, J.M. Corton, A.M. El-Nahas, R.F. Wood, A.G. Pockley, Elevated levels of circulating heat shock protein 70 (Hsp70) in peripheral and renal vascular disease, Heart Vessels 15 (1) (2000) 18–22.
[69] A.D. Blann, C.N. McCollum, von Willebrand factor, endothelial cell damage and atherosclerosis, Eur. J. Vasc. Surg. 8 (1) (1994) 10–15.
[70] A. Kol, G.K. Sukhova, A.H. Lichtman, P. Libby, Chlamydial heat shock protein 60 localizes in human atheroma and regulates macrophage tumor necrosis factor-alpha and matrix metalloproteinase expression, Circulation 98 (4) (1998) 300–307.
[71] Q. Xu, G. Schett, H. Perschinka, et al., Serum soluble heat shock protein 60 is elevated in subjects with atherosclerosis in a general population, Circulation 102 (1) (2000) 14–20.
[72] J.P. Halcox, J. Deanfield, A. Shamaei-Tousi, et al., Circulating human heat shock protein 60 in the blood of healthy teenagers: a novel determinant of endothelial dysfunction and early vascular injury? Arterioscler. Thromb. Vasc. Biol. 25 (11) (2005) e141–e142.
[73] M. Knoflach, S. Kiechl, M. Kind, et al., Cardiovascular risk factors and atherosclerosis in young males: ARMY study (Atherosclerosis Risk-Factors in Male Youngsters), Circulation 108 (9) (2003) 1064–1069.
[74] A.G. Pockley, R. Wu, C. Lemne, R. Kiessling, U. de Faire, J. Frostegard, Circulating heat shock protein 60 is associated with early cardiovascular disease, Hypertension 36 (2) (2000) 303–307.
[75] A.G. Pockley, A. Georgiades, T. Thulin, U. de Faire, J. Frostegard, Serum heat shock protein 70 levels predict the development of atherosclerosis in subjects with established hypertension, Hypertension 42 (3) (2003) 235–238.
[76] B. Dybdahl, A. Wahba, E. Lien, et al., Inflammatory response after open heart surgery: release of heat-shock protein 70 and signaling through toll-like receptor-4, Circulation 105 (6) (2002) 685–690.
[77] A.D. Johnson, P.A. Berberian, M. Tytell, M.G. Bond, Atherosclerosis alters the localization of HSP70 in human and macaque aortas, Exp. Mol. Pathol. 58 (3) (1993) 155–168.
[78] Y.V. Bobryshev, R.S. Lord, Expression of heat shock protein-70 by dendritic cells in the arterial intima and its potential significance in atherogenesis, J. Vasc. Surg. 35 (2) (2002) 368–375.
[79] J. Zhu, A.A. Quyyumi, H. Wu, et al., Increased serum levels of heat shock protein 70 are associated with low risk of coronary artery disease, Arterioscler. Thromb. Vasc. Biol. 23 (6) (2003) 1055–1059.
[80] W. Zhu, P. Roma, F. Pellegatta, A.L. Catapano, Oxidized-LDL induce the expression of heat shock protein 70 in human endothelial cells, Biochem. Biophys. Res. Commun. 200 (1) (1994) 389–394.

[81] S. Genth-Zotz, A.P. Bolger, P.R. Kalra, et al., Heat shock protein 70 in patients with chronic heart failure: relation to disease severity and survival, Int. J. Cardiol. 96 (3) (2004) 397–401.
[82] F. Hoppichler, M. Lechleitner, C. Traweger, et al., Changes of serum antibodies to heat-shock protein 65 in coronary heart disease and acute myocardial infarction, Atherosclerosis 126 (2) (1996) 333–338.
[83] Q. Xu, J. Willeit, M. Marosi, et al., Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis, Lancet 341 (8840) (1993) 255–259.
[84] M. Knoflach, S. Kiechl, B. Mayrl, et al., T-cell reactivity against HSP60 relates to early but not advanced atherosclerosis, Atherosclerosis 195 (2) (2007) 333–338.
[85] S. Kiechl, J. Willeit, The natural course of atherosclerosis. Part I: incidence and progression, Arterioscler. Thromb. Vasc. Biol. 19 (6) (1999) 1484–1490.
[86] S. Kiechl, J. Willeit, The natural course of atherosclerosis. Part II: vascular remodeling. Bruneck Study Group, Arterioscler. Thromb. Vasc. Biol. 19 (6) (1999) 1491–1498.
[87] J.M. Ramage, J.L. Young, J.C. Goodall, J.S. Gaston, T cell responses to heat-shock protein 60: differential responses by CD4+ T cell subsets according to their expression of CD45 isotypes, J. Immunol. 162 (2) (1999) 704–710.
[88] M.G. Danieli, M. Candela, A.M. Ricciatti, et al., Antibodies to mycobacterial 65 kDa heat shock protein in systemic sclerosis (scleroderma), J. Autoimmun. 5 (4) (1992) 443–452.
[89] S. Ishibashi, M.S. Brown, J.L. Goldstein, R.D. Gerard, R.E. Hammer, J. Herz, Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery, J. Clin. Invest. 92 (2) (1993) 883–893.
[90] A.S. Plump, J.D. Smith, T. Hayek, et al., Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells, Cell 71 (2) (1992) 343–353.
