

Chronic Infections and Atherosclerosis

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Abstract The immune response against heat shock protein 60 (HSP60) derived from pathogens causing chronic infections is thought to be an important pro-atherogenic mechanism because high serum levels of antibodies against HSP60 have been associated with atherosclerotic diseases, such as coronary artery diseases, or cerebro-vascular events. Furthermore, the presence of HSP60-specific T lymphocytes in circulation may increase the risk of atherosclerosis. Our recent in vitro and in vivo studies have also shown an association of *Helicobacter pylori*-HSP60 (Hp-HSP60) specific Th1 immune responses elicited by *H.*

pylori infection with the progression of atherosclerosis in a hyperlipidemic mouse model. These Th1 dominant immune responses may cross-react with endogenous HSP60 expressed on stressed cells of the vascular endothelium, likely due to molecular mimicry. However, the exact mechanisms by which endothelial cells display their HSP60 molecule or present HSP60 antigenic epitopes on the surface are still unclear.

Keywords Atherosclerosis · Heat shock protein 60 · *Helicobacter pylori*

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Introduction

Atherosclerosis is a chronic inflammatory process of the arteries resulting in angina pectoris, myocardial infarction, and cerebral infarction. In the last decade, experimental evidence has demonstrated an active involvement of inflammatory and immune mechanisms in atherogenesis [1, 2]. Several epidemiological studies have revealed that host immune reaction against persistent infectious pathogens such as *Chlamydia pneumoniae*, *Porphyromonas gingivalis*, and *cytomegalovirus*, may promote the development of atherosclerosis [3–6]. Notably, that immune response against pathogen-derived heat shock protein 60 (HSP60) contribute to atherogenesis [7, 8].

HSP60 is a highly conserved protein found in both eukaryotic and prokaryotic organisms. Analysis of the crystal structure of *Escherichia coli* HSP60 (GroEL) showed a porous cylinder of 14 subunits made of two nearly sevenfold rotationally symmetrical rings stacked back-to-back with dyad symmetry [9]. The GroEL complex functioned as the chaperone together with GroES [10]. Further, the high degree of homology between microbial and human HSP60

has led to the concept that molecular mimicry between the microbial and endogenous HSP60 may be involved in the pathogenesis of some autoimmune disease [11–13]. In atherogenesis, cellular and humoral immunity against HSP60 derived from infectious pathogens could promote the development of atherosclerosis because the immune systems may recognize endogenous HSP60 expressed on the surface of endothelial cells [14–16].

Helicobacter pylori, a known pathogen for chronic gastritis, gastroduodenal ulcer, gastric cancer, and MALT lymphoma, is thought to be one of the pro-atherogenic agents [17, 18]. Half of the human population is actually infected with *H. pylori* and most of them just have asymptomatic chronic gastritis. Local inflammatory and immune responses against *H. pylori* in the stomach can induce systemic (including atherosclerotic lesions) immune reactions due to the chronic nature of the infection. In fact, chronic *H. pylori* infection generates IgG anti-*H. pylori*-HSP60 (*Hp*-HSP60) antibodies [19], and induces Th1-polarization of T lymphocytes [20]. Patients with atherosclerosis occasionally have circulating IgG that cross-react with human (endogenous) HSP60 [21], with Th1-dominant T lymphocytes infiltrating their atherosclerotic lesions [22]. Indeed, our in vivo and in vitro investigations have revealed that immune responses against *Hp*-HSP60 could promote atherogenesis with chronic *H. pylori* infection [23, 24].

However, several previous studies have shown controversial results regarding the relationship between *H. pylori* infections and development of atherosclerosis [18, 25–27]. One hypothesis claims that *H. pylori* may be indirectly involved in the development of atherosclerosis due to chronic permanence in the gastrointestinal tract, while *C. pneumoniae* and CMV can directly disseminate into the arterial vessel walls [17]. We focused our attention on the immuno-reactive participants and how they interact with HSP60 on endothelial cells.

