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Heat-shock or stress proteins (HSPs) are intracellular molecules that are expressed under cellular stress and have housekeeping and cytoprotective functions. Many of them act also as molecular chaperones, assisting the correct folding, stabilization, and translocation of proteins. In pathological situations, such as necrotic cell death, they can be released into the extracellular environment complexed with intact or fragmented cellular proteins. Evidence is now accumulating to indicate that, under certain circumstances, these complexes can contribute to induction of autoimmunity by receptor-mediated activation of the innate immune response (signaling the “danger”) and by participation in the presentation of autoantigens for the adaptive immune response (acting as natural adjuvants). In addition, the conservation of HSPs through prokaryotes and eukaryotes, together with the increased production of host and microbial HSPs at the site of infection, has led to the proposition that these proteins may provide a link between infection and autoimmunity. This review outlines the mechanisms for the potential involvement of chaperones in the induction of autoimmune disease.

The Role of Chaperone Proteins in Autoimmunity

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ABSTRACT: Heat-shock or stress proteins (HSPs) are intracellular molecules that are expressed under cellular stress and have housekeeping and cytoprotective functions. Many of them act also as molecular chaperones, assisting the correct folding, stabilization, and translocation of proteins. In pathological situations, such as necrotic cell death, they can be released into the extracellular environment complexed with intact or fragmented cellular proteins. Evidence is now accumulating to indicate that, under certain circumstances, these complexes can contribute to induction of autoimmunity by receptor-mediated activation of the innate immune response (signaling the "danger") and by participation in the presentation of autoantigens for the adaptive immune response (acting as natural adjuvants). In addition, the conservation of HSPs through prokaryotes and eukaryotes, together with the increased production of host and microbial HSPs at the site of infection, has led to the proposition that these proteins may provide a link between infection and autoimmunity. This review outlines the mechanisms for the potential involvement of chaperones in the induction of autoimmune disease.

KEYWORDS: autoimmunity; chaperones; heat shock stress proteins; epitopes; calreticulin; HSP70; HSP60; Bip

HEAT-SHOCK / STRESS PROTEINS AND MOLECULAR CHAPERONES

Cell stressors including heat, irradiation, reactive oxygen species, hypoxia, pH shift, infection, inflammation, nutritional deficiency, and exposure to chemical agents cause modifications of the intracellular milieu. They include downregulation of many housekeeping genes and activation of stress genes that are usually transcribed at low levels in the absence of stress.[1,2] The products of these genes are called heat-shock stress proteins (HSPs) and have the capacity

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to stabilize and refold the partially denatured proteins or mediate the degradation of nonreversibly damaged proteins under stress conditions. Under physiological conditions, some of these proteins function as molecular chaperones. They assist a nascent polypeptide chain to attain a functional conformation and then bring the protein to the cellular site where it carries out its functions.[3] The term "heat shock proteins" is somewhat of a misnomer, as they are not induced solely by heat shock. However, for historical reasons, the term "HSP" is used even if the parent gene is induced by other than heat-shock stressors. We should also note that many HSPs are not chaperones and, conversely, only a fraction of chaperones are encoded in genes that are inducible by stressors and thus belong to the stress proteins. Therefore, the terms "HSPs" and "chaperones" have to be used carefully to avoid misunderstanding (e.g., a HSP is not necessarily induced by heat shock and a chaperone is not necessarily a HSP).

Chaperones and HSPs are classified into groups according their localization and molecular mass in kilodaltons (kDa). The best-understood HSPs are those with a molecular weight of 110, 90, 70, and 60 kDa, respectively, called also major HSPs. They are constitutively expressed primarily in the cytosol and mitochondria in the absence of heat stress and their expression can be upregulated by various stressors. The second group comprises the "minor" HSPs, which are located primary in the rough endoplasmic reticulum (ER) and are induced by glucose deprivation. This group includes glucose-regulated proteins (grp) with molecular weights of 34, 47, 56, 75, 78, 94, and 174 kDa. A third group consists of low (about 20 kDa) molecular mass HSPs.

HSP60 and HSP70 families are the major chaperones of the cytosol. HSP70s are highly conserved and demonstrate a 60–78% base identity among eukaryotic cells and a 40–60% identity between eukaryotic HSP70 and *Escherichia coli* DNAK.[4,5] HSP70 family members are equipped with two major functional domains, including a C-terminal region that binds peptides and denatured proteins, and an N-terminal ATPase domain that controls the opening and closing of the peptide-binding domain.[6] The HSP60 family comprises HSP60 in mammals, mycobacterial homologue mHSP65, chlamydial HSP60, and the *E. coli* homologue GroEL.[7] HSP60 family members bind to partially folded polypeptides and assist in their correct folding as well as in their assembly in multimeric complexes.[6]

