PLoS PATHOGENS

Review

Chlamydia trachomatis Infection and Anti-Hsp60 Immunity: The Two Sides of the Coin

Francesco Cappello[1,2]*, Everly Conway de Macario[3], Valentina Di Felice[1], Giovanni Zummo[1], Alberto J. L. Macario[3]

1 Dipartimento di Medicina Sperimentale, Sezione di Anatomia Umana "Emerico Luna", Università degli Studi di Palermo, Palermo, Italy, 2 Istituto EuroMEditerraneo di Scienza e Tecnologia (IEMEST), Palermo, Italy, 3 Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, Maryland, United States of America

Abstract: *Chlamydia trachomatis* (CT) infection is one of the most common causes of reproductive tract diseases and infertility. CT-Hsp60 is synthesized during infection and is released in the bloodstream. As a consequence, immune cells will produce anti-CT-Hsp60 antibodies. Hsp60, a ubiquitous and evolutionarily conserved chaperonin, is normally sequestered inside the cell, particularly into mitochondria. However, upon cell stress, as well as during carcinogenesis, the chaperonin becomes exposed on the cell surface (sf-Hsp60) and/or is secreted from cells into the extracellular space and circulation. Reports in the literature on circulating Hsp and anti-Hsp antibodies are in many cases short on details about Hsp60 concentrations, and about the specificity spectra of the antibodies, their titers, and their true, direct, pathogenetic effects. Thus, more studies are still needed to obtain a definitive picture on these matters. Nevertheless, the information already available indicates that the concurrence of persistent CT infection and appearance of sf-Hsp60 can promote an autoimmune aggression towards stressed cells and the development of diseases such as autoimmune arthritis, multiple sclerosis, atherosclerosis, vasculitis, diabetes, and thyroiditis, among others. At the same time, immunocomplexes composed of anti-CT-Hsp60 antibodies and circulating Hsp60 (both CT and human) may form deposits in several anatomical locations, e.g., at the glomerular basal membrane. The opposite side of the coin is that pre-tumor and tumor cells with sf-Hsp60 can be destroyed with participation of the anti-Hsp60 antibody, thus stopping cancer progression before it is even noticed by the patient or physician.

Hsp60, a Ubiquitous Molecule with Multiple Roles in Health and Disease

Hsp60 is a Group I chaperonin highly conserved during evolution with essential roles in cells and tissues [1–4]. In eukaryotes, this chaperonin is usually described as a mitochondrial molecule that works together with its co-chaperonin, Hsp10, to assist in the correct folding of other mitochondrial proteins. The two chaperonins assemble and form an "American football–shaped" molecular complex, a structure that is efficient for correctly folding other proteins, i.e., "client polypeptides" [5]. The Hsp60/Hsp10 complex is typically formed of a double ring-shaped Hsp60 oligomer of 14 monomers and a dome-shaped Hsp10 single ring of seven monomers. Each Hsp60 monomer displays three domains: apical, intermediate, and equatorial [6].

Mammalian Hsp60 has been well characterized [7] and, in humans, its gene resides on Chromosome 2 [8]. Hsp60 proteins are highly conserved in evolution and, therefore, those of

eukaryotes and prokaryotes share numerous identical amino acids [2,7]. This high similarity in primary structure implies common antigenic sites (henceforth called epitopes) that elicit and react with crossreactive antibodies [9,10]. This is the reason why exogenous Hsp60 from a microbe can elicit an immune response in humans, a response that although directed primarily against the microbial molecule also reacts with the endogenous chaperonin [11], providing a link between infection and development of autoimmune diseases, as postulated for arthritis [12–14], multiple sclerosis [15–17], and diabetes [9,18,19]. These findings have stimulated interest in Hsp60 in physicians willing to understand the molecular basis of disease. However, from the literature it appears that research on circulating Hsp60 and anti-Hsp60 antibodies has been marred by a lack of rigorous quantification of the chaperonin concentrations and antibody titers in plasma or serum. Furthermore, the specificity spectrum of the anti-Hsp60 antibodies has not usually been determined and, therefore, it is difficult if not impossible to know the range of antigens, namely human and bacterial chaperonins, recognized by the antibodies and with what avidity. Because of the two limitations mentioned above, and because of the inherent difficulty in devising reliable strategies to obtain direct correlations between antibody levels and extension of pathological lesions and clinical status in representative samples of pathological specimens and patients and adequate controls, conclusions about the role of anti-Hsp60 antibodies in the onset and progression of disease must be taken as provisory and subject to challenge with further investigation. The main aim of this review is to make pathologists and clinicians fully aware of the existence and pathogenetic potential, which we assume is quite high, of anti-Hsp60 antibodies, but at the same time the review intends to raise awareness on the limitations of previous studies and, thus, encourage new ones more quantitative and accurate in terms of specificity and avidity of the antibodies. In summary, this review presents a field with exciting prospects but

full of traps that ought to be recognized within what has been done in the past and what should be avoided in future research.

Surface and Secreted Hsp60 Can Activate the Immune System

Although Hsp60 is primarily considered a mitochondrial protein, in mammals 20% to 40% of cellular Hsp60 occurs in extra-mitochondrial sites (Table 1) [20–23]. The presence of Hsp60 on the cell membrane's surface (sfHsp60) has been noted in normal [24], stressed [25], and tumor cells [26–28] and was thought to be associated with membrane transport and signaling [24,29]. An increase in sfHsp60 levels is considered a danger signal for the immune system in as much as it leads to activation and maturation of dendritic cells and generation of an antitumor T cell response [27,30]. Hsp60 is a ligand of Toll-like receptor 4, part of the innate immune system, and sfHsp60 expression positively correlates with the triggering of apoptotic phenomena [31]. In addition, the expression of sfHsp60 on the lymphocytic membrane has been associated with spontaneous apoptosis and cell lysis [32,33]. Therefore, Hsp60 translocation through the plasma membrane should not be considered just as a passive, inconsequential event, but as a key step in the pathogenesis of immune system–mediated disorders (Table 2).

Hsp60 is also secreted from cells and thus reaches the interstitial fluid and the bloodstream [34]. The levels of Hsp60 in plasma of healthy subjects vary over a wide range from undetectable up to over 1,000 ng/mL; nevertheless, plasma levels in any single individual are rather stable, probably because they are under genetic control [35]. Because of this individual stability of Hsp60 plasma levels when changes appear in any given person, they most probably indicate that something is abnormal. This is one important reason why circulating Hsp60 has recently become a potentially useful marker for clinicians, worth measuring in sera of patients affected by a variety of diseases, as will be discussed later. However, due to the variations in Hsp60 levels among individuals, quantification of circulating chaperonin in populations of patients and controls must be carefully done and repeated to obtain representative samples of data amenable to rigorous statistical analysis that, in turn, will provide a satisfactory basis for assessing correlations of Hsp60 levels with pathology.

Hsp60 Can Be Elevated in Tumors and Cardiovascular Diseases

Previous studies have revealed that the levels of cytosolic Hsp60 in vivo gradually increase during carcinogenetic steps, from normal tissue to dysplasia to fully developed carcinoma, in various organs: uterine exocervix [36], large bowel [37], and prostate [38]. In contrast, in other malignancies cytosolic Hsp60 was found to decrease during carcinogenesis as compared with normal tissue in tumors of the tongue [39], bladder [40], and airways [41,42].

Table 1. Hsp60 Locations and Functions.

Location	Function(s)	Reference
Mitochondrion	Protein folding	[5,6]
Cytosol	Control of signal transduction, apoptosis, senescence, glycolysis	[20–23]
Cell membrane	Membrane transport, cell–cell signaling, immune system alerting	[24–33]
Intercellular interstitium	Either pro- or anti-inflammatory	[25,49,50,59,61–63]

doi:10.1371/journal.ppat.1000552.t001

sfHsp60 occurs in the cell membrane of certain types of tumors [28], where it is associated with p21ras protein [43] and also with alpha-3-beta-1 integrin, which is involved in the adhesion of metastatic breast cancer cells to lymph nodes and bone tissue [44]. It has also been shown that sfHsp60 plays a role in the metastatization of pancreatic carcinoma [26]. sfHsp60 occurs on the membrane of oral tumor cells and seemingly participates in the mechanism of the tumor cell lysis induced by gammadelta T lymphocytes [45]. Experiments in vitro with a number of tumor cells have shown that photodynamic therapy can induce an increase in Hsp60 [46,47] and membrane surface localization [48].