[91] S.H. Zhang, R.L. Reddick, J.A. Piedrahita, N. Maeda, Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E, Science 258 (5081) (1992) 468–471.
[92] M.S. Marber, R. Mestril, S.H. Chi, M.R. Sayen, D.M. Yellon, W.H. Dillmann, Overexpression of the rat inducible 70-kD heat stress protein in a transgenic mouse increases the resistance of the heart to ischemic injury, J. Clin. Invest. 95 (4) (1995) 1446–1456.
[93] R.W. Currie, M. Karmazyn, M. Kloc, K. Mailer, Heat-shock response is associated with enhanced postischemic ventricular recovery, Circ. Res. 63 (3) (1988) 543–549.
[94] A. Afek, J. George, B. Gilburd, et al., Immunization of low-density lipoprotein receptor deficient (LDL-RD) mice with heat shock protein 65 (HSP-65) promotes early atherosclerosis, J. Autoimmun. 14 (2) (2000) 115–121.
[95] J. George, Y. Shoenfeld, A. Afek, et al., Enhanced fatty streak formation in C57BL/6J mice by immunization with heat shock protein-65, Arterioscler. Thromb. Vasc. Biol. 19 (3) (1999) 505–510.
[96] L.B. Kirby, J.S. Mondy, C.M. Brophy, Balloon angioplasty induces heat shock protein 70 in human blood vessels, Ann. Vasc. Surg. 13 (5) (1999) 475–479.
[97] J. George, S. Greenberg, I. Barshack, I. Goldberg, G. Keren, A. Roth, Immunity to heat shock protein 65—An additional determinant in intimal thickening, Atherosclerosis 168 (1) (2003) 33–38.
[98] J. George, A. Afek, B. Gilburd, Y. Shoenfeld, D. Harats, Cellular and humoral immune responses to heat shock protein 65 are both involved in promoting fatty-streak formation in LDL-receptor deficient mice, J. Am. Coll. Cardiol. 38 (3) (2001) 900–905.
[99] P. Keren, J. George, A. Shaish, et al., Effect of hyperglycemia and hyperlipidemia on atherosclerosis in LDL receptor-deficient mice: establishment of a combined model and association with heat shock protein 65 immunity, Diabetes 49 (6) (2000) 1064–1069.

[100] Q. Xu, H. Dietrich, H.J. Steiner, et al., Induction of arteriosclerosis in normocholesterolemic rabbits by immunization with heat shock protein 65, Arterioscler. Thromb. 12 (7) (1992) 789–799.
[101] Q. Xu, R. Kleindienst, G. Schett, et al., Regression of arteriosclerotic lesions induced by immunization with heat shock protein 65-containing material in normocholesterolemic, but not hypercholesterolemic, rabbits, Atherosclerosis 123 (1–2) (1996) 145–155.
[102] Q. Xu, R. Kleindienst, W. Waitz, H. Dietrich, G. Wick, Increased expression of heat shock protein 65 coincides with a population of infiltrating T lymphocytes in atherosclerotic lesions of rabbits specifically responding to heat shock protein 65, J. Clin. Invest. 91 (6) (1993) 2693–2702.
[103] D.J. Lamb, L.J. Eales, G.A. Ferns, Immunization with bacillus Calmette-Guerin vaccine increases aortic atherosclerosis in the cholesterol-fed rabbit, Atherosclerosis 143 (1) (1999) 105–113.
[104] D.J. Lamb, G.A. Ferns, The magnitude of the immune response to heat shock protein-65 following BCG immunisation is associated with the extent of experimental atherosclerosis, Atherosclerosis 165 (2) (2002) 231–240.
[105] M. Ghayour-Mobarhan, D.J. Lamb, S. Tavallaie, G.A. Ferns, Relationship between plasma cholesterol, von Willebrand factor concentrations, extent of atherosclerosis and antibody titres to heat shock proteins-60, -65 and -70 in cholesterol-fed rabbits, Int. J. Exp. Pathol. 88 (4) (2007) 249–255.
[106] R.K. Kanwar, J.R. Kanwar, D. Wang, D.J. Ormrod, G.W. Krissansen, Temporal expression of heat shock proteins 60 and 70 at lesion-prone sites during atherogenesis in ApoE-deficient mice, Arterioscler. Thromb. Vasc. Biol. 21 (12) (2001) 1991–1997.
[107] G. Pfister, C.M. Stroh, H. Perschinka, et al., Detection of HSP60 on the membrane surface of stressed human endothelial cells by atomic force and confocal microscopy, J. Cell Sci. 118 (Pt 8) (2005) 1587–1594.
[108] G. Schett, Q. Xu, A. Amberger, et al., Autoantibodies against heat shock protein 60 mediate endothelial cytotoxicity, J. Clin. Invest. 96 (6) (1995) 2569–2577.
[109] C. Jamin, C. Dugue, J.E. Alard, et al., Induction of endothelial cell apoptosis by the binding of anti-endothelial cell antibodies to Hsp60 in vasculitis-associated systemic autoimmune diseases, Arthritis Rheum. 52 (12) (2005) 4028–4038.
[110] A.D. Johnson, P.A. Berberian, M. Tytell, M.G. Bond, Differential distribution of 70-kD heat shock protein in atherosclerosis. Its potential role in arterial SMC survival, Arterioscler. Thromb. Vasc. Biol. 15 (1) (1995) 27–36.