In the ER the major chaperones include Grp78, Grp58, calnexin, and calreticulin. Grp78 (also referred to as BiP, the immunoglobulin binding protein)[8] interacts with many secretory and membrane proteins within the ER during the course of their maturation. Grp58 belongs to the family of disulfide isomerases, the folding enzymes of the ER, which catalyze the formation of disulfide bonds.[9] Calnexin and calreticulin are thought to function as chaperones monitoring the folding and assembly of glycoproteins.[10] Calreticulin is also considered to recognize specific oligosaccharides in the carbohydrate portion of glycoproteins, acting like a lectin.[11]

CHAPERONES AND THE IMMUNE SYSTEM

The most appropriate stimulus for innate immunity is the exposure to a foreign molecule in a "dangerous" milieu. In this context, "danger" is signaled by certain conserved molecules of invading pathogens (pathogen-associated molecular pattern, PAMP). The essential decision for responding to or ignoring a particular antigen is made by innate immune recognition receptors upon activation by PAMP molecules, such as lipopolysaccharide (LPS) or bacterial CpG DNA.[12] Receptors of this type, called pattern recognition receptors (PRRs) have been identified in large numbers. Many recently cloned PRRs belong to the expanding family of toll-like receptors (TLRs). An alternative pathway for the activation of innate immunity was proposed by Matzinger. The so-called "danger theory" states that, in addition, innate immunity can be activated by endogenous substances released by damaged or stressed tissue.[13] Thus, stressed mammalian cells can messenger stress to other (immune or nonimmune) cells. Potential candidates for signaling tissue damage or cellular stress are heat-shock proteins. In this regard, HSP60 and HSP70 have been found capable of signaling through CD14, TLR-2, and TLR-4.[14–16]

In addition to having a function as stimulators of the innate immune system, HSPs have been also shown to play a role in generating antigen-specific T cell responses.[17,18] The proposed mechanism is that peptides, complexed with the HSPs—including HSP70, Gp96, and calreticulin—are delivered to antigen-presenting cells (APCs) by receptor-mediated internalization of the HSPs, making them available for processing and presentation on major histocompatibility complex (MHC) molecules. Specific receptor-mediated mechanisms exist for the capture and internalization of HSPs,[19] suggesting that cross-presentation of HSP-derived antigenic determinants is a legitimate mechanism for cross-priming by professional APCs.

Moreover, HSPs can be overexpressed in different pathologic conditions and serve as specific targets of the adaptive immune response. All these discrete immunological functions of HSPs are discussed in detail in the following paragraphs.

HSPs as Targets of the Immune Response

Infection is a stressful process—for both the pathogen and the host—and therefore inevitably results in increased production of molecular chaperones by the pathogen as well as by the host. The conservation of HSPs through prokaryotes and eukaryotes, together with the increased production of host and microbial HSPs at the site of infection, has led to suggestions that cross-reactivity between host and pathogen HSPs might be responsible for a variety of autoreactive disorders that are associated with high frequency recognition of HSPs.[7,20] In this context, the possible involvement of mycobacterial HSP70 in

the autoantibody production in systemic lupus erythematosus (SLE) has been indicated in one study.[21]

Regardless of the participation of HSPs in the pathogenesis of autoimmunity via antigenic cross-reactivity, HSPs are capable of eliciting immune responses.[22] Autoantibodies and cells reactive to HSP have been detected in patients with rheumatoid arthritis,[23] SLE,[24] inflammatory bowel disease,[25] multiple sclerosis,[26,27] Bechet's disease,[28] ocular inflammation,[29] and development of vascular lesions.[30] The role of this autoimmune response to HSPs in various diseases has not been yet identified. In one case autoantibodies to HSP90 have been correlated with elevated levels of IL-6 in SLE.[31] In another study, a cross-recognition between the HSP60 and the Ro60 autoantigen, has been demonstrated.[32,33]

Besides molecular mimicry, a process known as intermolecular spreading of humoral autoimmunity has been implicated for the induction of autoantibodies against HSPs. As a model for epitope spreading, the Ro/La ribonucleoprotein complex (RNP) was used. This autoantigenic complex is formed by the noncovalent association of the Ro52, La, and Ro60 autoantigens with a small cytoplasmic RNA (hYRNA).[34] The chaperone calreticulin has been also identified as an additional component of the complex.[35] Immunization experiments showed spreading of the immune response from Ro52 and Ro60—but not La—to calreticulin in murine experimental autoimmunity, consistent with the notion that calreticulin may associate with the subpopulation of Ro particles from which La has already dissociated.[36] Subsequent work from the same group demonstrated additional spreading of autoimmunity to the Bip and HSP70 chaperones after immunization with Ro52 and Ro60 autoantigens, suggesting that these components may co-localize and physically associate under certain conditions. The potential importance of the ER-resident chaperones, Bip and calreticulin, in autoimmune response against Ro/La RNP is further supported by their co-localization with Ro in small apoptotic membrane blebs[37] and the finding that Bip interacts with the Ro52 autoantigen.[38]