In this paper, we review previous reports regarding the relationship between humoral or cellular immunity against HSP60 and atherosclerosis, and also describe recent observations regarding alternative antigen presenting mechanisms in which cellular immunity to *Hp*-HSP60 may be involved.

Humoral immunity to HSP60 and atherosclerosis

There have been many arguments put forward that humoral immune reactions against HSP60 are related to atherogenesis. In 1993, Xu et al. [28] demonstrated the correlation between intima-media thickness of carotid arteries and antibody titers against mycobacterial HSP65 (mHSP65), which paradigmatically was included as bacterial HSP60. They also confirmed the association between high antibody titers against mHSP60 and mortality from coronary artery

disease (CAD) [29]. Since then, many groups have verified the atherogenic role of antibodies against different HSP60s (Table 1) [30]. Presently, antibodies against HSP60s have been well established as risk factors for CAD prevalence, incidence, and mortality. According to the hypothesis of autoantibody-mediated atherogenesis, these antibodies would potentially cross-react with human HSP60 and disrupt endothelial cells. Several in vitro studies have demonstrated that HSP60 molecules are expressed on the endothelium and complement-dependent cytotoxicity against endothelial cells activated by anti-HSP60 antibodies [15, 16, 31]. However, not all studies demonstrated positive cytotoxicity against endothelial cells [32–34]. This discrepancy might be due to limited subclasses of antibodies, which react with certain epitopes shared by human and infectious-derived HSP60, and that could activate complement to disrupt endothelial cells. Our clinical studies showed that the titers of IgG2 antibodies against specific peptides of *Hp*-HSP60 (Glu¹⁴¹-Leu¹⁶⁰) share the amino acid sequences with human HSP60. In a recent mice study, we also found that anti-*Hp*-HSP60 IgG2a antibody titers correlated with the severity of atherosclerosis. The endothelium in atherosclerotic lesions was strongly stained with monoclonal and polyclonal antibodies generated by immunization with *Hp*-HSP60. Nevertheless, sera obtained from mice infected with *H. pylori* were no cytotoxic for mice endothelial cells. Thus, we assumed that autoantibodies to HSP60s have little effect on atherogenesis and were merely immunological “bystanders” produced in *H. pylori* infected mice.

Table 1 Clinical studies on anti-HSP60/65 antibodies and atherosclerosis

Investigators	Disease	Cases	Odds ratio/ <i>P</i>
Xu et al. 1993	Carotid AS	867	1.52 (1.09–2.02)
Gruber et al. 1996	Vasculitis	107	<i>P</i> <0.001
Hoppichler et al. 1996	CAD, MI	203	<i>P</i> <0.05
Mukherjee et al. 1996	CAD	28	<i>P</i> =0.036
Frostegard et al. 1997	Hypertension	66	<i>P</i> =0.034
Birnie et al. 1998	CAD	136	<i>P</i> =0.012
Prohaszka et al. 1999	CAD	74	<i>P</i> <0.0001
Xu et al. 1999	Carotid AS	750	1.42 (1.02–1.98)
Zhu et al. 2001	CAD	274	1.86 (1.13–3.04)
Burian et al. 2001	CAD	276	2.6 (1.3–5.0)
Gromadzka et al. 2001	stroke	180	<i>P</i> <0.0001
Prohaszka et al. 2001	CAD	424	<i>P</i> <0.007
Ciervo et al. 2002	CAD	179	<i>P</i> <0.05
Huittinen et al. 2002	CAD	239	2.0 (1.1–3.6)
Veres et al. 2002	CV	386	2.1 (1.2–3.9)
Biasucci et al. 2003	CAD	100	<i>P</i> <0.0001
Huittinen et al. 2003	CAD	241	2.1(1.08–4.3)

AS Atherosclerosis, CAD coronary artery disease, CV cardiovascular events, MI myocardial infarction

