

Role of Heat Shock Proteins in Protection from and Pathogenesis of Infectious Diseases

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INTRODUCTION

Heat shock proteins (hsp) are widely distributed in nature and are among the most highly conserved molecules of the biosphere. hsp perform important functions in the folding and unfolding or translocation of proteins, as well as in the assembly and disassembly of protein complexes. Because of these helper functions, hsp have been termed molecular chaperones. The molecules involved in antigen recognition, i.e., immunoglobulins (Ig), T-cell receptors (TCR), and gene products of the major histocompatibility complex (MHC), are all multimeric complexes, and their assembly is promoted by distinct chaperones. Several lines of evidence also favor an important role for members of the hsp family in intracellular antigen-processing pathways. The first part of this review describes the biological roles of hsp as they relate to the assembly of protein complexes and participation in different processing and presentation steps of antigens.

hsp synthesis is increased to protect prokaryotic or eukaryotic cells from various insults during periods of stress caused by infection, inflammation, or similar events. Consistent with this abundance, in several infections and autoimmune diseases, hsp represent prominent antigens in the humoral and cellular im-

mune response mediated by antibodies and T cells, respectively. The second part of this review summarizes the diseases, in both experimental-animal models and humans, where evidence has been obtained for a unique role of hsp as antigens. Although hsp play an important role in several infectious and autoimmune diseases, evidence arguing against the direct involvement of hsp in protection or autoaggression has been gathered. At present, initiation of protective immunity against infectious agents or autoimmune disorders by hsp alone appears unlikely. Rather, it seems more likely that they become important antigens during infection and inflammation and in this way influence and sustain anti-infectious and autoimmune responses. Thus, hsp act as chaperones, not only during the biogenesis of other proteins but also during the immune response to other antigens.

ACQUIRED IMMUNE RESPONSE TO INFECTIOUS AGENTS

The vertebrate immune system encounters an enormous variety of pathogens. Specific identification and elimination of such a multitude of potentially infectious agents depends on a broad array of detection and execution systems. The specific recognition of foreign invaders is effected by clonally distributed receptors expressed on lymphocytes, such as antibodies produced by B lymphocytes and TCR expressed on the surface of T lymphocytes. These receptors specifically recognize structures termed antigens that encompass subunits named epitopes.

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While antibodies directly recognize peptide or carbohydrate epitopes, T cells interact only with antigens presented by products of the MHC complex expressed on the surface of target cells. In the extracellular space, antibodies specifically recognize pathogens and neutralize microbial products, for example bacterial toxins.

In contrast to the humoral response, the cellular immune response—mediated by T lymphocytes—possesses the capacity to recognize antigens of intracellular microbes, which are hidden from antibody detection (127). Whenever T or B cells are confronted with an antigen, a lymphocyte clone expressing unique receptor specificity is expanded. Frequent encounters with the same antigen result in immunological memory, which enables the immune system to respond to repeated microbial confrontation more potently both in qualitative and in quantitative terms.

B cells produce antibodies which further segregate into five different Ig classes (208). T cells can be subdivided into at least three major classes according to the expression of specific surface molecules (127). In humans and mice the majority of T cells (>90%) express a TCR composed of an α -chain and a β -chain. In addition to the highly variable TCR, these T cells express a diverse set of accessory CD4 or CD8 molecules. Most mature $\alpha\beta$ T cells express either the CD4 or the CD8 molecule in a mutually exclusive way, and the expression of these molecules correlates with a characteristic recognition pattern.

CD4 T cells recognize peptides presented by MHC class II molecules, which consist of an α -chain and a β -chain with a peptide binding groove composed of polymorphic domains of both chains (35). The expression of MHC class II molecules is restricted to a few antigen-presenting cells (APC), such as mononuclear phagocytes, dendritic cells, and B cells. Epitopes which are recognized by CD4 T cells are generally of extracellular origin and are derived from the endosomal compartment (147). Thus, antigens of intracellular microbes which are localized in the endosome are processed primarily through the MHC class II pathway. The main feature of CD4 T cells is their helper function. After antigen stimulation, they produce various cytokines which induce the secretion of distinct Ig isotypes by B cells or activate antimicrobial effector mechanisms in professional phagocytes (195). According to their cytokine expression pattern, CD4 T cells can be further subdivided into two subclasses. CD4 T cells producing gamma interferon and interleukin-2 (IL-2) are typified as T helper (Th) 1 cells and primarily activate professional phagocytes and cytolytic T cells. In contrast, Th2 cells typically produce IL-4 and IL-5 and act primarily on B cells.