In addition to carcinogenesis, Hsp60 has been associated with several other pathologies, for example, with atherosclerosis (ATS), a disease that can be serologically monitored by measuring Hsp60 and anti-Hsp60 antibodies [49]. It has been shown that soluble Hsp60 plays a role in activating vascular and immune cells during ATS development [49], and that the levels of complement-activating anti-Hsp60 antibodies are elevated in ATS-related diseases [50]. Hsp60 has been detected on the surface of stressed endothelial cells [51,52] and, therefore, these cells become susceptible to complement-dependent lysis by anti-Hsp60 antibodies. In light of these findings, ATS has been proposed as an "autoimmune disease due to an immune reaction against Hsp60" [53]. Nevertheless, for the reasons mentioned earlier about quantification of Hsp60 and anti-Hsp60 antibodies, one has to be aware that the role of Hsp60 in ATS pathogenesis is still under scrutiny. For example, while in teenagers a positive correlation was revealed between early ATS and Hsp60 levels [54], the picture is not clear in older individuals with symptomatic ATS, because in these older patients there are so many other variables that must be considered in relation to ATS that to make reliable correlations between plasma Hsp60 levels and disease is practically impossible.

Recently, it has also been shown that stressed myocardiocytes excrete Hsp60 by the exosomal pathway [34], which may reflect the increase in myocardial levels of Hsp60 that double by the end-stage of heart failure [55]. A chronic injury of progressive heart failure resulted from the localization of Hsp60 in the plasma membrane [25]. Moreover, levels of sfHsp60 were positively correlated with myocardiocyte apoptosis and with the release of the chaperonin into circulation, resulting in the activation of the innate immune system with generation of a pro-inflammatory process in the myocardial interstitium [25].

Since sfHsp60 might be involved in the pathogenesis of ATS, by extension one can infer that this chaperonin is implicated in the pathogenesis of cerebrovascular disorders, such as stroke. In this regard it is noteworthy that Hsp60 levels and distribution are altered in various central nervous system conditions that are not primarily due to the failure of blood circulation, such as Alzheimer, Parkinson, and Huntington diseases, which suggests a participation of the chaperonin in the pathogenesis of these diseases unrelated to vascular pathology [56]. It has been shown that Hsp60 expression in cultured human adult astrocytes is induced by cytokines, i.e., interleukins IL-1β, IL-4, IL-6, and IL-10, and TNF-alpha, which leads to the suggestion that Hsp60 plays an important role also in the pathogenesis of autoimmune diseases of the nervous system, like multiple sclerosis [57].

Anti-Hsp60 antibodies have been found to occur in a number of systemic autoimmune disease–associated vasculitides, such as Takayasu arteritis, polyarteritis nodosa, Wegener granulomatosis, and systemic lupus erythematosus [58]. In all of these conditions, however, for the reasons mentioned earlier, the exact nature (specificity) and role of the anti-Hsp60 antibodies in pathogenesis is still incompletely understood.

Table 2. Pathologic Conditions in Which Surface Hsp60 Has Been Correlated with Pathogenesis.

Condition	Cell	Role	Reference
Cancer	Tumor	Involved in metastatization to lymph nodes and bones and antitumor immune response activation	[26,44–48]
Atherosclerosis	Endothelial	Confers susceptibility to complement-dependent cell lysis	[50–52]
Heart failure	Myocardiocyte	Promotes myocyte apoptosis and pro-inflammatory status in myocardium	[25,34,53,55]
Diabetes	Beta, (insulitis)	Becomes target for T cell–mediated beta-cell destruction	[66]

doi:10.1371/journal.ppat.1000552.t002

Other Diseases Potentially Related to Hsp60

Hsp60 has also been implicated in the pathogenesis of degenerative joint diseases such as rheumatoid arthritis (RA) [59]. This is an autoimmune disorder with pathogenesis and outcome influenced by the balance between the activities of Th-1 and Th-2 cells. Th-1 activation induces secretion by RA synovial-fluid mononuclear cells of pro-inflammatory cytokines such as IL-1 and TNF-alpha, with consequent cartilage damage, whereas Th-2 activation promotes secretion of IL-4, inhibiting Th-1 activity and diminishing inflammation and cartilage damage [60]. In this respect, it is interesting that mycobacterial Hsp60 activates Th-1 production of IL-1 and TNF-alpha, which suppress cartilage proteoglycan synthesis and contribute to cartilage damage [61]. In contrast, human Hsp60 stimulates Th-2 production of IL-4 and determines a lower release of IL-1 and TNF-alpha by Th-1 cells in comparison to non-human Hsp60-stimulated Th-1 cells [62]. It has been postulated that a humoral response against bacterial chaperonin can elicit a crossreaction against the infected host's Hsp60, thus perpetuating local inflammation and destructive processes in cartilage [59,63]. All of these data, confirmed also in experimental models of adjuvant arthritis [64], suggest that human, but not bacterial, Hsp60 contributes to suppressing inflammation. Unfortunately, the human chaperonin can also serve as an autoantigen in pathological lesions that may attract antibodies, thus contributing to inflammation and tissue destruction. Such considerations have to be taken into account when thinking of therapeutic uses of Hsp60.

Under physiologic conditions, pancreatic beta-cells show Hsp60 only in mitochondria and secretory granules [65], but in pancreatic islets affected by insulitis the chaperonin migrates towards the cytoplasm as well as to the plasma membrane, in which it can be detected by the immune system that, consequently, mounts an immune response [66]. Since it was realized that Hsp60 is one of the most relevant self-antigens for diabetogenic T cell clones, the chaperonin peptide DiaPep277 has been used to slow down beta-cell damage after the clinical onset of diabetes, both in non-obese diabetic mice and human adults [67,68]. The first results seem encouraging, but further clinical trials are currently in progress to complete the validation of this therapeutic approach.

It has also been hypothesized that Hsp60 plays a role in the pathogenesis of thyroid and adrenal immune diseases characterized by a proliferation of oncocytes, i.e., intensely eosinophilic cells with granular cytoplasm and a very large number of mitochondria [69]. Lately, the chaperonin has been described as a relevant disease-related autoantigen in autoimmune glomerulonephritis [70], juvenile dermatomyositis [71], and both plaque and guttate psoriasis [72].

Microbial Hsp60, a Strong and Potentially Harmful Antigen

Numerous infections caused by bacteria, fungi, and mycobacteria can trigger an immune reaction against the microbial Hsp60 with the generation of anti-Hsp60 antibodies [73]. In this regard, it is important to bear in mind that anti-Hsp60 antibodies can also be found in healthy subjects (likely elicited by the chaperonin from microbes in the normal digestive tract flora for instance), representing an early non-specific defense mechanism against pathogens [10]. The sharing of considerable similarity in the primary structure of microbial and host Hsp60 predicts antigenic crossreactivity and development of immune reactions against both proteins [11].

Humoral immune reactions to bacterial Hsp60, such as those from *Chlamydia pneumonia* (CP) and *Escherichia coli* have been suggested to be involved in the process of vascular endothelial injury during ATS pathogenesis [74]. It has been stated that the risk of crossreactivity between the microbial chaperonin and the human counterpart on the surface of stressed cells of the arterial endothelium is the cost the organism has to pay for protective immunity against microbial molecules [75]. Infections by *Porphyromonas gingivalis*[76–78] and *Helicobacter pylori*[79] have been correlated with a higher risk of development of coronary ATS, due to the high crossreactivity of anti-microbial Hsp60 antibodies with human Hsp60. For similar reasons, *E. coli* Hsp60 has also been implicated in the pathogenesis of autoimmune rheumatic [80] and pancreatic [81] diseases, and Sjogren syndrome [82]. Moreover, an increase in the levels of autoantibodies against endogenous Hsp60 precedes the onset of diabetes in cystic fibrosis patients; hyperimmunization with bacterial Hsp60 caused an increase in anti-Hsp60 autoantibodies that was followed by glucose intolerance [83].