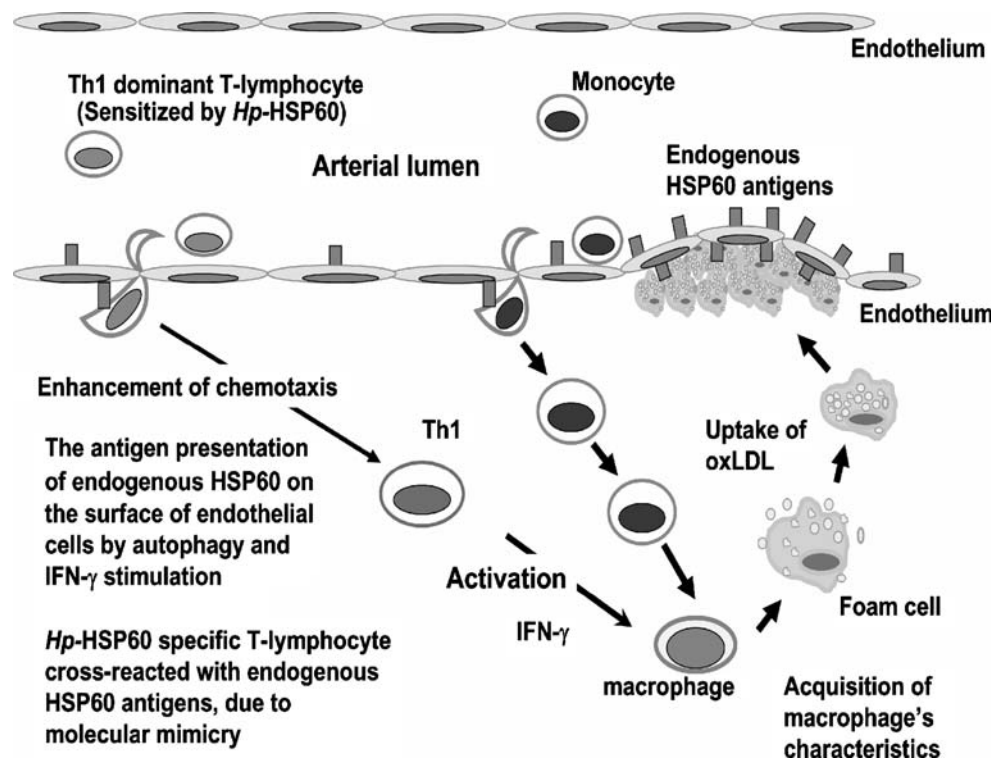
Cellular immune response to HSP60 and atherosclerosis

The pathogenicity of HSP60 reactive T lymphocytes is still the subject of intensive research. Epidemiologically, the ARMY study [35] demonstrated that specific T cell reactivity against HSP60 is an independent major risk factor for early intima-media thickening in clinically healthy young males. In animal models, George et al. [36] reported that mHSP65 specific T lymphocytes contribute to atherogenesis by T-cell transfer experiments. Since then, several animal models have been used to assess the influence of cellular immune response in atherogenesis. Recent studies have clarified that Th1 differentiation of naïve T lymphocytes contributed to atherogenesis [37]. In this context, we examined *H. pylori* infected *apoe*^{+/-+/-} mice. In contrast to Mach et al. [26], who did not find increased atherosclerotic plaques in *H. pylori* infection in atherosclerosis-prone *ldlr*^{-/-} mice, we clearly showed that *H. pylori* infection resulted in significantly expanded atherosclerotic lesions in *apoe*^{+/-}*ldlr*^{+/-} mice [23].

Our studies also indicate that systemic Th1-immune response against *Hp*-HSP60 induced by *H. pylori* infection in the gastric mucosa may promote atherosclerosis through circulating *Hp*-HSP60-reactive T cells (Th1). Due to extremely high homology in amino acid sequences between *Hp*-HSP60 and mammalian endogenous HSP60 [38], *Hp*-HSP60-specific Th1 lymphocytes sensitized in the gastric mucosa could be activated and be attracted by endogenous HSP60 on the endothelium. Endothelial cells ubiquitously