[111] Q. Xu, G. Schett, C. Li, Y. Hu, G. Wick, Mechanical stress-induced heat shock protein 70 expression in vascular smooth muscle cells is regulated by Rac and Ras small G proteins but not mitogen-activated protein kinases, Circ. Res. 86 (11) (2000) 1122–1128.
[112] M. Amrani, J. Corbett, S.Y. Boateng, M.J. Dunn, M.H. Yacoub, Kinetics of induction and protective effect of heat-shock proteins after cardioplegic arrest, Ann. Thorac. Surg. 61 (5) (1996) 1407–1411.
[113] R.W. Currie, R.M. Tanguay, J.G. Kingma, Jr, Heat-shock response and limitation of tissue necrosis during occlusion/reperfusion in rabbit hearts, Circulation 87 (3) (1993) 963–971.
[114] D.V. Cumming, R.J. Heads, A. Watson, D.S. Latchman, D.M. Yellon, Differential protection of primary rat cardiocytes by transfection of specific heat stress proteins, J. Mol. Cell Cardiol. 28 (12) (1996) 2343–2349.
[115] D.V. Cumming, R.J. Heads, R.S. Coffin, D.M. Yellon, D.S. Latchman, Pharmacological preconditioning of primary rat cardiac myocytes by FK506, Basic Res. Cardiol. 91 (5) (1996) 367–373.

[116] R.J. Heads, D.S. Latchman, D.M. Yellon, Stable high level expression of a transfected human HSP70 gene protects a heart-derived muscle cell line against thermal stress, J. Mol. Cell Cardiol. 26 (6) (1994) 695–699.
[117] A.A. Knowlton, The role of heat shock proteins in the heart, J. Mol. Cell Cardiol. 27 (1) (1995) 121–131.
[118] M.M. Hutter, R.E. Sievers, V. Barbosa, C.L. Wolfe, Heat-shock protein induction in rat hearts. A direct correlation between the amount of heat-shock protein induced and the degree of myocardial protection, Circulation 89 (1) (1994) 355–360.
[119] W.H. Dillmann, H.B. Mehta, A. Barrieux, B.D. Guth, W.E. Neeley, J. Ross, Jr, Ischemia of the dog heart induces the appearance of a cardiac mRNA coding for a protein with migration characteristics similar to heat-shock/stress protein 71, Circ. Res. 59 (1) (1986) 110–114.
[120] O.N. Demidov, V.V. Tyrenko, A.S. Svistov, et al., Heat shock proteins in cardiosurgery patients, Eur. J. Cardiothorac. Surg. 16 (4) (1999) 444–449.
[121] G. Valen, G.K. Hansson, A. Dumitrescu, J. Vaage, Unstable angina activates myocardial heat shock protein 72, endothelial nitric oxide synthase, and transcription factors NFkappaB and AP-1, Cardiovasc. Res. 47 (1) (2000) 49–56.
[122] R. Mestril, W.H. Dillmann, Heat shock proteins and protection against myocardial ischemia, J. Mol. Cell Cardiol. 27 (1) (1995) 45–52.
[123] A.D. Johnson, P.A. Berberian, M.G. Bond, Effect of heat shock proteins on survival of isolated aortic cells from normal and atherosclerotic cynomolgus macaques, Atherosclerosis 84 (2–3) (1990) 111–119.
[124] J.L. Martin, R. Mestril, R. Hilal-Dandan, L.L. Brunton, W.H. Dillmann, Small heat shock proteins and protection against ischemic injury in cardiac myocytes, Circulation 96 (12) (1997) 4343–4348.
[125] J.L. Martin, E. Hickey, L.A. Weber, W.H. Dillmann, R. Mestril, Influence of phosphorylation and oligomerization on the protective role of the small heat shock protein 27 in rat adult cardiomyocytes, Gene. Expr. 7 (4–6) (1999) 349–355.
[126] I. Schimke, G. Lutsch, U. Schernes, et al., Increased level of HSP27 but not of HSP72 in human heart allografts in relation to acute rejection, Transplantation 70 (12) (2000) 1694–1697.
[127] W. Chen, U. Syldath, K. Bellmann, V. Burkart, H. Kolb, Human 60-kDa heat-shock protein: a danger signal to the innate immune system, J. Immunol. 162 (6) (1999) 3212–3219.
[128] J.M. Bruey, C. Ducasse, P. Bonniaud, et al., Hsp27 negatively regulates cell death by interacting with cytochrome c, Nat. Cell Biol. 2 (9) (2000) 645–652.
[129] C. Garrido, J.M. Bruey, A. Fromentin, A. Hammann, A.P. Arrigo, E. Solary, HSP27 inhibits cytochrome c-dependent activation of procaspase-9, FASEB J. 13 (14) (1999) 2061–2070.
[130] B.K. Brar, A. Stephanou, M.J. Wagstaff, et al., Heat shock proteins delivered with a virus vector can protect cardiac cells against apoptosis as well as against thermal or hypoxic stress, J. Mol. Cell Cardiol. 31 (1) (1999) 135–146.