Chlamydia trachomatis Hsp60 Is Abundantly Produced during Persistent Infections

Among pathogens, *Chlamydia trachomatis* (CT) is an intracellular bacterium responsible for a sexually transmitted disease. Approximately 4 million cases of CT infections are estimated to occur annually in the United States, although only about one quarter of those cases are diagnosed and treated [84].

CT includes three human biovars: trachoma (serovars A, B, and Ba or C), urethritis (serovars D-K), and lymphogranuloma venereum (LGV; serovars L1, 2, and 3) [85]. Each CT biovar can cause from mild to severe symptoms. Some infected individuals are asymptomatic, which allows spread from person to person before the infection is detected. In the US in 1990, the direct costs of treating CT infections and its complications were estimated at US$4.2 billion [86], while projected costs for the years 2000s were US$10 billion [87]. These figures clearly demonstrate the magnitude of the health and social problems caused by CT.

CT penetrates into epithelial cells as an elementary body and then converts to a reticulate body, the replicating form of the pathogen. During persistent infections, CT produces a large quantity of Hsp60 (CT-Hsp60) [88], which has been implicated in the pathogenesis of autoimmune disorders such as reactive arthritis

[89,90]. CT-Hsp60 might exert an antiapoptotic effect in nascent tumor cells, which would contribute to female genital tract oncogenesis [91,92].

There are three CT-Hsp60 isoforms: CT-Hsp60-1, mainly found in the reticulate bodies, and CT-Hsp60-2 and CT-Hsp60-3, which are released extracellularly [93]. Prolonged exposure of the immune system to any of the CT-Hsp60 isoforms leads to immune system activation and antibody formation [94]. CT-Hsp60 is able to stimulate production of pro-inflammatory cytokines in endothelial and smooth-muscle cells and macrophages [95], and it can also promote the activation of specific immune cells via a Toll-like receptor [96].

In a recent study, our group compared the amino acid sequences of human-Hsp60 and CT-Hsp60-1 (serovar D), and we found four epitopes with a 100% identity and 13 other peptides of various lengths with identities between 33% and 75% [97]. These epitopes are present in all three domains of the molecule (Figure 1).

Previous comparisons of human versus CT (serovars B, C, and L2) Hsp60 sequences identified 13 major epitopes, seven of which showed crossreactive antibody binding with homologous peptide sequences in human Hsp60 [98]. These data should draw the attention of clinicians towards an often ignored pathogenetic factor, namely, the crossreactive anti-Hsp60 antibodies formed during CT infection. One of the major objectives of this article is to inform clinicians about the occurrence of these antibodies and about their considerable pathogenetic potential.

Chlamydia trachomatis Infection and Antihuman-Hsp60 Antibodies: Negative and Positive Impact

CT infections can persist for very long periods because, usually, the human immune system cannot eliminate pathogens that remain hidden but virulent at focal sites; thus, these silent foci represent a high risk for complications [99].

People affected by CT develop high titres of serum antibodies anti-CT-Hsp60 [93,99]. These antibodies also recognize homologous epitopes on human Hsp60 [100], so the more prolonged the infection the greater the increase in the risk that crossreactive antibodies will react against host cells expressing sfHsp60 (Figure 2). These examples illustrate the importance of assessing the specificity spectrum of anti-Hsp60 antibodies as mentioned earlier, to obtain a defined picture of the antibody populations at play in pathology, a consideration that must also be applied when considering CT versus CP infections (see below).

Eukaryotic Hsp60 is a ubiquitous, multifaceted, versatile molecule in as much as it has been classically described in mitochondria, but it has lately been found in extramitochondrial sites [101]. In addition, during carcinogenesis Hsp60 may be augmented or diminished [102]. If augmented, it can have either pro- [103,104] or anti-apoptotic effects [23,105] or both [106]. Likewise, when Hsp60 is released outside cells, it can exert either pro- [25,53] or anti-inflammatory roles [62].

High levels of Hsp60 in plasma of healthy subjects have been positively correlated with low socioeconomic status, social isolation, and psychological distress [107]. Moreover, serum levels of Hsp60, but not those of anti-Hsp60 auto-antibodies, decline with age [108]. These autoantibodies could bind Hsp60 and, thus, produce immunoprecipitates with pathological impact on cells and tissues. There is a report on one case of immune complex glomerulonephritis in a 24-year-old individual with a CT infection of the fallopian tube [109]. Cases like this are probably frequent but missed in clinical practice. Therefore, the occurrence of anti-Hsp60 autoantibodies in individuals with CT infections should always be investigated, paying especial attention to aged patients, since both prevalence of autoimmunity and titres of autoantibodies tend to increase with age [110–113].

Anti-Hsp60 autoantibodies recognize Hsp60 epitopes exposed on stressed endothelial cell membranes [51,52]. This would represent the initial event triggering the formation of ATS lesions
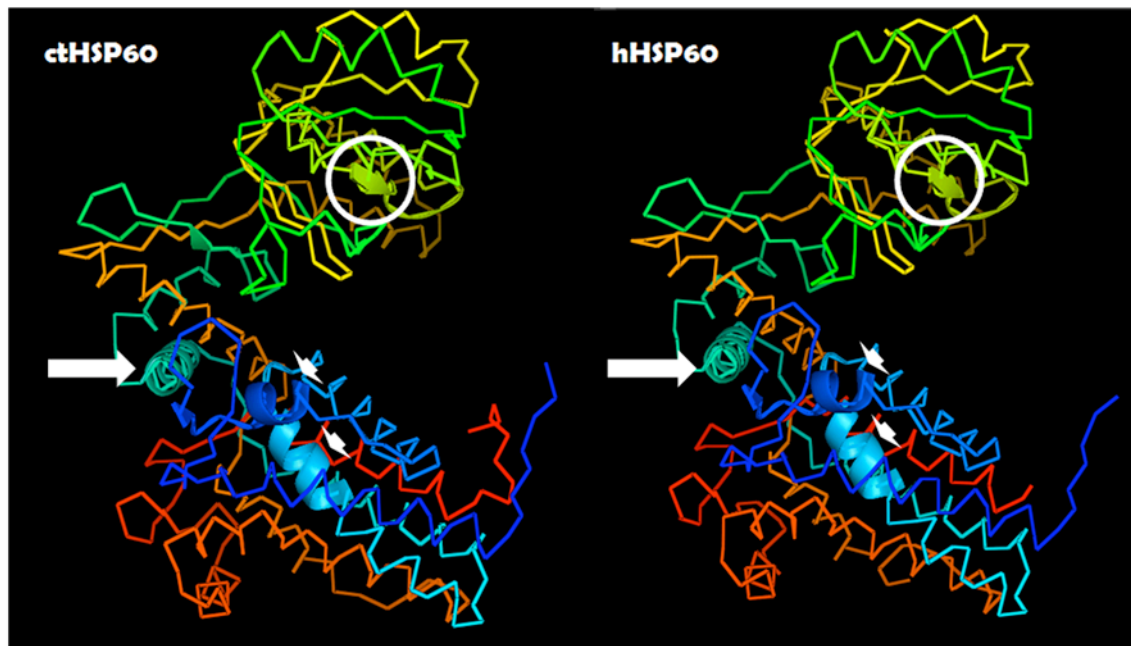