HSPs as Pro-inflammatory Mediators

The "danger theory" asserts that—in addition to infectious non-self-agents—professional APCs can be activated by endogenous substances released by damaged, infected, stressed, or transformed cells.[13,39] The first piece of evidence demonstrating that HSP60 and HSP70 stimulate human monocytes to release proinflammatory cytokines was presented in 1993.[40–42] Later, many authors provided further evidence for signaling and activation of different cells by HSP60 and HSP70.[43] However, it was found that LPS contamination has biased the results of these early experiments. The remarkable findings of Bausinger *et al*. call more attention to the interpretation of the

results obtained before 2002.[44] These authors reported that endotoxin-free recombinant HSP70 fails to activate dendritic cells. In line with these observations, Gao *et al*. published two papers on the meticulous investigation of HSP-induced cytokine production by murine macrophages. HSP70 or HSP60 preparations with very low endotoxin contamination had no stimulatory effect on murine macrophages.[45,46] For this reason, researchers introduced more appropriate controls to rule out the potential LPS contamination present in recombinant preparations used for cell stimulation. In recent years, convincing evidence from well-documented studies indicate that the recognition of HSPs by different cells and the activation of targeted cells is a receptor-mediated process. Extracellular HSP60 from both bacteria and humans can stimulate a proinflammatory phenotype in various cells including monocytes/macrophages, dendritic cells, and vascular endothelial cells. These cells present increased expression of cell-surface adhesion molecules and release proinflammatory cytokines. The receptor(s) for human HSP60 is CD14 and/or TLR2 and/or TLR4.[47] Exogenous human and bacterial HSP70 proteins also stimulate monocytes/macrophages and dendritic cells via a plethora of receptors including CD14,[48] TLR2, TLR4,[49] CD40,[50,51] CD91,[52] and LOX-1.[53] The stimulation of proinflammatory behavior in various cells can also trigger the generation of adaptive immune response. Thus, both HSP60 and HSP70 seem to initiate the MyD88-dependent, Th1-type response–inducing pathway.[54]

HSPs as Potent Antigen Carriers

HSPs such as HSP70, HSP90, gp96, HSP110, grp170, and calreticulin (CRT) associate with a broad array of peptides generated within the cells.[55,56] These peptides include normal self-peptides as well as antigenic peptides derived from tumor,[57] bacterial antigens,[58] or viral antigens.[59] The cross-presentation hypothesis suggests that the complexes of HSP-chaperoned peptides released from the cells, after stress or cell death, are taken up by the APCs, resulting in representation (cross-presentation) of the peptides by MHC molecules of the APCs[56] A large body of evidence suggests that HSP–peptide complexes, which are generated within cells, can be used to immunize mice and elicit antigen-specific $CD8^+$ T cell responses. Immunization with femtomolar quantities of antigenic peptides chaperoned by HSPs (but not other proteins) is effective in eliciting such T cell responses.[60] On theoretical grounds, immunization with such a small quantity can be effective only after receptor-mediated endocytosis of the HSP-chaperoned peptides.[61] In attempting to identify the HSP receptor, Binder *et al*.[62] applied solubilized membranes of APCs to gp96 affinity columns and eluted and sequenced a gp96-binding protein. This turned out to be the previously known receptor of α2-macroglobulin, CD91. The CD91 molecule appears to function as the receptor not only for gp96, but also for

HSP90, HSP70, and calreticulin–peptide complexes,[52] although other receptors have also been proposed, including CD40[50] and LOX-1.[53]

It is now well accepted that HSPs act to chaperone peptides present in the cytosol for presentation and processing via the MHC class I molecule-loading pathway.[61,63] It has also been shown that gp96-associated peptides, besides MHC class I molecule-loading pathway, can enter an acidic compartment and load onto MHC class II molecules, implicating gp96 in MHC class II presentation.[64] Previous studies showed that exogenous bacterial HSPs enhance the class II MHC antigen processing and presentation of chaperoned peptides to $CD4^+$ T cells. Roth *et al*. demonstrated an enhancing effect of bacterial DNAK, the *E. coli* analogue of HSP70, in the MHC class II-dependent presentation of recombinant human acetylcholine receptor alpha-subunit Ag.[65] Similarly, Tobian *et al.* showed that exogenous bacterial HSPs (*E. coli* DNAK and *Mycobacterium tuberculosis* HSP70) delivered an extended OVA peptide for processing and MHC-II presentation, as detected by hybridoma T cells. These HSPs enhanced MHC-II presentation only if the peptide was chaperoned by the HSP. This process was found to be independent of TLR-induced induction of accessory molecules since it was intact in MyD88 knockout cells, which lack most TLR signaling.[66]