[131] A.K. De, K.M. Kodys, B.S. Yeh, C. Miller-Graziano, Exaggerated human monocyte IL-10 concomitant to minimal TNF-alpha induction by heat-shock protein 27 (Hsp27) suggests Hsp27 is primarily an antiinflammatory stimulus, J. Immunol. 165 (7) (2000) 3951–3958.
[132] R.M. Vabulas, P. Ahmad-Nejad, S. Ghose, C.J. Kirschning, R.D. Issels, H. Wagner, HSP70 as endogenous stimulus of the Toll/interleukin-1 receptor signal pathway, J. Biol. Chem. 277 (17) (2002) 15107–15112.
[133] K. Mandal, M. Jahangiri, Q. Xu, Autoimmunity to heat shock proteins in atherosclerosis, Autoimmun. Rev. 3 (2) (2004) 31–37.

[134] A. Kol, T. Bourcier, A.H. Lichtman, P. Libby, Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells, and macrophages, J. Clin. Invest. 103 (4) (1999) 571–577.
[135] W.L. Beatty, R.P. Morrison, G.I. Byrne, Persistent chlamydiae: from cell culture to a paradigm for chlamydial pathogenesis, Microbiol. Rev. 58 (4) (1994) 686–699.
[136] C.M. Boulanger, A. Scoazec, T. Ebrahimian, et al., Circulating microparticles from patients with myocardial infarction cause endothelial dysfunction, Circulation 104 (22) (2001) 2649–2652.
[137] Z. Mallat, B. Hugel, J. Ohan, G. Leseche, J.M. Freyssinet, A. Tedgui, Shed membrane microparticles with procoagulant potential in human atherosclerotic plaques: a role for apoptosis in plaque thrombogenicity, Circulation 99 (3) (1999) 348–353.
[138] Z. Mallat, A. Tedgui, Current perspective on the role of apoptosis in atherothrombotic disease, Circ. Res. 88 (10) (2001) 998–1003.
[139] M. Mesri, D.C. Altieri, Leukocyte microparticles stimulate endothelial cell cytokine release and tissue factor induction in a JNK1 signaling pathway, J. Biol. Chem. 274 (33) (1999) 23111–23118.
[140] A. Kol, A.H. Lichtman, R.W. Finberg, P. Libby, E.A. Kurt-Jones, Cutting edge: heat shock protein (HSP) 60 activates the innate immune response: CD14 is an essential receptor for HSP60 activation of mononuclear cells, J. Immunol. 164 (1) (2000) 13–17.
[141] Q. Xu, Role of heat shock proteins in atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 22 (10) (2002) 1547–1559.
[142] A. Asea, S.K. Kraeft, E.A. Kurt-Jones, et al., HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine, Nat. Med. 6 (4) (2000) 435–442.
[143] P. Matzinger, Tolerance, danger, and the extended family, Annu. Rev. Immunol. 12 (1994) 991–1045.
[144] P.K. Srivastava, A. Menoret, S. Basu, R.J. Binder, K.L. McQuade, Heat shock proteins come of age: primitive functions acquire new roles in an adaptive world, Immunity 8 (6) (1998) 657–665.
[145] G. Wick, M. Romen, A. Amberger, et al., Atherosclerosis, autoimmunity, and vascular-associated lymphoid tissue, FASEB J. 11 (13) (1997) 1199–1207.
[146] Q. Xu, G. Wick, The role of heat shock proteins in protection and pathophysiology of the arterial wall, Mol. Med. Today 2 (9) (1996) 372–379.
[147] M. Mayr, B. Metzler, S. Kiechl, et al., Endothelial cytotoxicity mediated by serum antibodies to heat shock proteins of *Escherichia coli* and *Chlamydia pneumoniae*: immune reactions to heat shock proteins as a possible link between infection and atherosclerosis, Circulation 99 (12) (1999) 1560–1566.
[148] H.M. Dansky, S.A. Charlton, M.M. Harper, J.D. Smith, T and B lymphocytes play a minor role in atherosclerotic plaque formation in the apolipoprotein E-deficient mouse, Proc. Natl. Acad. Sci. USA 94 (9) (1997) 4642–4646.
[149] A. Daugherty, E. Pure, D. Defel-Butteiger, et al., The effects of total lymphocyte deficiency on the extent of atherosclerosis in apolipoprotein E-/-mice, J. Clin. Invest. 100 (6) (1997) 1575–1580.
[150] A.I. Fyfe, J.H. Qiao, A.J. Lusis, Immune-deficient mice develop typical atherosclerotic fatty streaks when fed an atherogenic diet, J. Clin. Invest. 94 (6) (1994) 2516–2520.
[151] S. Gupta, A.M. Pablo, X. Jiang, N. Wang, A.R. Tall, C. Schindler, IFN-gamma potentiates atherosclerosis in ApoE knock-out mice, J. Clin. Invest. 99 (11) (1997) 2752–2761.
[152] J. Frostegard, A.K. Ulfgren, P. Nyberg, et al., Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines, Atherosclerosis 145 (1) (1999) 33–43.

[153] M. Knoflach, D. Bernhard, G. Wick, Anti-HSP60 immunity is already associated with atherosclerosis early in life, Ann. N. Y. Acad. Sci. 1051 (2005) 323–331.
[154] R. Rigano, E. Profumo, B. Buttari, et al., Heat shock proteins and autoimmunity in patients with carotid atherosclerosis, Ann. N. Y. Acad. Sci. 1107 (2007) 1–10.