Although CD8 T cells can produce different cytokines, their major task is the lysis of target cells. CD8 T cells recognize peptides that are presented by MHC class I molecules (127). Unlike MHC class II gene products, MHC class I molecules are expressed by virtually all cells of the mammalian host and consist of a polymorphic α -chain noncovalently bound to β_2 -microglobulin (β_2m) (231). Endogenously synthesized proteins, e.g., viral, tumor, and self antigens, have access to the MHC class I processing pathway. These proteins are degraded into peptides by a multimeric enzymatic complex in the cytoplasm—the proteasome—and are translocated into the endoplasmic reticulum (ER), where they bind to MHC class I molecules (122, 202).

By using a novel approach, it was finally possible to identify naturally processed peptides presented by MHC molecules. Acid elution was used to dissociate these peptides from MHC molecules, and their sequences were analyzed by mass spectrometry (129, 231). Typical peptides presented by MHC class I molecules have thus been characterized as 8 to 10 amino

acids (aa) in length, with characteristic MHC allele-specific residues that are essential for anchoring the peptide in the MHC class I peptide binding cleft (230, 231). In contrast, MHC class II peptides vary between 12 and 25 aa in length. MHC class II peptides are also held in the groove by anchoring via two or three residues to the groove of the MHC class II molecules but in a less restricted manner.

Antigens from bacteria which remain in the endosome, e.g., *Mycobacterium bovis* or *Salmonella typhi*/*S. typhimurium*, are processed primarily through the MHC class II pathway, whereas antigens from pathogenic organisms which enter the cytoplasm, such as *Listeria monocytogenes*, are also presented by MHC class I molecules (147). The identification of CD8 T cells specific for antigens derived from bacteria which remain in the phagosome suggests that some exogenous antigens can reach the cytosol and thus have access to the MHC class I processing pathway (102, 103, 234, 280). Conversely, endogenous antigens can be reintroduced into the MHC class II processing pathway and presented to CD4 T cells (34, 151, 300).

In addition to the major T-cell population expressing an $\alpha\beta$ TCR, a minor population of T cells expresses a TCR consisting of a γ -chain and a δ -chain (127, 148). Usually these cells express neither the CD4 nor the CD8 molecule, and they function like $\alpha\beta$ TCR-expressing T cells. Although these cells frequently contribute to immunity against pathogens (63, 147, 163), antigen recognition by such cells is still incompletely understood. Some $\gamma\delta$ T cells, for instance, can recognize antigen in the context of MHC class I and class II molecules, but the majority of $\gamma\delta$ T cells see their antigenic ligands differently. Direct recognition of surface molecules, including MHC gene products, has also been reported (248, 299). Moreover, restriction elements other than classical MHC molecules are used frequently by these cells. Some $\gamma\delta$ T cells recognize antigenic ligands presented by the MHC class I-like molecule Qa-1, and others recognize the CD1 molecule (221, 266). Other studies suggest that a member of the hsp70 family, grp75, presents antigenic ligands to $\gamma\delta$ T cells (153). Recently, recognition by intestinal human $\gamma\delta$ T cells of stress-induced MHC class I-related molecules was described (96). In addition, direct recognition of surface-expressed antigen independent of antigen processing and presentation by MHC molecules similar to antibodies was described for $\gamma\delta$ T cells (172). Nonpeptide ligands containing phosphate were documented for human but not mouse $\gamma\delta$ T cells (148). In fact, stimulation by phospholigands was demonstrated for the major subset of human $\gamma\delta$ T cells expressing the V γ 2V δ 2 TCR (which is identical to the V γ 9V δ 2 TCR used in another nomenclature system). These ligands were originally isolated from *Mycobacterium tuberculosis* (218) but were later also found in numerous other bacteria (148). It has been shown that phosphorylation of ligands is critical for $\gamma\delta$ T-cell stimulation (46, 251, 282, 283). The isopentenyl pyrophosphate represents the first natural phospholigand described for $\gamma\delta$ T cells. This structure is found in all cells from bacteria to humans and represents a metabolite of vitamins, lipids, and steroids of prokaryotes and eukaryotes.