Recently, evidence that endogenous HSPs are involved in MHC class II antigen presentation has been provided. Mycko *et al.*, using MHC class II APCs, overexpressing HSP70 and an MBP-specific TCR hybridoma as well as T cell lines, demonstrated that HSP70 binds MBP peptides in an ATP/ADP-dependent manner and is actively involved in MHC class II-dependent autoantigen processing by the APCs.[67] Doody *et al.* compared the ability of the ER-resident HSP gp96 to prime CD4 and CD8 cells using TCR transgenic adoptive transfer systems and soluble gp96–peptide complexes. It was found that gp96 facilitated the *in vivo* cross-presentation of both class-I and class-II restricted peptides to CD8 and CD4 T cells, respectively. However, gp96 preferentially primed CD8—but not CD4—cell effector function.[68] In another study, using a soluble heat-shock fusion protein (Hsfp) having peptide sequences capable of inducing both $CD4^+$ and $CD8^+$ cells, it was found that many more class-II MHC peptide than class-I MHC peptide complexes are displayed on dendritic cells. In this study, the CD4 cells were found to respond far more vigorously than the CD8 cells.[69] In a similar way, taking advantage of an identified region in HIV Gag p24 that contains overlapping CTL and Th epitopes, Sen *et al.* studied the simultaneous presentation of these epitopes from a single precursor peptide complexed to HSP gp96. They demonstrated an efficient HSP-mediated presentation of at least eight different CTL and Th epitopes from the same precursor peptide sequence and induction of both $CD8^+$ and $CD4^+$T-cell responses, respectively.[70]

Taken together, internalized HSP–peptide complexes can enter the MHC class I- and class II-enriched compartments by a receptor-mediated uptake and be presented to CD8 and CD4 T cells, eliciting a peptide-specific response.

HSPs as Natural Adjuvants

HSPs chaperone peptides, enchancing their antigenicity. One of the best-studied examples is the ER-resident chaperone calreticulin. Calreticulin is upregulated in response to various types of ER stress and has the ability to bind to glycoproteins containing monoglucosylated core glycans as well as to several nonglycosylated peptides. The ability of calreticulin to interact with nonglycosylated polypeptide substrates *in vitro* is highly influenced by environmental parameters, including the temperature and the presence of ATP and divalent cations. It has been suggested that exposure at high temperatures permits the exchange of naturally bound peptides to calreticulin with those added exogenously.[71,72] ATP binding to calreticulin increases its hydrophobicity, resulting in significant conformational alterations of the molecule.[73] Similarly, divalent cations appear to play a regulatory role on its activity as chaperone, most probably by neutralizing its negative charges, thereby inducing specific conformational changes.[74] A recent study demonstrated that the polypeptide-binding conformation of calreticulin is induced by heat shock, calcium depletion, or by deletion of the C-terminal acidic region.[75] Thus, cell stress conditions that generate nonnative substrates of calreticulin also affect the conformational properties of calreticulin itself, and enhance its binding to substrates, independent of substrate glucosylation.

We observed previously that calreticulin can be complexed with specific epitopes, inducing conformation-dependent recognition by autoantibodies from autoimmune human sera.[76] In our study calreticulin was isolated from human spleen, using a multistep purification method, and allowed to interact with seven biotinylated epitopes of Ro60, La, and Sm autoantigens. Among the synthetic peptides tested, only the two epitopes of Ro60 kDa autoantigen, spanning the sequences 175–184aa and 216–232aa, exhibited a substantial binding to calreticulin. Our results indicated that calreticulin–Ro60 kD peptide interaction was favored by heating at 40°C, the presence of ATP, and optimum concentrations of divalent ions. These conditions not only increased the interaction of calreticulin with Ro60 kDa epitopes, but also the antigenicity of the complex that exhibited a much more stronger anti-Ro60 kDa reactivity compared to that observed when calreticulin and peptides were tested individually. In fact, all anti-Ro60 kDa–positive sera of patients with autoimmune rheumatic diseases recognized the complex calreticulin–peptide, while the same sera displayed very low reactivity when tested individually with calreticulin or the Ro epitopes alone (TABLE 1). It was therefore proposed that calreticulin may play a more active role for the generation of autoimmune response against Ro60 kDa. According to our hypothesis, calreticulin can be released into the extracellular space, together with Ro fragments, after necrosis or cell lysis by cytotoxic T cells.[77,78] Under certain physicochemical conditions favored by the microenviromental milieu, calreticulin can bind Ro peptides, eventually increasing their antigenicity. The complex is then transported to professional APCs and the peptides are

TABLE 1. Reactivity of autoimmune sera against calreticulin (Crtc), Ro60 epitopes (10p and 17p), and calreticulin peptide complexes (Crtc-17p and Crtc-10p)

	Crtc-17p (%)	Crtc-10p (%)	Crtc (%)	17p (%)	10p
anti-Ro (+)	100	95	8	29	11%
anti-Ro (−)	8	4	4	0	4%

presented to autoreactive Th cells. In this regard calreticulin is used as a vehicle to deliver peptides in the immune system, augmenting the specific autoimmune response.