[155] J. George, N. Yacov, E. Breitbart, et al., Suppression of early atherosclerosis in LDL-receptor deficient mice by oral tolerance with beta 2-glycoprotein I, Cardiovasc. Res. 62 (3) (2004) 603–609.
[156] H. Perschinka, M. Mayr, G. Millonig, et al., Cross-reactive B-cell epitopes of microbial and human heat shock protein 60/65 in atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 23 (6) (2003) 1060–1065.
[157] J. Zhu, A.A. Quyyumi, D. Rott, et al., Antibodies to human heat-shock protein 60 are associated with the presence and severity of coronary artery disease: evidence for an autoimmune component of atherogenesis, Circulation 103 (8) (2001) 1071–1075.
[158] K. Buhlin, A. Gustafsson, A.G. Pockley, J. Frostegard, B. Klinge, Risk factors for cardiovascular disease in patients with periodontitis, Eur. Heart J. 24 (23) (2003) 2099–2107.
[159] Y.C. Chan, N. Shukla, M. Abdus-Samee, et al., Anti-heat-shock protein 70 kDa antibodies in vascular patients, Eur. J. Vasc. Endovasc. Surg. 18 (5) (1999) 381–385.
[160] M. Ghayour-Mobarhan, A. Sahebkar, S.M. Parizadeh, et al., Antibody titres to heat shock protein 27 are elevated in patients with acute coronary syndrome, Int. J. Exp. Pathol. 89 (3) (2008) 209–215.
[161] A. Ciervo, P. Visca, A. Petrucca, L.M. Biasucci, A. Maseri, A. Cassone, Antibodies to 60-kilodalton heat shock protein and outer membrane protein 2 of *Chlamydia pneumoniae* in patients with coronary heart disease, Clin. Diagn. Lab. Immunol. 9 (1) (2002) 66–74.
[162] J. Mollenhauer, A. Schulmeister, The humoral immune response to heat shock proteins, Experientia 48 (7) (1992) 644–649.
[163] A. Schattner, B. Rager-Zisman, Virus-induced autoimmunity, Rev. Infect. Dis. 12 (2) (1990) 204–222.
[164] U. Feige, W. van Eden, Infection, autoimmunity and autoimmune disease, EXS 77 (1996) 359–373.
[165] A. Veres, Z. Prohaszka, S. Kilpinen, M. Singh, G. Fust, M. Hurme, The promoter polymorphism of the IL-6 gene is associated with levels of antibodies to 60-kDa heat-shock proteins, Immunogenetics 53 (10–11) (2002) 851–856.
[166] Z. Prohaszka, J. Duba, G. Lakos, et al., Antibodies against human heat-shock protein (hsp) 60 and mycobacterial hsp65 differ in their antigen specificity and complement-activating ability, Int. Immunol. 11 (9) (1999) 1363–1370.
[167] Q. Xu, G. Schett, C.S. Seitz, Y. Hu, R.S. Gupta, G. Wick, Surface staining and cytotoxic activity of heat-shock protein 60 antibody in stressed aortic endothelial cells, Circ. Res. 75 (6) (1994) 1078–1085.
[168] A. Veres, T. Szamosi, M. Ablonczy, et al., Complement activating antibodies against the human 60 kDa heat shock protein as a new independent family risk factor of coronary heart disease, Eur. J. Clin. Invest. 32 (6) (2002) 405–410.
[169] D.H. Birnie, E.R. Holme, I.C. McKay, S. Hood, K.E. McColl, W.S. Hillis, Association between antibodies to heat shock protein 65 and coronary atherosclerosis. Possible mechanism of action of *Helicobacter pylori* and other bacterial infections in increasing cardiovascular risk, Eur. Heart J. 19 (3) (1998) 387–394.
[170] K. Heltai, Z. Kis, K. Burian, et al., Elevated antibody levels against Chlamydia pneumoniae, human HSP60 and mycobacterial HSP65 are independent risk factors in myocardial infarction and ischaemic heart disease, Atherosclerosis 173 (2) (2004) 339–346.
[171] K. Burian, Z. Kis, D. Virok, et al., Independent and joint effects of antibodies to human heat-shock protein 60 and *Chlamydia pneumoniae* infection in the development of coronary atherosclerosis, Circulation 103 (11) (2001) 1503–1508.

[172] S. Hoshida, M. Nishino, J. Tanouchi, T. Kishimoto, Y. Yamada, Acute *Chlamydia pneumoniae* infection with heat-shock-protein-60-related response in patients with acute coronary syndrome, Atherosclerosis 183 (1) (2005) 109–112.
[173] T. Huittinen, M. Leinonen, L. Tenkanen, et al., Synergistic effect of persistent *Chlamydia pneumoniae* infection, autoimmunity, and inflammation on coronary risk, Circulation 107 (20) (2003) 2566–2570.
[174] V.Y. Hoymans, J.M. Bosmans, P.L. Van Herck, M.M. Ieven, C.J. Vrints, Implications of antibodies to heat-shock proteins in ischemic heart disease, Int. J. Cardiol. 123 (3) (2008) 277–282.