HEAT SHOCK PROTEINS AS MOLECULAR CHAPERONES

Stress Response

Reversing polypeptide unfolding and preventing protein aggregation are major functions of hsp, especially under stress (21, 47, 48, 106, 107). In nonstressed cells hsp are present in low concentrations, while in stressed cells they accumulate at high levels. In *Escherichia coli*, for example, the hsp60 homolog

TABLE 1. Association of chaperones with molecules involved in antigen recognition

Chaperone	Most likely association	Reference
Bip	Human MHC class I molecules	54
	Incompletely assembled MHC class II molecules	29
	Early-folding intermediates of Ig chains	192
	TCR α -chains	279
gp96	MHC class II molecules in the absence of invariant chain	246
	Unassembled but not assembled Ig	193
Calnexin	Unassembled MHC class I molecules; retaining unassembled MHC class I α -chains in the ER	227
	Assembly of intermediates of MHC class II molecules	5
	Incompletely assembled TCR; retaining incompletely assembled forms of TCR in the ER	228

GroEL, which was first described by Hendrix et al. (111), represents 1 to 2% of the total protein content under normal conditions. Under stress conditions, however, its concentration is increased four- to fivefold (260). Although hsp do not accumulate to such high levels in eukaryotes, their concentrations are also increased after heat shock (260, 301).

When entering the host from the environment, a microbial pathogen is confronted by several changes, some of which are highly stressful. These include alterations in temperature, pH, and pO_2 (141, 142). Moreover, the pathogen is exposed to natural host resistance mechanisms such as phagocytosis by professional phagocytes (147). Once engulfed by phagocytes, the pathogen is confronted with reactive oxygen and nitrogen intermediates, attack by lysosomal enzymes, and depletion of Fe^{2+} . To protect itself against the host, the pathogen activates various evasion mechanisms including hsp synthesis. The importance of hsp for pathogen survival in this stressful environment is illustrated by experiments with a mutant of the intracellular pathogen *S. typhimurium* which overexpresses hsp (39). This mutant was shown to be resistant to a variety of oxidizing agents and heat. Conversely, mutants of *S. typhimurium* with specific hsp gene defects are highly susceptible to killing by activated macrophages and also express decreased virulence in vivo (79, 133).

While the importance of hsp for survival in the host holds true for a variety of intracellular pathogens, hsp induction seems to be less relevant for some other microbes, including *L. monocytogenes*. The ability of *L. monocytogenes* to survive in macrophages in the absence of increased hsp synthesis could be explained by the potential of this pathogen to evade the stressful endosomal environment at an early phase after phagocytosis (99). Thus, the impact of hsp on microbial survival in the host varies in different infections.

Infection is a bimodal process determined by the host and pathogen. During infection, the pathogen as well as the host increases hsp production (124, 145, 271, 273). Induction of host hsp synthesis in response to encounter with a pathogen has at least two major causes. First, infected macrophages are confronted with antimicrobial mechanisms which they have activated themselves during infection. Efficient protection against their own effector molecules (e.g., reactive radicals) becomes vital for macrophage survival. Second, once inside a phagocyte, many microbes, especially those which persist in the host, interfere with intracellular host cell metabolism. Not surprisingly, many of these pathogens are potent inducers of hsp synthesis in mammalian cells.

How Heat Shock Proteins Function as Chaperones

Proper folding and assembly of polypeptides depends on a set of conserved proteins known as molecular chaperones.

Although many chaperones are classified as stress proteins, they also perform essential functions under normal physiological conditions. Some hsp temporarily stabilize unfolded or partially folded proteins and thus promote the generation of the correct tertiary structure (21, 48, 88, 93, 106, 107).