Recently, evidence for a similar mechanism has been provided for HSP70.[79] The heat-shock protein HSP70 was found to enhance antigen-specific proliferation of human $CD4^+$ memory T cells and to increase the immunogenicity of presented peptides. At low doses of antigen, stimulation with HSP70–peptide complexes was found to be far superior to stimulation with peptide alone. The complex formation of the antigenic peptide with HSP70 was found to be absolutely required in order to elicit an antigen-specific amplification. In this scenario, the induction of HSP70 decreases the threshold of activation of human $CD4^+$ T cells by the antigenic peptide. The increased reactivity of T cells against a peptide chaperoned by HSP indicates a putative involvement of HSP in the pathogenesis of autoimmune diseases. Thus, the induction of HSP by certain stress factors, such as infection, would facilitate an immune response

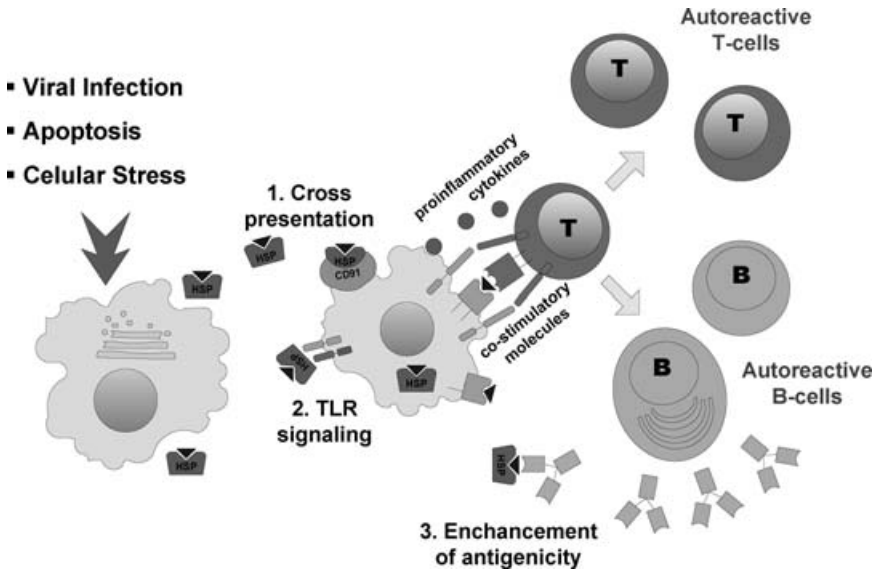

FIGURE 1. Proposed mechanism for the role of chaperones in the pathogenesis of autoimmunity.

to a given peptide of the self that would not be immunogenic under conditions where HSP is not available.

CHAPERONES AND AUTOIMMUNITY

HSPs seem to be directly involved in the pathogenesis of autoimmunity. They can also be directly implicated for the induction of autoimmunity if we summarize the functions of HSPs and consider the following series of events: Under specific conditions HSPs can meet self proteins, which are normally resident in different compartments of the cell. For example after induction of apoptosis or after a viral infection, which is accompanied by ER remodeling, the ER-resident chaperones can interact with intact or fragmented cytoplasmic autoantigens, thus enchancing their antigenicity.[76,38] Following subsequent secondary necrosis, extracellular HSPs may be released that can act as both proinflammatory mediators (signaling the "danger") and antigen carriers, facilitating cross-presentation of their "antigenic cargo" to sensitized Th and CTL cells. The activation of autoreactive T cells in the "proinflammatory milieu" of their microenvironment seems to be sufficient for the induction of humoral and cellular autoimmunity (FIG. 1).

REFERENCES

1. VERBEKE, P. *et al*. 2001. Heat shock response and ageing: mechanisms and applications. Cell Biol. Int. **25:** 845–857.
2. GAO, H. *et al*. 2004. Global transcriptome analysis of the heat shock response of *Shewanella oneidensis*. J. Bacteriol. **186:** 7796–7803.
3. KLEIZEN, B. & I. BRAAKMAN. 2004. Protein folding and quality control in the endoplasmic reticulum. Curr. Opin. Cell Biol. **16:** 343–349.
4. LINDQUIST, S. 1986. The heat-shock response. Annu. Rev. Biochem. **55:** 1151–1191.
5. CAPLAN, A.J., D.M. CYR & M.G. DOUGLAS. 1993. Eukaryotic homologues of *Escherichia coli* dnaj: a diverse protein family that functions with HSP70 stress proteins. Mol. Biol. Cell. **4:** 555–563.
6. BUKAU, B. & A.L. HORWICH. 1998. The HSP70 and HSP60 chaperone machines. Cell **92:** 351–366.
7. YOUNG, R.A. & T.J. ELLIOTT. 1989. Stress proteins, infection, and immune surveillance. Cell **59:** 5–8.
8. HAAS, I.G. 1991. BiP—a heat shock protein involved in immunoglobulin chain assembly. Curr. Top. Microbiol. Immunol. **167:** 71–82.
9. MAZZARELLA, R.A. *et al*. 1994. Erp61 is grp58, a stress-inducible luminal endoplasmic reticulum protein, but is devoid of phosphatidylinositide-specific phospholipase C activity. Arch. Biochem. Biophys. **308:** 454–460.
10. MICHALAK, M. *et al*. 1992. Calreticulin. Biochem. J. **285:** 681–692.
11. HELENIUS, A. 1994. How N-linked oligosaccharides affect glycoprotein folding in the endoplasmic reticulum. Mol. Biol. Cell. **5:** 253–265.