Figure 1. Comparison between the structures of *Chlamydia trachomatis* **(ct-) and human- (h-) Hsp60.** Shown are the positions of the four epitopes with 100% homologies. Circle: apical domain; arrow: intermediate domain; arrowhead: equatorial domain. See text and reference [94] for further details. The images were created with PyMol (http://pymol.sourceforge.net).
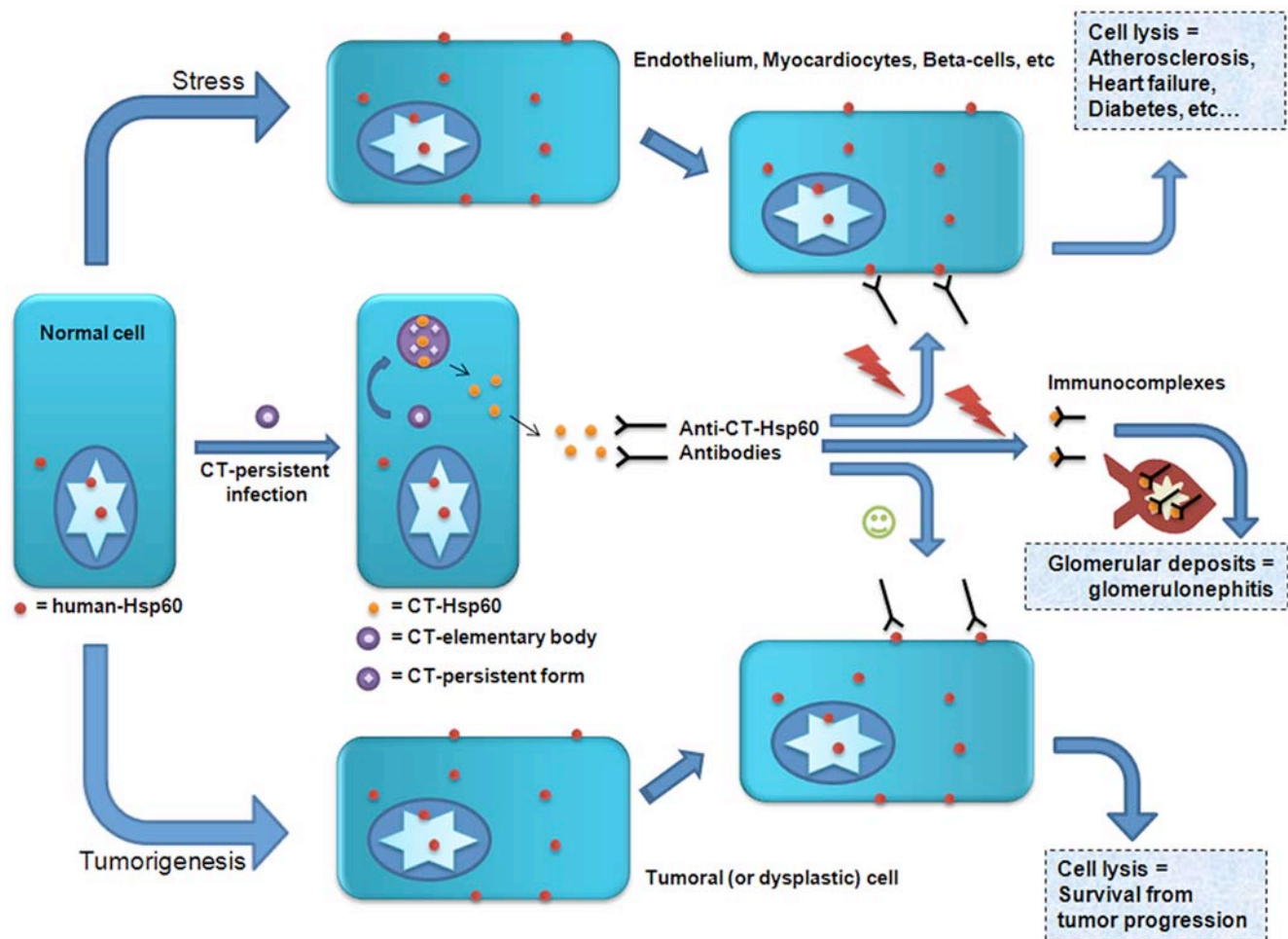doi:10.1371/journal.ppat.1000552.g001

Figure 2. Potential effects of anti-CT-Hsp60 antibodies generated during persistent CT infections. CT-Hsp60 is released from cells infected with *Chlamydia trachomatis* (CT), and anti-CT-Hsp60 antibodies are produced by the host's immune system. In turn, these antibodies recognize surface Hsp60 on either stressed or tumor cells and, consequently, cell lysis and organ destruction can occur, determining pathogenesis of a number of diseases (see text for further details). Likewise, immunocomplexes formed by anti-CT-Hsp60 antibodies and CT- (or human-, not shown) Hsp60 can form deposits in the glomerular basal membrane, causing an idiopathic form of glomerulonephritis. Tumor cell lysis can arrest tumorigenesis, in which case it is likely that the infected individual escapes from cancer without having experienced a detectable pathology or symptom.
doi:10.1371/journal.ppat.1000552.g002

and vasculitis. Autoantibodies are also thought to induce apoptosis in cells of the vascular endothelium and to generate renal vasculopathy in systemic lupus erythematosus [114].

When sfHsp60 is exposed on the myocardiocyte plasma membrane, anti-Hsp60 autoantibodies can trigger myocyte destruction via macrophage and/or neutrophil Fc recognition, accelerating heart failure [25]. Studies on Hsp60 autoimmunity also indicate that autoimmune diabetes can begin after bacterial hyperimmunization [83].

Cautionary Notes on Anti-CT-Hsp60 Antibody Measurements

Although several studies have been carried out to investigate the presence of Hsp60 and anti-Hsp60 in CT-infected individuals (and in various autoimmune diseases, as discussed elsewhere in this article), the results must be subjected to scrutiny and, if possible, to confirmatory research. This is pertinent, for example, to detection of Hsp60 in human plasma.

First, in many of these studies, undiluted plasma was used to measure Hsp60 or antibodies to Hsp60 by ELISA. As a consequence, it becomes very difficult to be certain as to what was actually detected by the antigen–antibody binding assay because undiluted plasma is rich in a wide variety of proteins, which makes non-specific binding likely. In addition, in a considerable number of cases it is not clear what internal controls were used and how the ELISA was calibrated to avoid false positives and false negatives.

Second, since antibodies to Hsp60 are also found in normal, healthy individuals and since their levels could vary widely (see above [35]), the possible significance of such antibodies, if they are detected in patients, in regard to development of autoimmune diseases can be very difficult to establish.

Third, despite the fact that antibodies to Hsp60 could be induced by a variety of bacteria in human niches, including those present in periodontal pockets and the gastrointestinal tract [10], the antigenic specificity of the antibodies was not, as a rule, determined. Thus, antibodies found to react with CT Hsp60 could very well have been elicited by chaperonins from other microbes, a possibility that must not be ignored due to the known high degree of similarity of Hsp60 across species. Consequently, efforts must be made to obtain and prepare panels of well-characterized antigens

to assess the specificity spectrum of the antibodies and identify those most likely to be the relevant ones for any bacterial infection, including CT infection.

Finally, it is of the utmost importance to be aware that CP infections are more widespread in the human population than are CT infections [115]. More than half of all adults in North America have antibodies to CP. As a consequence, one can expect that antibodies to CP Hsp60, which are most likely directed against similar epitopes as those in CT, should be of common occurrence. Here again, the challenge is to determine the specificity spectrum of the antibodies with a panel of Hsp60 molecules from pertinent species and, thus, determine with accuracy the true specificity of the antibodies as well as their titers with regard to the antigen under investigation (e.g., CT or CP Hsp60).

How, and When, to Defend from CT-Hsp60 Production?

Despite all the suggestive information already in the literature, sfHsp60 has not yet been sufficiently studied in human tissues under normal or pathological conditions, or after stress when Hsp60 translocates to the plasma membrane. sfHsp60 becomes recognizable by the immune system, leading to the generation of autoantibodies and other manifestations of the immune response accompanied by cell destruction, inflammation, and organ damage (Table 3). This series of concatenated events typical of autoimmune disorders due to autoantigens that share sequence homology with human Hsp60 very likely occur in various diseases, for example, Hashimoto disease, thyroiditis, scleroderma, pemphigoid, multiple sclerosis, chronic active hepatitis, primary biliary cirrhosis, and Addison disease [116]. It is imperative to ask the question whether all those diseases, and perhaps others, are not due at least in part to the invasion of the host by microbial Hsp60 from undiagnosed or misdiagnosed (and therefore untreated) bacterial infections, among which CT could be one of the prime suspects. The invading Hsp60 would elicit antibodies primarily directed to the microbial chaperonin but crossreactive with the host's counterpart (Figure 2). If that were the case, an essential distinction should be made between these antibodies elicited by a foreign antigen but reactive also with autoantigens and true autoantibodies elicited by autoantigens. This essential difference could very well mean other important dissimilarities pertaining to structural, functional, and biological molecular aspects, all of which deserve investigation in order to understand pathogenetic mechanisms, and to devise adequate diagnostic and therapeutic strategies.