Many molecular chaperones described so far are members of the hsp60 and hsp70 families. hsp70 cognate proteins in the cytosol associate with newly formed polypeptides during ribosomal translation (88) and are directly involved in protein transport processes between different intracellular compartments that lie across membranes (106). The mitochondrial hsp70 Ssc, for example, promotes protein translocation into mitochondria and is required for subsequent folding of newly translocated proteins (93, 107). In *E. coli*, the hsp70 homolog DnaK stabilizes newly synthesized proteins and promotes the assembly of proteins into multimeric complexes as well as their disassembly. In eukaryotes, one hsp70 (hsc73) participates in lysosomal degradation of cytosolic proteins while another hsp70 cognate protein plays an essential role in protein translocation into the ER (106).

In the ER, Bip (binding protein), also known as grp78, plays a somewhat wide-ranging role in the assembly of imported proteins. This protein binds intermediates of multimeric polypeptide complexes and controls their proper assembly (82, 83). Direct involvement of Bip in the formation of multimeric complexes has been shown for many proteins, including Ig, TCR, and MHC molecules (60, 191) (Table 1). In addition to Bip, a member of the hsp90 family, gp96 (also known as grp94), participates in the assembly of antibody molecules (192, 193) (Table 1). After being transported into the ER lumen, the newly synthesized light and heavy chains of the Ig molecules sequentially bind to hsp70 and to gp96 in a chaperone pathway (192).

Peptide binding sites have been identified for some chaperones, but unlike the highly specific substrate binding sites of enzymes, they possess relatively promiscuous specificity (191). For example, Bip has a unique binding site at the carboxy-terminal domain for interaction with polypeptides that resembles the peptide binding groove of MHC class I molecules (82). This binding site allows binding to a wide variety of unrelated polypeptides through hydrophobic residues. As with MHC molecules, some substrate motif specificity has been described for Bip, which preferentially recognizes peptides of 7 to 8 aa, comprising a promiscuous motif (27, 84, 237). Transient binding of such sequences to Bip seems to occur before they become buried inside fully folded proteins (215).

Similarly to hsp70, members of the hsp60 family mediate intracellular folding and translocation of proteins. hsp60 chaperones have been preferentially found in mitochondria and the cytoplasm. In *E. coli*, the 60-kDa GroEL and 15-kDa GroES act together in protein folding and assembly. Preliminary data

suggest that this GroE complex participates in cell wall synthesis (189). An hsp60 homolog of GroEL which is present in the mitochondrial matrix in eukaryotes and which participates in the folding processes of newly imported proteins and in prevention of aggregation of stress-denatured polypeptides has been identified (21, 75, 106). It has been suggested that members of the hsp70 and hsp60 families cooperate in a chaperone-assisted protein-folding pathway (107).

Role of Heat Shock Proteins in Antigen Processing and Presentation

Formation of stable MHC complexes capable of presenting antigenic peptides to T cells depends on their proper folding and assembly in the ER, as well as on the availability of peptide ligands. Folding and assembly of both MHC class I and class II molecules is initiated in the ER, whereas the site of peptide loading depends on the intracellular compartment in which degraded protein fragments are sampled (202). MHC class I molecules are loaded in the ER with ligands derived from endogenous proteins present in the cytosol (viral, tumor, or self antigens). Peptides from the cytoplasm are transported into the ER by a specialized transport system, termed the transporter associated with antigen processing (TAP). In contrast, MHC class II molecules bind ligands of extracellular origin in the endosomal compartment. To prevent premature loading of the MHC class II molecule in the ER, its binding site is blocked by the invariant chain, which is released in the endosome so that loading of MHC class II molecules with endosomal peptides becomes possible (244).