12. JANEWAY, C.A. Jr. & R. MEDZHITOV. 2002. Innate immune recognition. Annu. Rev. Immunol. **20:** 197–216.
13. MATZINGER, P. 1998. An innate sense of danger. Semin. Immunol. **10:** 399–415.
14. ASEA, A. 2003. Chaperokine-induced signal transduction pathways. Exerc. Immunol. Rev. **9:** 25–33.
15. OHASHI, K. *et al*. 2000. Cutting edge: heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. J. Immunol. **164:** 558–561.
16. VABULAS, R.M. *et al*. 2001. Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. J. Biol. Chem. **276:** 31332–31339.
17. SRIVASTAVA, P. 2002. Roles of heat-shock proteins in innate and adaptive immunity. Nat. Rev. Immunol. **2:** 185–194.
18. POCKLEY, A.G. 2003. Heat shock proteins as regulators of the immune response. Lancet **362:** 469–476.
19. ARNOLD-SCHILD, D. *et al*. 1999. Cutting edge: receptor-mediated endocytosis of heat shock proteins by professional antigen-presenting cells. J. Immunol. **162:** 3757–3760.
20. ZUGEL, U. & S.H. KAUFMANN. 1999. Role of heat shock proteins in protection from and pathogenesis of infectious diseases. Clin. Microbiol Rev. **12:** 19–39.
21. TASNEEM, S., N. ISLAM & R. ALI. 2001. Crossreactivity of SLE autoantibodies with 70 kDa heat shock proteins of *Mycobacterium tuberculosis*. Microbiol. Immunol. **45:** 841–846.
22. WINFIELD, J.B. & W.N. JARJOUR. 1991. Stress proteins, autoimmunity, and autoimmune disease. Curr. Top. Microbiol. Immunol. **167:** 161–189.
23. PANCHAPAKESAN, J., M. DAGLIS & P. GATENBY. 1992. Antibodies to 65 kDa and 70 kDa heat shock proteins in rheumatoid arthritis and systemic lupus erythematosus. Immunol. Cell Biol. **70:** 295–300.
24. JARJOUR, W.N. *et al*. 1991. Autoantibodies to human stress proteins. A survey of various rheumatic and other inflammatory diseases. Arthritis Rheum. **34:** 1133–1138.
25. STEVENS, T.R. *et al*. 1992. Circulating antibodies to heat-shock protein 60 in Crohn's disease and ulcerative colitis. Clin. Exp. Immunol. **90:** 271–274.
26. SALVETTI, M. *et al*. 1992. T-lymphocyte reactivity to the recombinant mycobacterial 65- and 70-kDa heat shock proteins in multiple sclerosis. J. Autoimmun. **5:** 691–702.
27. GEORGOPOULOS, C. & H. MCFARLAND. 1993. Heat shock proteins in multiple sclerosis and other autoimmune diseases. Immunol. Today **14:** 373–375.
28. STANFORD, M.R. *et al*. 1994. Heat shock protein peptides reactive in patients with Behcet's disease are uveitogenic in Lewis rats. Clin. Exp. Immunol. **97:** 226–231.
29. DE SMET, M.D. & A. RAMADAN. 2001. Circulating antibodies to inducible heat shock protein 70 in patients with uveitis. Ocul. Immunol Inflamm. **9:** 85–92.
30. GROMADZKA, G. *et al*. 2001. Elevated levels of anti-heat shock protein antibodies in patients with cerebral ischemia. Cerebrovasc. Dis. **12:** 235–239.
31. RIPLEY, B.J., D.A. ISENBERG & D.S. LATCHMAN. 2001. Elevated levels of the 90 kDa heat shock protein (HSP90) in SLE correlate with levels of IL-6 and autoantibodies to HSP90. J. Autoimmun. **17:** 341–346.
32. TEZEL, G., J. YANG & M.B. WAX. 2004. Heat shock proteins, immunity and glaucoma. Brain Res. Bull. **62:** 473–480.
33. WAX, M.B. *et al*. 1998. Anti-Ro/SS-A positivity and heat shock protein antibodies in patients with normal-pressure glaucoma. Am. J. Ophthalmol. **125:** 145–157.