On the other side of the coin, since sfHsp60 can be present on the surface of tumor cells [26–28], the occurrence of an anti-Hsp60 immune response, including circulating autoantibodies, could be beneficial in as much as the response would have a strong negative impact on tumor growth [117]. In this scenario, patients with a chronic CT infection may inadvertently be protected from, or "vaccinated" against as it were, cancer (Figure 2). This is a plausible possibility deserving investigation, even more so because a comprehensive study in vivo on sfHsp60 localization in tumor cells is still lacking.

In regard to the above, in vitro experiments showed that photodynamic therapy could induce sfHsp60 localization in a number of tumor cells [48]. This is one reason why it is believed that the presence of anti-Hsp60 autoantibodies might have antitumor effects and that the use of such antibodies could be a means for cancer treatment.

In summary, the high prevalence of CT infection in humans and the high similarity in the primary structure of CT and human Hsp60 should keep physicians on the alert and drive them to make every possible effort to diagnose CT infection or rule it out. Thus, measuring serum for anti-Hsp60, and also anti-Hsp10, antibodies offers a promising approach if the proper methodology is used (as discussed earlier in various sections of this article). If this diagnostic conduct is abided by, it is likely that much light will be shed on a number of misdiagnosed, idiopathic autoimmune disorders. In addition, investigating autoimmunity elicited by CT Hsp60 could provide information on a suspected protective role of the autoimmune phenomena in cancer, specifically when the cancer cells bear sfHsp60.

Conclusions and Perspectives

The chaperonin of Group I, Hsp60, or Cpn60 has many important functions, and its alterations, whether genetic or acquired, can cause pathologic disorders [118,119]. Like other Hsp chaperones, Hsp60 is an evolutionarily conserved protein and, consequently, molecules from different species share sequences that can be antigenic and elicit crossreactive antibodies. This situation is particularly relevant to human diseases with an autoimmune mechanism, particularly in patients with chronic infections.

This article focuses on the potential pathogenetic effect of Hsp60 from *Chlamydia trachomatis* (CT-Hsp60) that shares various antigenic determinants with the human counterpart. Infection with CT leads to an immune response against the invader's chaperonin, but the response crossreacts with the host's Hsp60. The crossreactive effects are perpetuated, and possibly amplified,

Table 3. Anatomic Sites and Cells in Which Hsp60 Could Play the Role of Autoantigen during Persistent CT Infection and Anti-CT-Hsp60 Antibody Production.

Site	Cell	Pathology	Reference
Vessels	Endothelial cells	Vasculitis, atherosclerosis	[49,50,53,54,58]
Heart	Myocardiocyte	Myocarditis, infarct, heart failure	[25,34,55]
Joints	Synoviocyte	Rheumatoid arthritis	[59,61–63]
Pancreas	Beta-cells	Diabetes	[66,69]
Thyroid	Thyreocyte	Hashimoto thyroiditis	[69]
Liver	Hepatocyte, biliary duct cells	Chronic active hepatitis, primary biliary cirrhosis	[69]
Adrenal glands	Glomerular zone cells	Addison disease	[69]
Kidney	Glomerulus	Glomerulonephitis	[70]
Skin	Keratinocyte, fibroblast, endothelial cells	Scleroderma, pemphigoid, psoriasis, dermatomyositis	[71,72]

doi:10.1371/journal.ppat.1000552.t003

by the fact that the human chaperonin is present not only inside cells but also outside them, attached to the cell membrane or in circulation. Thus, antibody-antigen reactions can occur on the cell's surface, in the intercellular space, and in biological fluids with a variety of consequences, including modulation of the immune system and generation of pathological lesions with immunoprecipitates. If Hsp60 occurs on the surface of malignant cells, a fact already ascertained for some types of cancer, antibodies or immune cells that react with the chaperonin have the potential ability to damage the tumor.

Since CT infections may go undetected or be misdiagnosed, and may be long lasting, anti-Hsp60 antibodies are likely to be causing disease silently for a long time without the physician being aware of this potentially very damaging situation. Likewise, protracted infections with CT could protect against the growth of certain tumors. These examples demonstrate why Hsp60 is considered a multifaceted, versatile molecule difficult to understand in any particular situation. As a consequence, the physician ought to become aware of the various roles of Hsp60 and anti-Hsp60 antibodies and of the importance of measuring them in all cases with suspected or demonstrated autoimmune manifestations.

It would be important to determine at what stage of human development Hsp60 begins to appear in extracellular locations and whether it is tolerogenic. Elucidation of these aspects of Hsp60 biology is essential to determine if, why, and when anti-Hsp60 autoantibodies (elicited by the endogenous human chaperonin) emerge, and what is their distribution in any given population. Clarification of these points will help understand the mechanism involved in the generation of anti-Hsp60 antibodies and/or their increase in response to invasion by a crossreactive chaperonin from a microbe.

Accession Numbers

The Entrez Protein Data Bank (http://www.ncbi.nlm.nih.gov/sites/entrez?db = protein) accession codes for the proteins discussed in this paper are *Chlamydia trachomatis* Hsp60-1 (CAH04305, [97]); mitochondrial heat shock 60 kD protein 1 variant 1 [*Homo sapiens*] (ACE06961, [97]); mycobacterial Hsp60 (CAD95638, [120]); *Chlamydia pneumoniae* Hsp60 (AAF38748, [121]); *Escherichia coli* Hsp60 (Q1R3B6, [122]); *Helicobacter pylori* (ACD47477, Reference Kersulyte, D., et al., direct submission, unpublished).