Several lines of evidence suggest that hsp play a role in MHC-antigen processing (49, 60, 191, 302). Folding and assembly of MHC-peptide complexes are promoted by molecular chaperones, which holds true for many other proteins. Members of the hsp70 family are critically involved in the processing and presentation of antigens (60, 126, 249, 290, 302). Bip and another endoplasmic chaperone, calnexin, promote the assembly of both MHC class I and class II molecules in the ER (5, 125, 191, 227) (Table 1). Furthermore, for Bip and other chaperones such as gp96 and hsp70 (ERp72), an interaction with misfolded MHC class II molecules has been demonstrated, resulting in their retention in the ER (29, 246). In the murine system, association of calnexin-bound MHC class I α -chain- β_2m heterodimers with TAP has been observed (211). This finding raises the possibility that calnexin facilitates MHC class I-TAP interactions and thus controls peptide binding to MHC class I molecules.

Srivastava and coworkers have provided substantial evidence that peptide transport from the proteasome to the ER and subsequent peptide loading of MHC class I molecules in the ER depend on a battery of hsp including cytosolic and endoplasmic members of the hsp70 and hsp90 families (174, 267, 268). Recent studies have revealed that gp96 in the ER acts as a peptide acceptor, receiving peptides of cytosolic origin after their transport through the ER membrane by TAP molecules (165). Subsequently, gp96-peptide complexes bind to MHC and the peptides are then translocated from gp96 to MHC class I molecules in an ATP-dependent manner (268). Due to its proteolytic activity, gp96 may also participate in further trimming of MHC class I peptides in the ER (11, 174). Finally, circumstantial evidence suggests association of hsp with non-classical MHC products, which may be recognized by a subset of $\gamma\delta$ T cells (123).

HEAT SHOCK PROTEINS AND PROTECTIVE IMMUNITY

Pathogen-Derived Heat Shock Proteins as Targets for the Immune Response: Control of Infection

Both host cells and microbes are confronted with dramatic alterations in their living conditions during infection. With these changing conditions, induction of hsp synthesis is vital for pathogen survival. Subsequently, increased pathogen hsp levels in cells lead to rapid degradation of hsp by the host processing machinery. Pathogen-derived determinants may then be efficiently presented by host cells and promote recognition of infected cells by the immune system. Although the exact role of hsp in immunity to microbial infection is incompletely understood, hsp apparently serve as important antigens in defense against infectious agents (141, 142, 144). In fact, immune responses to hsp have been observed in infectious diseases caused by bacteria, protozoa, fungi, and nematodes, as well as in various experimental infection models (Table 2) (43, 143, 250, 260). Evidently, due to their high conservation among various microbial pathogens, hsp are major antigens. They are known to induce very strong humoral and cellular immune responses in numerous infections. Different hsp cognate proteins, e.g., hsp60 or hsp70, have a high degree of sequence homology among various pathogenic or nonpathogenic bacteria (260). For example, the hsp60 in mycobacteria is homologous to the common antigen of *Pseudomonas aeruginosa* and to hsp60 of other gram-negative bacteria, including GroEL of *E. coli* (261, 308). The recent finding that the GroE complex is involved in bacterial cell wall synthesis suggests the ready accessibility of these hsp molecules to antibodies (189).

At least two factors contribute to the fact that hsp represent major antigens in a wide spectrum of infections: first, these proteins are abundant in the pathogen, especially under stress conditions; and second, immunologic memory for cross-reactive determinants of conserved hsp is generated during life based on frequent restimulation by subsequent encounters with microbes of with different degrees of virulence (143). Under these conditions, infection of an individual with a virulent pathogen would enable the already prepared immune system to react quickly before the immune response to more pathogen-specific antigens develops. An immune response to conserved determinants of hsp shared by different microbes may, furthermore, prevent colonization of the host by microbial pathogens. Consistent with this notion, a preference for hsp of $\gamma\delta$ T cells has been described in the murine system. Generally, $\gamma\delta$ T cells are considered to contribute to the first line of defense.