[175] C.A. Jantos, C. Krombach, F.N. Wuppermann, et al., Antibody response to the 60-kDa heat-shock protein of *Chlamydia pneumoniae* in patients with coronary artery disease, J. Infect. Dis. 181 (5) (2000) 1700–1705.
[176] R.G. Deshpande, M.B. Khan, C.A. Genco, Invasion of aortic and heart endothelial cells by *Porphyromonas gingivalis*, Infect. Immun. 66 (11) (1998) 5337–5343.
[177] K. Tabeta, K. Yamazaki, H. Hotokezaka, H. Yoshie, K. Hara, Elevated humoral immune response to heat shock protein 60 (hsp60) family in periodontitis patients, Clin. Exp. Immunol. 120 (2) (2000) 285–293.
[178] J.I. Choi, S.W. Chung, H.S. Kang, et al., Epitope mapping of *Porphyromonas gingivalis* heat-shock protein and human heat-shock protein in human atherosclerosis, J. Dent. Res. 83 (12) (2004) 936–940.
[179] G. Schett, B. Metzler, R. Kleindienst, et al., Salivary anti-hsp65 antibodies as a diagnostic marker for gingivitis and a possible link to atherosclerosis, Int. Arch. Allergy Immunol. 114 (3) (1997) 246–250.
[180] M. Bongrazio, L. Comini, G. Gaia, T. Bachetti, R. Ferrari, Hypertension, aging, and myocardial synthesis of heat-shock protein 72, Hypertension 24 (5) (1994) 620–624.
[181] Q. Xu, D.G. Li, N.J. Holbrook, R. Udelsman, Acute hypertension induces heat-shock protein 70 gene expression in rat aorta, Circulation 92 (5) (1995) 1223–1229.
[182] D. Elias, D. Markovits, T. Reshef, R. van der Zee, I.R. Cohen, Induction and therapy of autoimmune diabetes in the non-obese diabetic (NOD/Lt) mouse by a 65-kDa heat shock protein, Proc. Natl. Acad. Sci. USA 87 (4) (1990) 1576–1580.
[183] Z. Szilvassy, P. Ferdinandy, J. Szilvassy, et al., The loss of pacing-induced preconditioning in atherosclerotic rabbits: role of hypercholesterolaemia, J. Mol. Cell Cardiol. 27 (12) (1995) 2559–2569.
[184] T. Csont, G. Balogh, C. Csonka, et al., Hyperlipidemia induced by high cholesterol diet inhibits heat shock response in rat hearts, Biochem. Biophys. Res. Commun. 290 (5) (2002) 1535–1538.
[185] A. Shamaei-Tousi, J.W. Stephens, R. Bin, et al., Association between plasma levels of heat shock protein 60 and cardiovascular disease in patients with diabetes mellitus, Eur. Heart J. 27 (13) (2006) 1565–1570.
[186] J. Frostegard, C. Lemne, B. Andersson, R. van der Zee, R. Kiessling, U. de Faire, Association of serum antibodies to heat-shock protein 65 with borderline hypertension, Hypertension 29 (1 Pt 1) (1997) 40–44.
[187] I.M. Rea, S. McNerlan, A.G. Pockley, Serum heat shock protein and anti-heat shock protein antibody levels in aging, Exp. Gerontol. 36 (2) (2001) 341–352.
[188] S. Shams, S. Shafi, K. Bodman-Smith, P. Williams, S. Mehta, G.A. Ferns, Anti-heat shock protein-27 (Hsp-27) antibody levels in patients with chest pain: association with established cardiovascular risk factors, Clin. Chim. Acta 395 (1–2) (2008) 42–46.
[189] I. Kardys, N. Rifai, O. Meilhac, et al., Plasma concentration of heat shock protein 27 and risk of cardiovascular disease: a prospective, nested case – control study, Clin. Chem. 54 (1) (2008) 139–146.

[190] A.G. Pockley, U. de Faire, R. Kiessling, C. Lemne, T. Thulin, J. Frostegard, Circulating heat shock protein and heat shock protein antibody levels in established hypertension, J. Hypertens 20 (9) (2002) 1815–1820.
[191] T. Wu, J. Ma, S. Chen, et al., Association of plasma antibodies against the inducible Hsp70 with hypertension and harsh working conditions, Cell Stress Chaperones 6 (4) (2001) 394–401.
[192] J. Kunes, M. Poirier, J. Tremblay, P. Hamet, Expression of hsp70 gene in lymphocytes from normotensive and hypertensive humans, Acta Physiol. Scand. 146 (3) (1992) 307–311.
[193] N. Vaidya, M. Ghayour-Mobarhan, D.J. Lamb, G.A. Ferns, Anti heat shock protein27 (Hsp27) titres and serum hs-CRP in patients with traditional coronary risk factors including metabolic syndrome (MS), Clin. Chim. Acta 355 (2005) S118.
[194] X. Zhang, M.A. He, L. Cheng, et al., Joint effects of antibody to heat shock protein 60, hypertension, and diabetes on risk of coronary heart disease in Chinese, Clin. Chem. 54 (6) (2008) 1046–1052.
[195] D. Bernhard, G. Pfister, C.W. Huck, et al., Disruption of vascular endothelial homeostasis by tobacco smoke: impact on atherosclerosis, FASEB J. 17 (15) (2003) 2302–2304.