References

1. Ranson NA, White HE, Saibil HR (1998) Chaperonins. Biochem J 333: 233–242.
2. Karlin S, Brocchieri L (2000) Heat shock protein 60 sequence comparisons: duplications, lateral transfer, and mitochondrial evolution. Proc Natl Acad Sci U S A 97: 11348–11353.
3. Levy-Rimler G, Bell RE, Ben-Tal N, Azem A (2002) Type I chaperonins: not all are created equal. FEBS Lett 529: 1–5.
4. Macario AJL, Conway de Macario E (2004) The pathology of anti-stress mechanisms: A new frontier. Stress 7: 243–249.
5. Azem A, Diamant S, Kessel M, Weiss C, Goloubinoff P (1995) The protein-folding activity of chaperonins correlates with the symmetric GroEL14 (GroES7)2 heterooligomer. Proc Natl Acad Sci U S A 92: 12021–12025.
6. Martin J, Langer T, Boteva R, Schramel A, Horwich AL, Hartl FU (1991) Chaperonin-mediated protein folding at the surface of GroEL through a molten globule-like intermediate. Nature 352: 36–42.
7. Jindal S, Dudani AK, Singh B, Harley CB, Gupta RS (1989) Primary structure of a human mitochondrial protein homologous to the bacterial and plant chaperonins and to the 65-kilodalton mycobacterial antigen. Mol Cell Biol 9: 2279–2283.
8. Hansen JJ, Bross P, Westergaard M, Nielsen MN, Eiberg H, et al. (2003) Genomic structure of the human mitochondrial chaperonin genes: HSP60 and HSP10 are localised head to head on chromosome 2 separated by a bidirectional promoter. Hum Genet 112: 71–77.
9. Elias D, Markovits D, Reshef T, van der Zee R, Cohen IR (1990) 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 U S A 87: 1576–1580.
10. Zügel U, Kaufmann SH (1999) Role of heat shock proteins in protection from and pathogenesis of infectious diseases. Clin Microbiol Rev 12: 19–39.
11. Pockley AG (2003) Heat shock proteins as regulators of the immune response. Lancet 362: 469–476.
12. Res PC, Schaar CG, Breedveld FC, van Eden W, van Embden JD, et al. (1988) Synovial fluid T cell reactivity against 65 kDa heat shock protein of mycobacteria in early chronic arthritis. Lancet 2: 478–480.
13. Gaston JSH, Life PF, Jenner PJ, Colston MJ, Bacon PA (1990) Recognition of a mycobacteria-specific epitope in the 65 kD heat shock protein by synovial fluid derived T cell clones. J Exp Med 171: 831–841.
14. De Graeff-Meeder ER, van der Zee R, Rijkers GT, Schuurman HJ, Kuis W, et al. (1991) Recognition of human 60 kD heat shock protein by mononuclear cells from patients with juvenile chronic arthritis. Lancet 337: 1368–1372.
15. Wuckerpfennig K, Newcombe J, Li H, Keddy C, Cuzner ML, et al. (1992) gd T cell receptor repertoire in acute multiple sclerosis lesions. Proc Nat Acad Sci U S A 89: 4588–4592.
16. Georgopoulos C, Mc Farland H (1993) Heat shock proteins in multiple sclerosis and other autoimmune diseases. Immunol Today 14: 373–375.
17. Stinissen P, Vandevyver C, Medaer R, Vandegaer L, Nies J, et al. (1995) Increased frequency of gd T cells in cerebrospinal fluid and peripheral blood of patients with multiple sclerosis: reactivity cytotoxicity and T cell receptor V gene rearrangements. J Immunol 154: 4883–4894.
18. Child D, Smith C, Williams C (1993) Heat shock protein and the double insult theory for the development of insulin-dependent diabetes. J Royal Soc Med (Eng) 86: 217–219.
19. Tun RYM, Smith MD, Lo SS, Rook GA, Lydyard P, et al. (1994) Antibodies to heat shock protein 65 kDa in Type 1 diabetes mellitus. Diabet Med 11: 66–70.
20. Soltys BJ, Gupta RS (1996) Immunoelectron microscopic localization of the 60-kDa heat shock chaperonin protein (Hsp60) in mammalian cells. Exp Cell Res 222: 16–27.
21. Soltys BJ, Gupta RS (1999) Mitochondrial proteins at unexpected cellular locations: export of proteins from mitochondria from an evolutionary perspective. Int Rev Cytol 194: 133–196.
22. Cechetto JD, Soltys BJ, Gupta RS (2000) Localization of mitochondrial 60-kD heat shock chaperonin protein (Hsp60) in pituitary growth hormone secretory granules and pancreatic zymogen granules. J Histochem Cytochem 48: 45–56.
23. Gupta S, Knowlton AA (2002) Cytosolic HSP60, hypoxia and apoptosis. Circulation 106: 2727–2733.
24. Soltys BJ, Gupta RS (1997) Cell surface localization of the 60 kDa heat shock chaperonin protein (hsp60) in mammalian cells. Cell Biol Int 21: 315–320.
25. Lin L, Kim SC, Wang Y, Gupta S, Davis B, et al. (2007) HSP60 in heart failure: abnormal distribution and role in cardiac myocyte apoptosis. Am J Physiol Heart Circ Physiol 293: H2238–H2247.
26. Piselli P, Vendetti S, Vismara D, Cicconi R, Poccia F, et al. (2000) Different expression of CD44, ICAM-1, and HSP60 on primary tumor and metastases of a human pancreatic carcinoma growing in scid mice. Anticancer Res 20: 825–831.
27. Feng H, Zeng Y, Graner MW, Katsanis E (2002) Stressed apoptotic tumor cells stimulate dendritic cells and induce specific cytotoxic T cells. Blood 100: 4108–4115.
28. Shin BK, Wang H, Yim AM, Le Naour F, Brichory F, et al. (2003) Global profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with chaperone function. J Biol Chem 278: 7607–7616.
29. Dziewanowska K, Carson AR, Patti JM, Deobold CF, Bayles KW, et al. (2000) Staphylococcal fibronectin binding protein interacts with heat shock protein 60 and integrins: role in internalization by epithelial cells. Infect Immun 68: 6321–6328.
30. Osterloh A, Meier-Stiegen F, Veit A, Fleischer B, von Bonin A, et al. (2004) Lipopolysaccharide-free heat shock protein 60 activates T cells. J Biol Chem 279: 47906–47911.
31. Ohashi K, Burkart V, Flohe S, Kolb H (2000) Cutting edge: heat shock protein 60 is a putative endogenous ligand of the Toll-like receptor-4 complex. J Immunol 164: 558–561.
32. Sapozhnikov AM, Ponomarev ED, Tarasenko TN, Telford WG (1999) Spontaneous apoptosis and expression of cell surface heat-shock proteins in cultured EL-4 lymphoma cells. Cell Prolif 32: 363–378.
33. Thomas ML, Samant UC, Deshpande RK, Chiplunkar SV (2000) T cells lyse autologous and allogenic oesophageal tumours: involvement of heat shock proteins in the tumour cell lysis. Cancer Immunol Immunother 48: 653–659.

34. Gupta S, Knowlton AA (2007) HSP60 trafficking in adult cardiac myocytes: role of the exosomal pathway. Am J Physiol Heart Circ Physiol 292: H3052–3056.

35. Shamaei-Tousi A, Steptoe A, O'Donnell K, Palmen J, Stephens JW, et al. (2007) Plasma heat shock protein 60 and cardiovascular disease risk: the role of psychosocial, genetic, and biological factors. Cell Stress Chaperon 12: 384–392.

36. Cappello F, Bellafiore M, Palma A, Marcianò V, Martorana G, et al. (2002) Expression of 60-kD heat shock protein increases during carcinogenesis in the uterine exocervix. Pathobiology 70: 83–88.

37. Cappello F, Bellafiore M, Palma A, David S, Marcianò V, et al. (2003) 60 KDa chaperonin (HSP60) is over-expressed during colorectal carcinogenesis. Eur J Histochem 47: 105–110.

38. Cappello F, Rappa F, David S, Anzalone R, Zummo G (2003) Immunohistochemical evaluation of PCNA, p53, HSP60, HSP10 and MUC-2 presence and expression in prostate carcinogenesis. Anticancer Res 23: 1325–1331.

39. Ito T, Kawabe R, Kurasono Y, Hara M, Kitamura H, et al. (1998) Expression of heat shock proteins in squamous cell carcinoma of the tongue: an immunohistochemical study. J Oral Pathol Med 27: 18–22.

40. Lebret T, Watson RW, Molinié V, O'Neill A, Gabriel C, et al. (2003) Heat shock proteins HSP27, HSP60, HSP70, and HSP90: expression in bladder carcinoma. Cancer 98: 970–977.

41. Cappello F, Di Stefano A, D'Anna SE, Donner CF, Zummo G (2005) Immunopositivity of heat shock protein 60 as a biomarker of bronchial carcinogenesis. Lancet Oncol 6: 816.

42. Cappello F, Di Stefano A, David S, Rappa F, Anzalone R, et al. (2006) Hsp60 and Hsp10 down-regulation predicts bronchial epithelial carcinogenesis in smokers with chronic obstructive pulmonary disease. Cancer 107: 2417–2424.

43. Ikawa S, Weinberg RA (1992) An interaction between p21ras and heat shock protein hsp60, a chaperonin. Proc Natl Acad Sci U S A 89: 2012–2016.

44. Barazi HO, Zhou L, Templeton NS, Krutzsch HC, Roberts DD (2002) Identification of heat shock protein 60 as a molecular mediator of alpha 3 beta 1 integrin activation. Cancer Res 62: 1541–1548.

45. Laad AD, Thomas ML, Fakih AR, Chiplunkar SV (1999) Human gamma delta T cells recognize heat shock protein-60 on oral tumor cells. Int J Cancer 80: 709–714.

46. Hanlon JG, Adams K, Rainbow AJ, Gupta RS, Singh G (2001) Induction of Hsp60 by Photofrin-mediated photodynamic therapy. J Photochem Photobiol B 64: 55–61.

47. Jalili A, Makowski M, Switaj T, Nowis D, Wilczynski GM, et al. (2004) Effective photoimmunotherapy of murine colon carcinoma induced by the combination of photodynamic therapy and dendritic cells. Clin Cancer Res 10: 4498–4508.

48. Korbelik M, Sun J, Cecic I (2005) Photodynamic therapy-induced cell surface expression and release of heat shock proteins: relevance for tumor response. Cancer Res 65: 1018–1026.

49. Xu Q, Schett G, Perschinka H, Mayr M, Egger G, et al. (2000) Serum soluble heat shock protein 60 is elevated in subjects with atherosclerosis in a general population. Circulation 102: 14–20.