express endogenous HSP60 on their cell membrane in response to mechanical stress such as blood pressure, hypercholesterolemia, cytokines, or low shear-stress [39]. However, the exact character of HSP60-specific T-cell clones and their epitopes are now intensively researched by many groups. Detail mechanisms of how pathogen-derived HSP60-specific T cells could penetrate into the intima and differentiate into Th1 lymphocytes are also being investigated. In some studies, HSP60-specific T cells from atherosclerotic lesions were mainly major histocompatibility complex (MHC) class II-restricted CD4⁺ T-cell clones regardless of the pathogen [40]. And, some putative peptides from human HSP60 were detected as part of CD4⁺ T-cell epitopes that could generate autoimmune atherogenesis [41]. In one of our recent studies, immunization with *Hp*-HSP60 modulated the Th1/Th2 balance (i.e., reduction in Th1-immune response) resulting in reduced atherosclerotic plaque size (i.e., anti-atherogenic effect). Eradication therapy to *H. pylori*-infected mice also successfully reduced atherosclerosis by the modulation of the Th1/Th2 immune balance. Thus, reduction of *Hp*-HSP60 specific Th1 (CD4⁺ T cell) prevented atherosclerosis. In other recent in vitro study, re-activated *Hp*-HSP60-specific Th1 lymphocytes from *H. pylori*-infected mice migrated beyond endothelial cell barrier, while non-activated lymphocytes from *H. pylori*-infected mice did not. When the endothelial cell barrier was treated with anti-HSP60 IgG antibodies from mice immunized with *Hp*-HSP60, re-activated *Hp*-HSP60-specific Th1 lymphocytes did not have the ability of transendothelial migration. These observations

Fig. 1 Schematic representation of possible pathogenic mechanisms atherosclerosis caused by *H. pylori* infection



implied that Th1 differentiation and antigen presentation were indispensable for T lymphocytes to penetrate endothelial cell barrier (preparing for submission). The discrepancy that pathogen HSP60-specific CD4⁺ T cells are not usually re-activated by endogenous antigens (human HSP60) in MHC class II-dependent manner, but CD8⁺ cells in MHC class I do, remains unexplained.

HSP60 antigen presentation on endothelial cells

Endothelial cells usually express HSP60 intracellularly and on their cell membrane in response to mechanical stress such as blood pressure or low shear-stress [42]. Therefore, previous investigations have focused on the interaction between anti-HSP60 antibodies and endothelial cells and between $\gamma\delta$ T lymphocyte and endothelial cells, which were evoked without MHC restriction [43]. HSP60 intrinsically forms giant complexes and functions as chaperon intracellularly. So, the whole molecule of HSP60 with native conformation could not be presented on the surface of endothelial cells. Zal et al. [44] demonstrated that CD4⁺ CD28^{null} T cells increased in unstable plaques and acted like natural killer cells by up-regulating IFN- γ and perforin with HSP60 stimulation. This evidence impacts the cytotoxicity of endothelium prior to MHC class II-restricted HSP60-specific CD4⁺ T-cell activation. Furthermore, our latest investigations revealed the ability of endothelial cells to present HSP60 antigens on MHC class II molecules by autophagy and stimulation with IFN- γ (preparing for submission). Anyway, further studies should be necessary to resolve the mechanisms of HSP60 antigen presentation by endothelial cells.

Conclusions

Many studies had shed some light on *H. pylori* infection causing not only chronic disease in the stomach, such as gastritis ulcer and MALT lymphoma, but also atherosclerosis. We propose a possible mechanism (Fig. 1) by which *H. pylori* infection of the gastric mucosa accelerates atherosclerosis: (1) *H. pylori* activates resident macrophages and lymphocytes in the gastric mucosa, followed by induction of systemic immune responses against *Hp*-HSP60. (2) Circulating *Hp*-HSP60-specific T lymphocytes which have Th1 dominance affect the endothelium of arteries by releasing IFN- γ and promote endothelial cells to present HSP60 antigens on their surface. The re-activated HSP60-specific T lymphocytes penetrate in the intima. (3) They contribute to the macrophage-derived foam cell formation by activating macrophages. (4) Antibiotic therapy and passive transfer of anti-*Hp*-HSP60 antibodies may

be most effective treatment for atherosclerosis. However, many issues still remain to be clarified in future studies.

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