In mycobacterial infections, reactivity to hsp predominates, with hsp60 as an immunodominant target of the antibody and T-cell response in mice and humans (144, 309). hsp60-specific antibodies have been detected in patients with tuberculosis and leprosy, and also in mice after infection with *M. tuberculosis* (260, 308). Interestingly, in mice, hsp60-specific antibodies cross-react with hsp60 homologs from other prokaryotes (e.g., GroEL from *E. coli*) but not with the murine hsp homolog. In patients with leprosy or in persons vaccinated with *M. bovis* BCG, CD4 $\alpha\beta$ T cells specific for the mycobacterial hsp60 have been found (201). Surprisingly, about 20% of all mycobacterium-reactive CD4 $\alpha\beta$ T cells in mice immunized with killed *M. tuberculosis* were specific for hsp60 (146). This finding points to an important role for hsp60-specific T cells in mycobacterial infection. A protective role for hsp60-specific T cells in mycobacterial infection is further supported by other studies. Both in vitro stimulation of murine splenocytes and immunization of mice with mycobacterial hsp60 induce the expansion of CD8

TABLE 2. Immune responses to hsp in infections

Pathogen	Disease	hsp family	Reference(s)
Helminths			
<i>Schistosoma mansoni</i>	Schistosomiasis	hsp70, hsp90	110, 134
<i>Onchocerca volvulus</i>	Onchocercosis	hsp70	239
<i>Brugia malayi</i>	Filiariasis	hsp70	255
Protozoa			
<i>Plasmodium falciparum</i>	Malaria	hsp70, hsp90	131, 185
<i>Trypanosoma cruzi</i>	Chagas' disease	hsp70, hsp90	62, 70
<i>Leishmania major</i>	Leishmaniasis	hsp70	168
<i>Leishmania donovani</i>	Leishmaniasis	hsp70	182
<i>Leishmania braziliensis</i>	Leishmaniasis	hsp70	4
<i>Toxoplasma gondii</i>	Toxoplasmosis	hsp60	116
Fungi			
<i>Candida albicans</i>	Candidiasis	hsp90	187
<i>Histoplasma capsulatum</i>	Histoplasmosis	hsp60, hsp70	53, 94, 95
Bacteria			
<i>Mycobacterium tuberculosis</i>	Tuberculosis	hsp60, hsp70	261, 308
<i>Mycobacterium leprae</i>	Leprosy	hsp10, hsp60, hsp70	1, 92, 261, 308
<i>Chlamydia trachomatis</i>	Trachoma	hsp60, hsp70	51, 194, 213
<i>Borrelia burgdorferi</i>	Lyme disease	hsp60, hsp70	9, 100
<i>Helicobacter pylori</i>	Gastritis	hsp60	78
<i>Yersinia enterocolitica</i>	Yersiniosis	hsp60	206, 207
<i>Legionella pneumophila</i>	Legionnaires' disease	hsp60	120
<i>Treponema pallidum</i>	Syphilis	hsp60	117
<i>Bordetella pertussis</i>	Pertussis	hsp60	59
<i>Listeria monocytogenes</i>	Listeriosis	hsp60, hsp70	119, 155

$\alpha\beta$ T cells specific for mycobacterial hsp60 (160, 272, 310, 311). When adoptively transferred, isolated CD8 T-cell clones from mice immunized with mycobacterial hsp60 mediate partial protection against infection with *M. bovis* BCG in T-cell recipients (310). Additionally, hsp60-specific CD8 $\alpha\beta$ T cells which confer protection against infection with *M. tuberculosis* have been identified in mice infected with *M. tuberculosis* (263).

Immune responses to hsp60 are also frequently found in other microbial infections. In a murine model of yersiniosis, for example, direct involvement of hsp60-specific T cells in the anti-pathogenic immune response has been demonstrated (206). Here, numbers of CD4 $\alpha\beta$ T cells specific for hsp were increased in infected animals and mediated significant protection against infection with *Yersinia enterocolitica* when adoptively transferred. Similarly, in infants, levels of antibodies against hsp60 were significantly increased after vaccination with a trivalent vaccine against tetanus, diphtheria, and pertussis (59). These findings further suggest that priming of the immune system to hsp60 is a common phenomenon, occurring at an early stage of life.