50. Prohaszka Z, Duba J, Lakos G, Kiss E, Varga L, et al. (1999) Antibodies against human heat-shock protein (hsp) 60 and mycobacterial hsp65 differ in their antigen specificity and complement-activating ability. Int Immunol 11: 1363–1370.

51. Xu Q, Schett G, Seitz CS, Hu Y, Gupta RS, et al. (1994) Surface staining and cytotoxic activity of heat-shock protein 60 antibody in stressed aortic endothelial cells. Circ Res 75: 1078–1085.

52. Pfister G, Stroh CM, Perschinka H, Kind M, Knoflach M, et al. (2005) Detection of HSP60 on the membrane surface of stressed human endothelial cells by atomic force and confocal microscopy. J Cell Sci 118: 1587–1594.

53. Wick G (2000) Atherosclerosis-an autoimmune disease due to an immune reaction against heat-shock protein 60. Herz 25: 87–90.

54. Knowlton AA, Srivatsa U (2008) Heat shock protein 60 and cardiovascular disease:a paradoxical role. Future Cardiol 4: 151–161.

55. Knowlton AA, Kapadia S, Torre-Amione G, Durand JB, Bies R, et al. (1998) Differential expression of heat shock proteins in normal and failing human hearts. J Mol Cell Cardiol 30: 811–818.

56. Martin JE, Swash M, Mather K, Leigh PN (1993) Expression of the human groEL stress-protein homologue in the brain and spinal cord. J Neurol Sci 118: 202–206.

57. Bajramovic JJ, Bsibsi M, Geutskens SB, Hassankhan R, Verhulst KC, et al. (2000) Differential expression of stress proteins in human adult astrocytes in response to cytokines. J Neuroimmunol 106: 14–22.

58. Alard JE, Dueymes M, Youinou P, Jamin C (2008) HSP60 and anti-HSP60 antibodies in vasculitis: they are two of a kind. Clin Rev Allergy Immunol 35: 66–71.

59. Krenn V, Vollmers HP, von Landenberg P, Schmausser B, Rupp M (1996) Immortalized B-lymphocytes from rheumatoid synovial tissue show specificity for bacterial HSP60. Virchows Arch 427: 511–518.

60. Van Roon JA, van Roy JL, Duits A, Lafeber FP, Bijlsma JW (1995) Proinflammatory cytokine production and cartilage damage due to rheumatoid synovial T helper-1 activation is inhibited by interleukin-4. Ann Rheum Dis 54: 836–840.

61. Wilbrink B, Holewijn M, Bijlsma JW, van Roy JL, den Otter W, van Eden W (1993) Suppression of human cartilage proteoglycan synthesis by rheumatoid synovial fluid mononuclear cells activated with mycobacterial 60-kd heat-shock protein. Arthritis Rheum 36: 514–518.

62. Van Roon JA, van Eden W, van Roy JL, Lafeber FP, Bijlsma JW (1997) Stimulation of suppressive T cell responses by human but not bacterial 60-kD heat-shock protein in synovial fluid of patients with rheumatoid arthritis. J Clin Invest 100: 459–463.

63. Rudolphi U, Rzepka R, Batsford S, Kaufmann SH, von der Mark K, et al. (1997) The B cell repertoire of patients with rheumatoid arthritis. II. Increased frequencies of IgG+ and IgA+ B cells specific for mycobacterial heat-shock protein 60 or human type II collagen in synovial fluid and tissue. Arthritis Rheum 40: 1409–1419.

64. Ramage JM, Gaston JS (1999) Depressed proliferative responses by peripheral blood mononuclear cells from early arthritis patients to mycobacterial heat shock protein 60. Rheumatology 38: 631–635.

65. Brudzynski K, Martinez V, Gupta RS (1992) Immunocytochemical localization of heat-shock protein 60-related protein in beta-cell secretory granules and its altered distribution in non-obese diabetic mice. Diabetologia 35: 316–324.

66. Brudzynski K (1993) Insulitis-caused redistribution of heat-shock protein HSP60 inside beta-cells correlates with induction of HSP60 autoantibodies. Diabetes 42: 908–913.

67. Raz I, Elias D, Avron A, Tamir M, Metzger M, et al. (2001) Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. Lancet 358: 1749–1753.

68. Lazar L, Ofan R, Weintrob N, Avron A, Tamir M, et al. (2007) Heat-shock protein peptide DiaPep277 treatment in children with newly diagnosed type 1 diabetes: a randomised, double-blind phase II study. Diabetes Metab Res Rev 23: 286–291.

69. Mallard K, Jones DB, Richmond J, McGill M, Foulis AK (1996) Expression of the human heat shock protein 60 in thyroid, pancreatic, hepatic and adrenal autoimmunity. J Autoimmun 9: 89–96.

70. Slot MC, Theunissen R, van Paassen P, Damoiseaux JG, Cohen Tervaert JW (2006) Evaluation of antibodies against human HSP60 in patients with MPO-ANCA associated glomerulonephritis: a cohort study. J Autoimmune Dis 5: 3–4.

71. Elst EF, Klein M, de Jager W, Kamphuis S, Wedderburn LR, et al. (2008) Hsp60 in inflamed muscle tissue is the target of regulatory autoreactive T cells in patients with juvenile dermatomyositis. Arthritis Rheum 58: 547–555.

72. Seung NR, Park EJ, Kim CW, Kim KH, Kim KJ, et al. (2007) Comparison of expression of heat-shock protein 60, Toll-like receptors 2 and 4, and T-cell receptor gammadelta in plaque and guttate psoriasis. J Cutan Pathol 34: 903–911.

73. Alard JE, Dueymes M, Youinou P, Jamin C (2007) Modulation of endothelial cell damages by anti-Hsp60 autoantibodies in systemic autoimmune diseases. Autoimm Rev 6: 438–443.

74. Mayr M, Metzler B, Kiechl S, Willeit J, Schett G, Xu Q, Wick G (1999) 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: 1560–1566.

75. Knoflach M, Mayrl B, Mayerl C, Sedivy R, Wick G (2003) Atherosclerosis as a paradigmatic disease of the elderly: role of the immune system. Immunol Allergy Clin North Am 23: 117–132.

76. Choi JI, Chung SW, Kang HS, Rhim BY, Kim SJ, et al. (2002) Establishment of Porphyromonas gingivalis heat-shock-protein-specific T-cell lines from atherosclerosis patients. J Dent Res 81: 344–348.

77. Chung SW, Kang HS, Park HR, Kim SJ, Kim SJ, et al. (2003) Immune responses to heat shock protein in Porphyromonas gingivalis-infected periodontitis and atherosclerosis patients. J Periodontal Res 38: 388–393.

78. Yamazaki K, Ohsawa Y, Itoh H, Ueki K, Tabeta K, et al. (2004) T-cell clonality to Porphyromonas gingivalis and human heat shock protein 60s in patients with atherosclerosis and periodontitis. Oral Microbiol Immunol 19: 160–167.

79. Okada T, Ayada K, Usui S, Yokota K, Cui J, et al. (2007) Antibodies against heat shock protein 60 derived from Helicobacter pylori: diagnostic implications in cardiovascular disease. J Autoimmun 29: 106–115.

80. Yokota SI, Hirata D, Minota S, Higashiyama T, Kurimoto M, et al. (2000) Autoantibodies against chaperonin CCT in human sera with rheumatic autoimmune diseases: comparison with antibodies against other Hsp60 family proteins. Cell Stress Chaperon 5: 337–346.

81. Szebeni A, Schloot N, Kecskemeti V, Hosszufalusi N, Panczel P, et al. (2005) Th1 and Th2 cell responses of type 1 diabetes patients and healthy controls to human heat-shock protein 60 peptides AA437–460 and AA394–408. Inflamm Res 54: 415–419.

82. Shovman O, Sherer Y, Gilbourd B, Gerli R, Bocci EB, et al. (2005) Low levels of heat shock proteins-60 and -65 autoantibodies in Sjogren's syndrome. Isr Med Assoc J 7: 778–780.