34. SLOBBE, R.L. *et al*. 1992. Ro ribonucleoprotein assembly in vitro. Identification of RNA-protein and protein-protein interactions. J. Mol. Biol. **227:** 361–366.
35. CHENG, S.T. *et al*. 1996. Calreticulin binds hYRNA and the 52-kDa polypeptide component of the Ro/SS-A ribonucleoprotein autoantigen. J. Immunol. **156:** 4484–4491.
36. KINOSHITA, G. *et al*. 1998. Spreading of the immune response from 52 kDaRo and 60 kDaRo to calreticulin in experimental autoimmunity. Lupus **7:** 7–11.
37. CASCIOLA-ROSEN, L.A., G. ANHALT & A. ROSEN. 1994. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. J. Exp. Med. **179:** 1317–1330.
38. PURCELL, A.W. *et al*. 2003. Association of stress proteins with autoantigens: a possible mechanism for triggering autoimmunity? Clin. Exp. Immunol. **132:** 193–200.
39. GALLUCCI, S. & P. MATZINGER. 2001. Danger signals: SOS to the immune system. Curr. Opin. Immunol. **13:** 114–119.
40. FRIEDLAND, J.S. *et al*. 1993. Mycobacterial 65-kD heat shock protein induces release of proinflammatory cytokines from human monocytic cells. Clin. Exp. Immunol. **91:** 58–62.
41. PEETERMANS, W.E. *et al*. 1993. Murine peritoneal macrophages activated by the mycobacterial 65-kilodalton heat shock protein express enhanced microbicidal activity in vitro. Infect. Immun. **61:** 868–875.
42. BEAGLEY, K.W. *et al*. 1993. The *Mycobacterium tuberculosis* 71-kDa heat-shock protein induces proliferation and cytokine secretion by murine gut intraepithelial lymphocytes. Eur. J. Immunol. **23:** 2049–2052.
43. RANFORD, J.C., A.R. COATES & B. HENDERSON. 2000. Chaperonins are cell-signalling proteins: the unfolding biology of molecular chaperones. Expert Rev. Mol. Med. **2000:** 1–17.
44. BAUSINGER, H. *et al*. 2002. Endotoxin-free heat-shock protein 70 fails to induce APC activation. Eur. J. Immunol. **32:** 3708–3713.
45. GAO, B. & M.F. TSAN. 2003. Recombinant human heat shock protein 60 does not induce the release of tumor necrosis factor alpha from murine macrophages. J. Biol. Chem. **278:** 22523–22529.
46. GAO, B. & M.F. TSAN. 2003. Endotoxin contamination in recombinant human heat shock protein 70 (HSP70) preparation is responsible for the induction of tumor necrosis factor alpha release by murine macrophages. J. Biol. Chem. **278:** 174–179.
47. TSAN, M.F. & B. GAO. 2004. Cytokine function of heat shock proteins. Am. J. Physiol Cell Physiol. **286:** C739-C744.
48. ASEA, A. *et al*. 2000. HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. Nat. Med. **6:** 435–442.
49. ASEA, A. *et al*. 2002. Novel signal transduction pathway utilized by extracellular HSP70: role of toll-like receptor (TLR) 2 and TLR4. J. Biol. Chem. **277:** 15028–15034.
50. BECKER, T., F.U. HARTL & F. WIELAND. 2002. CD40, an extracellular receptor for binding and uptake of HSP70-peptide complexes. J. Cell Biol. **158:** 1277–1285.
51. WANG, Y. *et al*. 2001. CD40 is a cellular receptor mediating mycobacterial heat shock protein 70 stimulation of CC-chemokines. Immunity **15:** 971–983.
52. BASU, S. *et al*. 2001. CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. Immunity **14:** 303–313.