[196] D. Bernhard, C.W. Huck, T. Jakschitz, et al., Development and evaluation of an *in vitro* model for the analysis of cigarette smoke effects on cultured cells and tissues, J. Pharmacol. Toxicol. Methods 50 (1) (2004) 45–51.
[197] D. Bernhard, A. Csordas, B. Henderson, A. Rossmann, M. Kind, G. Wick, Cigarette smoke metal-catalyzed protein oxidation leads to vascular endothelial cell contraction by depolymerization of microtubules, FASEB J. 19 (9) (2005) 1096–1107.
[198] G. Schett, B. Metzler, R. Kleindienst, et al., Myocardial injury leads to a release of heat shock protein (hsp) 60 and a suppression of the anti-hsp65 immune response, Cardiovasc. Res. 42 (3) (1999) 685–695.
[199] H. Kervinen, T. Huittinen, O. Vaarala, et al., Antibodies to human heat shock protein 60, hypertension and dyslipidemia. A study of joint effects on coronary risk, Atherosclerosis 169 (2) (2003) 339–344.
[200] A. Figueredo, J.L. Ibarra, A. Rodriguez, et al., Increased serum levels of IgA antibodies to hsp70 protein in patients with diabetes mellitus: their relationship with vascular complications, Clin. Immunol. Immunopathol. 79 (3) (1996) 252–255.
[201] T.J. Sims, A. Lernmark, L.A. Mancl, R.E. Schifferle, R.C. Page, G.R. Persson, Serum IgG to heat shock proteins and *Porphyromonas gingivalis* antigens in diabetic patients with periodontitis, J. Clin. Periodontol. 29 (6) (2002) 551–562.
[202] A. Shamaei-Tousi, A. Steptoe, K. O'Donnell, et al., Plasma heat shock protein 60 and cardiovascular disease risk: the role of psychosocial, genetic, and biological factors, Cell Stress Chaperones 12 (4) (2007) 384–392.
[203] H.K. Park, E.C. Park, S.W. Bae, et al., Expression of heat shock protein 27 in human atherosclerotic plaques and increased plasma level of heat shock protein 27 in patients with acute coronary syndrome, Circulation 114 (9) (2006) 886–893.
[204] M. Ghayour-Mobarhan, S.A. New, D.J. Lamb, et al., Dietary antioxidants and fat are associated with plasma antibody titers to heat shock proteins 60, 65, and 70 in subjects with dyslipidemia, Am. J. Clin. Nutr. 81 (5) (2005) 998–1004.
[205] T. Huittinen, M. Leinonen, L. Tenkanen, et al., Autoimmunity to human heat shock protein 60, *Chlamydia pneumoniae* infection, and inflammation in predicting coronary risk, Arterioscler. Thromb. Vasc. Biol. 22 (3) (2002) 431–437.
[206] M. Ghayour-Mobarhan, D.J. Lamb, N. Vaidya, C. Livingstone, T. Wang, G.A. Ferns, Heat shock protein antibody titers are reduced by statin therapy in dyslipidemic subjects: a pilot study, Angiology 56 (1) (2005) 61–68.

[207] Y.O. Shin, J.S. Bae, J.B. Lee, et al., Effect of cardiac rehabilitation and statin treatment on anti-HSP antibody titers in patients with coronary artery disease after percutaneous coronary intervention, Int. Heart J. 47 (5) (2006) 671–682.
[208] J. Kocsis, A. Veres, A. Vatay, et al., Antibodies against the human heat shock protein hsp70 in patients with severe coronary artery disease, Immunol. Invest. 31 (3–4) (2002) 219–231.
[209] B. Kwak, F. Mulhaupt, S. Myit, F. Mach, Statins as a newly recognized type of immunomodulator, Nat. Med. 6 (12) (2000) 1399–1402.
[210] A. Rosengren, S. Hawken, S. Ounpuu, et al., Association of psychosocial risk factors with risk of acute myocardial infarction in 11119 cases and 13648 controls from 52 countries (the INTERHEART study): case-control study, Lancet 364 (9438) (2004) 953–962.
[211] J. Lewthwaite, N. Owen, A. Coates, B. Henderson, A. Steptoe, Circulating human heat shock protein 60 in the plasma of British civil servants: relationship to physiological and psychosocial stress, Circulation 106 (2) (2002) 196–201.
[212] J. Yuan, M. Yang, H. Yao, et al., Plasma antibodies to heat shock protein 60 and heat shock protein 70 are associated with increased risk of electrocardiograph abnormalities in automobile workers exposed to noise, Cell Stress Chaperones 10 (2) (2005) 126–135.
[213] J. Zhu, R.J. Katz, A.A. Quyyumi, et al., Association of serum antibodies to heat-shock protein 65 with coronary calcification levels: suggestion of pathogen-triggered autoimmunity in early atherosclerosis, Circulation 109 (1) (2004) 36–41.
[214] M. Mukherjee, B.C. De, D. Jewitt, V.V. Kakkar, Association of antibodies to heat-shock protein-65 with percutaneous transluminal coronary angioplasty and subsequent restenosis, Thromb. Haemost. 75 (2) (1996) 258–260.
[215] Q. Xu, G. Luef, S. Weimann, R.S. Gupta, H. Wolf, G. Wick, Staining of endothelial cells and macrophages in atherosclerotic lesions with human heat-shock protein-reactive antisera, Arterioscler. Thromb. 13 (12) (1993) 1763–1769.