Similarly to hsp60, other members of the hsp family have been described as dominant antigens in several infectious diseases. Increased antibody levels to hsp70, for example, have been identified in sera of patients suffering from malaria, leishmaniasis, schistosomiasis, filariasis, and candidiasis (260). In contrast to hsp60, responses to pathogen-derived hsp70 seem to be more restricted, sometimes exclusively species specific. An important role of the humoral response against hsp90 was demonstrated in systemic candidiasis (260). The hsp90-specific antibodies contributed directly to protection against *Candida albicans* infection (186). Peptide mapping revealed that a neutralizing antibody as well as patient sera recognized a highly conserved, self-reactive determinant of hsp90 (187). Antibodies specific for this conserved determinant of hsp90 of *C. albicans* have also been identified in healthy individuals, implying

that hsp of nonpathogenic commensal organisms can activate hsp90-reactive antibodies.

Several lines of evidence indicate that hsp also represent unique targets for $\gamma\delta$ T cells (105, 121, 209). In experimental listeriosis of mice, for example, hsp60-reactive $\gamma\delta$ T cells and hsp70-reactive $\gamma\delta$ T cells are specifically activated, and a protective role of these cells in immunity against infection with *L. monocytogenes* has been proposed (119, 154, 155). $\gamma\delta$ T cells reactive with hsp60 accumulate at the site of *L. monocytogenes* infection, and depletion of $\gamma\delta$ T cells with monoclonal antibodies increases listerial multiplication (119). Similarly, in *Plasmodium yoelii*-infected mice, hsp60-reactive $\gamma\delta$ T cells which confer partial protection against parasites are induced when adoptively transferred into recipients (289). hsp70 reactivity among $\gamma\delta$ T cells has been described as well (20). In infection with *Leishmania major* in both man and mice, $\gamma\delta$ T cells are specifically activated (238, 241). However, so far, little is known about the participation of hsp70-reactive $\gamma\delta$ T cells in resistance to infection.

Born and colleagues isolated $\gamma\delta$ T cells derived from the thymus of newborn mice that recognize a defined sequence of the mycobacterial hsp60 (31, 209, 210). Further characterization revealed that these $\gamma\delta$ T cells respond to a mammalian hsp60 peptide which shows partial homology to a mycobacterial hsp60 peptide. Based on these findings, it was originally proposed that cross-reactive $\gamma\delta$ T cells against hsp not only contribute to immunity against mycobacterial infection but also play a potential role in autoimmunity. Recent studies on hsp-reactive $\gamma\delta$ T cells indicate that a minimal peptide of the mycobacterial hsp60 which is not homologous to the mammalian hsp60 allows recognition by hsp60-reactive $\gamma\delta$ T cells (89). Interestingly, this hsp60 peptide activates all $\gamma\delta$ T cells expressing the V γ 1 chain. It is possible that $\gamma\delta$ T-cell stimulation by hsp results in the expansion of $\gamma\delta$ T cells with different epitope fine specificity but all expressing the V γ 1 chain. Thus, diverse

$\gamma\delta$ TCR respond to a single hsp60 peptide, and hence activation is oligoclonal and independent of the fine specificity of the $\gamma\delta$ TCR. It is conceivable that the hsp peptide is contacted by a conserved site in the V γ 1 chain of the TCR different from the more variable complementary region with unique epitope specificity. It remains to be determined whether such oligoclonally activated $\gamma\delta$ T cells play a biological role in infection.

Heat Shock Proteins Promote Antigen Delivery into the Major Histocompatibility Complex Class I Presentation Pathway

In an attempt to elicit optimal peptide-specific immune responses *in vivo*, various proteins such as ovalbumin, bovine serum albumin, and tetanus toxoid have been used in animal models as carriers for peptides in immunization protocols. These molecules improve the immunogenicity of defined epitopes in immunization experiments. In a similar approach, microbial hsp, e.g., hsp60 and hsp70, have been used as carrier molecules for peptide immunization (58). It has been proposed that hsp are particularly suited to be carriers because of their high affinity of binding to certain peptides and their involvement in various steps in antigen processing. Recently, peptide binding sites which are structurally similar to the Bip binding site have been identified within the mycobacterial hsp70 (237). Clusters of aliphatic residues are often characteristic of hsp70 binding sites, although some markedly hydrophobic peptides also bind.