53. DELNESTE, Y. *et al*. 2002. Involvement of LOX-1 in dendritic cell-mediated antigen cross-presentation. Immunity. **17:** 353–362.
54. BULUT, Y. *et al*. 2002. Chlamydial heat shock protein 60 activates macrophages and endothelial cells through Toll-like receptor 4 and MD2 in a MyD88-dependent pathway. J. Immunol. **168:** 1435–1440.
55. SRIVASTAVA, P.K. *et al*. 1998. Heat shock proteins come of age: primitive functions acquire new roles in an adaptive world. Immunity **8:** 657–665.
56. LI, Z., A. MENORET & P. SRIVASTAVA. 2002. Roles of heat-shock proteins in antigen presentation and cross-presentation. Curr. Opin. Immunol. **14:** 45–51.
57. CASTELLI, C. *et al*. 2001. Human heat shock protein 70 peptide complexes specifically activate antimelanoma T cells. Cancer Res. **61:** 222–227.
58. ZUGEL, U. *et al*. 2001. gp96-peptide vaccination of mice against intracellular bacteria. Infect. Immun. **69:** 4164–4167.
59. NAVARATNAM, M. *et al*. 2001. Heat shock protein-peptide complexes elicit cytotoxic T-lymphocyte and antibody responses specific for bovine herpesvirus 1. Vaccine. **19:** 1425–1434.
60. BLACHERE, N.E. *et al*. 1997. Heat shock protein-peptide complexes, reconstituted in vitro, elicit peptide-specific cytotoxic T lymphocyte response and tumor immunity. J. Exp. Med. **186:** 1315–1322.
61. SINGH-JASUJA, H. *et al*. 2000. Cross-presentation of glycoprotein 96-associated antigens on major histocompatibility complex class I molecules requires receptor-mediated endocytosis. J. Exp. Med. **191:** 1965–1974.
62. BINDER, R.J., D.K. HAN & P.K. SRIVASTAVA. 2000. CD91: a receptor for heat shock protein gp96. Nat. Immunol. **1:** 151–155.
63. CASTELLINO, F. *et al*. 2000. Receptor-mediated uptake of antigen/heat shock protein complexes results in major histocompatibility complex class I antigen presentation via two distinct processing pathways. J. Exp. Med. **191:** 1957–1964.
64. MATSUTAKE, T. & P.K. SRIVASTAVA. 2000. CD91 is involved in MHC class II presentation of gp96 chaperoned peptides. *In* Second International Conference on Heat Shock Proteins in Immune Response. Cahperone, C.S., Ed.: pp 8–12. Vol. 378. Cell Stress Society International. Farmington, CT, USA.
65. ROTH, S. *et al*. 2002. Major differences in antigen-processing correlate with a single Arg71<–>Lys substitution in HLA-DR molecules predisposing to rheumatoid arthritis and with their selective interactions with 70-kDa heat shock protein chaperones. J. Immunol. **169:** 3015–3020.
66. TOBIAN, A.A., D.H. CANADAY & C.V. HARDING. 2004. Bacterial heat shock proteins enhance class II MHC antigen processing and presentation of chaperoned peptides to $CD4^+$ T cells. J. Immunol. **173:** 5130–5137.
67. MYCKO, M.P. *et al*. 2004. Inducible heat shock protein 70 promotes myelin autoantigen presentation by the HLA class II. J. Immunol. **172:** 202–213.
68. DOODY, A.D. *et al*. 2004. Glycoprotein 96 can chaperone both MHC class I- and class II-restricted epitopes for in vivo presentation, but selectively primes $CD8^+$ T cell effector function. J. Immunol. **172:** 6087–6092.
69. PALLISER, D. *et al*. 2005. Multiple intracellular routes in the cross-presentation of a soluble protein by murine dendritic cells. J. Immunol. **174:** 1879–1887.
70. SENGUPTA, D. *et al*. 2004. Heat shock protein-mediated cross-presentation of exogenous HIV antigen on HLA class I and class II. J. Immunol. **173:** 1987–1993.
71. BASU, S. & P.K. SRIVASTAVA. 1999. Calreticulin, a peptide-binding chaperone of the endoplasmic reticulum elicits tumor- and peptide-specific immunity. J. Exp. Med. **189:** 797–802.

72. JORGENSEN, C.S. *et al*. 2003. Dimerization and oligomerization of the chaperone calreticulin. Eur. J. Biochem. **270:** 4140–4148.
73. CORBETT, E.F. *et al*. 2000. The conformation of calreticulin is influenced by the endoplasmic reticulum luminal environment. J. Biol. Chem. **275:** 27177–27185.
74. SVAERKE, C. & G. HOUEN. 1998. Chaperone properties of calreticulin. Acta Chem. Scand. **52:** 942–949.
75. RIZVI, S.M. *et al*. 2004. A polypeptide binding conformation of calreticulin is induced by heat shock, calcium depletion, or by deletion of the C-terminal acidic region. Mol. Cell. **15:** 913–923.
76. STAIKOU, E.V. *et al*. 2003. Calreticulin binds preferentially with B cell linear epitopes of Ro60 kD autoantigen, enhancing recognition by anti-Ro60 kD autoantibodies. Clin. Exp. Immunol. **134:** 143–150.
77. BASU, S. *et al*. 2000. Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. Int. Immunol. **12:** 1539–1546.
78. DUPUIS, M. *et al*. 1993. The calcium-binding protein calreticulin is a major constituent of lytic granules in cytolytic T lymphocytes. J. Exp. Med. **177:** 1–7.
79. HAUG, M. *et al*. 2005. The heat shock protein HSP70 enhances antigen-specific proliferation of human $CD4^+$ memory T cells. Eur. J. Immunol. **35:** 3163–3172.