83. Jensen P, Johansen HK, Carmi P, Høiby N, Cohen IR (2001) Autoantibodies to pancreatic hsp60 precede the development of glucose intolerance in patients with cystic fibrosis. J Autoimmun 17: 165–172.

84. Workowski KA, Berman SM (2006) Sexually transmitted diseases treatment guidelines. MMWR Recomm Rep 55: 1–94.

85. Fredlund H, Falk L, Jurstrand M, Unemo M (2004) Molecular genetic methods for diagnosis and characterisation of Chlamydia trachomatis and Neisseria

gonorrhoeae: impact on epidemiological surveillance and interventions. APMIS 112: 771–784.

86. Washington AE, Aral SO, Wolner-Hanssen P, Grimes DA, Holmes KK (1990) Assessing risk for pelvic inflammatory disease and its sequelae. JAMA 266: 2581–2586.

87. Washington AE, Katz P (1991) Cost of and payment source for pelvic inflammatory disease. Trends and projections, 1983 through 2000. JAMA 266: 2565–2569.

88. Bavoil P, Stephens RS, Falkow S (1990) A soluble 60 kiloDalton antigen of Chlamydia spp. is a homologue of Escherichia coli GROES. Mol Microbiol 4: 461–469.

89. Van Eden W, Thole JE, Van der Zee R, Noodrzij A, Van Embden JD, et al. (1988) Cloning the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. Nature 331: 171–173.

90. Anderton SM, Van der Zee R, Prakken B, Noordzij A, Van Eden W (1995) Activation of T cells recognizing self 60-kD heat shock protein can protect against experimental arthritis. J Exp Med 181: 943–952.

91. Di Felice V, Ardizzone N, Marcianò N, Bartolotta T, Cappello F, et al. (2005) Senescence-associated Hsp60 expression in normal human skin fibroblasts. Anat Rec 284A: 446–453.

92. Di Felice V, David S, Cappello F, Farina F, Zummo G (2005) Is chlamydial heat shock protein 60 a risk factor for oncogenesis? Cell Mol Life Sci 62: 4–9.

93. Raulston JE, Paul TR, Knight ST, Wyrick PB (1998) Localization of Chlamydia trachomatis heat shock protein 60 and 70 during infection of a human endometrial epithelial cell line in vitro. Infect Immun 66: 2323–2329.

94. Witkin SS (1999) Immunity to heat shock proteins and pregnancy outcome. Infect Dis Obstetr Gynecol 7: 35–38.

95. Kol A, Bourcier T, Lichtman AH, Libby P (1999) Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells and macriphages. J Clin Invest 103: 571–577.

96. Stephen RS (2003) The cellular paradigm of Chlamydia pathogenesis. Trends Microbiol 11: 44–51.

97. Campanella C, Marino-Gammazza A, Mularoni L, Cappello F, Zummo G, et al. (2009) A comparative analysis of the products of GROEL-1 gene from Chlamydia trachomatis serovar D and the Hsp60 var1 transcript from homo sapiens suggests a possible autoimmune response. Int J Immunogenet 36: 73–78.

98. Yi Y, Zhong G, Brunham RC (1993) Continuous B-cell epitopes in Chlamydia trachomatis heat shock protein 60. Infect Immun 61: 1117–1120.

99. Swanborg RH, Boros DL, Whittum-Hudson JA, Hudson AP (2006) Molecular mimicry and horror autotoxicus: do chlamydial infections elicit autoimmunity? Expert Rev Mol Med 8: 1–23.

100. Jakus S, Neuer A, Dieterle S, Bongiovanni AM, Witkin SS (2008) Antibody to the Chlamydia trachomatis 60 k Da heat shock protein in follicular fluid and in vitro fertilization outcome. Am J Reproduct Immunol 59: 85–89.

101. Cappello F, Conway de Macario E, Marasa L, Zummo G, Macario AJL (2008) Hsp60 expression, new locations, functions, and perspectives for cancer diagnosis and therapy. Cancer Biol Ther 7: 801–809.

102. Cappello F, Zummo G (2005) HSP60 expression during carcinogenesis: a molecular "Proteus" of carcinogenesis. Cell Stress Chaperon 10: 263–264.

103. Samali A, Cai J, Zhivotovsky B, Jones DP, Orrenius S (1999) Presence of pre-apoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of Jurkat cells. EMBO J 18: 2040–2048.

104. Xanthoudakis S, Roy S, Rasper D, Hennessey T, Aubin Y, et al. (1999) Hsp60 accelerate the maturation of pro-caspase-3 by upstream activator proteases during apoptosis. EMBO J 18: 2049–2056.

105. Campanella C, Bucchieri F, Ardizzone NM, Marino-Gammazza A, Montalbano A, et al. (2008) Upon oxidative stress, the antiapoptotic Hsp60/procaspase-3 complex persists in mucoepidermoid carcinoma cells. Eur J Histochem 52: 221–228.

106. Chandra D, Choy G, Tang DG (2007) Cytosolic accumulation of HSP60 during apoptosis with or without apparent mitochondrial release: evidence that its pro-apoptotic or pro-survival functions involve differential interactions with caspase-3. J Biol Chem 282: 31289–31301.

107. Lewthwaite J, Owen N, Coates A, Henderson B, Steptoe A (2002) Circulating human heat shock protein 60 in the plasma of British civil servants: relationship to physiological and psychosocial stress. Circulation 106: 196–201.

108. Rea IM, McNerlan S, Pockley AG (2001) Serum heat shock protein and anti-heat shock protein antibody levels in aging. Exp Gerontol 36: 341–352.

109. Ohsawa I, Ohi H, Endo M, Fujita T, Hidaka M, et al. (2001) A case of renal involvement in persistent immune activation caused by chlamydial salpingitis. Virchows Arch 438: 306–311.

110. Hasler P, Zouali M (2005) Immune receptor signaling, aging, and autoimmunity. Cell Immunol 233: 102–108.

111. Nilsson BO, Skogh T, Ernerudh J, Johansson B, Löfgren S, et al. (2006) Antinuclear antibodies in the oldest-old women and men. J Autoimmun 27: 281–288.

112. Prelog M (2006) Aging of the immune system: a risk factor for autoimmunity? Autoimmun Rev 5: 136–139.

113. Liang KP, Gabriel SE (2007) Autoantibodies: innocent bystander or key player in immunosenescence and atherosclerosis? J Rheumatol 34: 1203–1207.

114. Van Paassen P, Duijvestijn A, Debrus-Palmans L, Damoiseaux J, Vroomen M, et al. (2007) Induction of endothelial cell apoptosis by IgG antibodies from SLE patients with nephropathy: a potential role for anti-endothelial cell antibodies. Ann N Y Acad Sci 1108: 147–156.

115. Kumar S, Hammerschlag MR (2007) Acute respiratory infection due to Chlamydia pneumoniae: current status of diagnostic methods. Clin Infect Dis 44: 568–576.

116. Jones DB, Coulson AF, Duff GW (1993) Sequence homologies between hsp60 and autoantigens. Immunol Today 14: 115–118.

117. Cappello F, Czarnecka AM, La Rocca G, Di Stefano A, Zummo G, et al. (2007) Hsp60 and Hsp10 as antitumor molecular agents. Cancer Biol Ther 6: 487–489.

118. Macario AJL, Conway de Macario E (2005) Sick chaperones, cellular stress, and disease. N Engl J Med 353: 1489–1501.

119. Macario AJL, Conway de Macario E (2007) Chaperonopathies by defect, excess, or mistake. Ann NY Acad Sci 1113: 178–191.

120. Garnier T, Eiglmeier K, Camus JC, Medina N, Mansoor H, et al. (2003) The complete genome sequence of Mycobacterium bovis. Proc Natl Acad Sci U S A 100: 7877–7882.

121. Read TD, Brunham RC, Shen C, Gill SR, Heidelberg JF, et al. (2000) Genome sequences of Chlamydia trachomatis MoPn and Chlamydia pneumoniae AR39. Nucleic Acids Res 28: 1397–1406.

122. Chen SL, Hung CS, Xu J, Reigstad CS, Magrini V, et al. (2006) Identification of genes subject to positive selection in uropathogenic strains of Escherichia coli: a comparative genomics approach. Proc Natl Acad Sci U S A 103: 5977–5982.