[216] I. Herz, R. Rosso, A. Roth, G. Keren, J. George, Serum levels of anti heat shock protein 70 antibodies in patients with stable and unstable angina pectoris, Acute Card. Care 8 (1) (2006) 46–50.
[217] S. Vogt, I. Portig, B. Kusch, et al., Detection of anti-hsp70 immunoglobulin G antibodies indicates better outcome in coronary artery bypass grafting patients suffering from severe preoperative angina, Ann. Thorac. Surg. 78 (3) (2004) 883–889.
[218] J. Kramer, P. Harcos, Z. Prohaszka, et al., Frequencies of certain complement protein alleles and serum levels of anti-heat-shock protein antibodies in cerebrovascular diseases, Stroke 31 (11) (2000) 2648–2652.
[219] Z. Prohaszka, J. Duba, L. Horvath, et al., Comparative study on antibodies to human and bacterial 60 kDa heat shock proteins in a large cohort of patients with coronary heart disease and healthy subjects, Eur. J. Clin. Invest. 31 (4) (2001) 285–292.
[220] A. Veres, G. Fust, M. Smieja, et al., Relationship of anti-60 kDa heat shock protein and anti-cholesterol antibodies to cardiovascular events, Circulation 106 (22) (2002) 2775–2780.
[221] G. Gromadzka, J. Zielinska, D. Ryglewicz, U. Fiszer, A. Czlonkowska, Elevated levels of anti-heat shock protein antibodies in patients with cerebral ischemia, Cerebrovasc. Dis. 12 (3) (2001) 235–239.
[222] X. Jin, C. Xiao, R.M. Tanguay, et al., Correlation of lymphocyte heat shock protein 70 levels with neurologic deficits in elderly patients with cerebral infarction, Am. J. Med. 117 (6) (2004) 406–411.
[223] H.L. Staub, G.L. Norman, T. Crowther, et al., Antibodies to the atherosclerotic plaque components beta2-glycoprotein I and heat-shock proteins as risk factors for acute cerebral ischemia, Arq. Neuropsiquiatr. 61 (3B) (2003) 757–763.
[224] G.K. Hansson, Immunological markers of atherosclerosis, Lancet 341 (8840) (1993) 278.

[225] I.U. Khan, R. Wallin, R.S. Gupta, G.M. Kammer, Protein kinase A-catalyzed phosphorylation of heat shock protein 60 chaperone regulates its attachment to histone 2B in the T lymphocyte plasma membrane, Proc. Natl. Acad. Sci. USA 95 (18) (1998) 10425–10430.
[226] R. Gruber, S. Lederer, U. Bechtel, S. Lob, G. Riethmuller, H.E. Feucht, Increased antibody titers against mycobacterial heat-shock protein 65 in patients with vasculitis and arteriosclerosis, Int. Arch. Allergy Immunol. 110 (1) (1996) 95–98.
[227] D. Harats, N. Yacov, B. Gilburd, Y. Shoenfeld, J. George, Oral tolerance with heat shock protein 65 attenuates Mycobacterium tuberculosis-induced and high-fat-diet-driven atherosclerotic lesions, J. Am. Coll. Cardiol. 40 (7) (2002) 1333–1338.
[228] R. Maron, G. Sukhova, A.M. Faria, et al., Mucosal administration of heat shock protein-65 decreases atherosclerosis and inflammation in aortic arch of low-density lipoprotein receptor-deficient mice, Circulation 106 (13) (2002) 1708–1715.
[229] G. Foteinos, A.R. Afzal, K. Mandal, M. Jahangiri, Q. Xu, Anti-heat shock protein 60 autoantibodies induce atherosclerosis in apolipoprotein E-deficient mice via endothelial damage, Circulation 112 (8) (2005) 1206–1213.
[230] C. Barrios, A.R. Lussow, E.J. Van, et al., Mycobacterial heat-shock proteins as carrier molecules. II: the use of the 70-kDa mycobacterial heat-shock protein as carrier for conjugated vaccines can circumvent the need for adjuvants and Bacillus Calmette Guerin priming, Eur. J. Immunol. 22 (6) (1992) 1365–1372.
[231] R. Perraut, A.R. Lussow, S. Gavoille, et al., Successful primate immunization with peptides conjugated to purified protein derivative or mycobacterial heat shock proteins in the absence of adjuvants, Clin. Exp. Immunol. 93 (3) (1993) 382–386.
[232] B. Sander, U. Skansen-Saphir, O. Damm, L. Hakansson, J. Andersson, U. Andersson, Sequential production of Th1 and Th2 cytokines in response to live bacillus Calmette–Guerin, Immunology 86 (4) (1995) 512–518.
[233] A.S. Mustafa, K.E. Lundin, F. Oftung, Human T cells recognize mycobacterial heat shock proteins in the context of multiple HLA-DR molecules: studies with healthy subjects vaccinated with *Mycobacterium bovis* BCG and *Mycobacterium leprae*, Infect. Immun. 61 (12) (1993) 5294–5301.
[234] P.J. Ford, K. Yamazaki, G.J. Seymour, Cardiovascular and oral disease interactions: What is the evidence? Prim. Dent. Care 14 (2) (2007) 59–66.