Cross-linking of peptides to mycobacterial hsp60 or hsp70 induced peptide-specific immune responses against malaria that were independent of adjuvants in mice and monkeys (17, 18, 181, 216). Immunization of mice with an immunodominant viral peptide noncovalently associated with hsp70 was capable of eliciting strong peptide-specific T-cell responses (235, 236). Similarly, immunization of mice with a soluble fusion protein, consisting of an ovalbumin fragment covalently linked to mycobacterial hsp70, induced a strong MHC class I-restricted CD8 T-cell response against a dominant ovalbumin T-cell epitope (278). Moreover, complexes of recombinant human hsp70 with a peptide representing a CD8 T-cell epitope of lymphocytic choriomeningitis virus were found to induce protective immunity in mice against lymphocytic choriomeningitis virus challenge (41). In a further extension of this concept, defined peptides of foreign or self hsp60 have been used successfully as carriers for poorly immunogenic T-cell-independent carbohydrate antigens (161). Importantly, as documented in other studies, immunization of mice with mycobacterial hsp60 conjugated to peptide or carbohydrate antigens induced hsp-specific antibodies that cross-reacted only with hsp homologs from other prokaryotes but not with the mammalian hsp60 cognate (19).

The capacity of hsp to serve as carrier molecules has been studied intensively in murine tumor models. The observation that tumorigenicity of a murine macrophage tumor cell line was decreased after transfection with mycobacterial hsp60 led to the suggestion that hsp60 promoted the delivery of immunodominant tumor antigens to the cell surface and consequently facilitated the recognition and eradication of tumors by specific T cells (180). In other studies, gp96, which is involved in the loading of MHC class I molecules in the ER, has been conjugated with a viral T-cell epitope. These studies showed that exogenous antigens can be chaperoned by gp96 into the endogenous processing pathway, leading to MHC class I-restricted recognition of peptides by CD8 T lymphocytes (277). By applying this concept, purified gp96 was also able to cross-prime a CD8 T-cell response to a minor histocompatibility

antigen (11). The identity of a viral peptide bound to gp96 with a naturally MHC class I-presented peptide in virus-infected cells provides evidence that gp96 is capable of chaperoning immunodominant epitopes (205). Because association of gp96 with the peptide occurs independently of TAP (10), this finding suggests that the repertoire of peptides bound by gp96 encompasses all peptides present inside the ER, not only the peptides transported by TAP. Based on this finding, gp96 may be useful in generating CD8 T-cell responses against all kinds of intracellular antigens. In an extension of this concept, effective antitumor activity has been induced by treatment of mice with gp96 derived from autologous tumor cells without identifying the tumor-specific antigenic epitopes (281).

Use of either foreign or self-hsp as carrier molecules for antigenic determinants provides a basis for applying hsp in conjugate vaccines. However, due to immunogenicity and sequence similarity to self hsp, the potential of foreign hsp when used as carrier molecules to induce cross-reactive immune responses against self must be carefully evaluated.

Vaccination with Pathogen Heat Shock Proteins

Because hsp represent dominant antigens in numerous microbial infections, a potential use of pathogen-derived hsp for vaccination has been suggested. In fact, in various infectious disease models different vaccination strategies using hsp have induced significant protection (Table 3). For example, immunization of mice for example, with recombinant GroES and GroEL from *Helicobacter pylori* protected the animals against subsequent infection and development of gastroduodenal disease (78). Moreover, vaccination of mice with recombinant hsp60 from *Histoplasma capsulatum* induced protection against pulmonary histoplasmosis (95). Another example of a protective anti-hsp immune response has been shown in murine infection with *Y. enterocolitica*. Immunization of mice with yersinia-hsp60 induced a strong yersinia-hsp60-reactive T-cell response which conferred protection against a challenge with yersiniae (207). Similarly, studies by Lowrie and coworkers suggest a protective role of mycobacterial hsp60 in murine infection with *M. tuberculosis*. Mycobacterial hsp60 was first transfected into APC, which were then used successfully to vaccinate mice against subsequent infection with *M. tuberculosis* (262). Transfer experiments with immune spleen cells revealed that protection was T-cell dependent (263). Analysis of lymphocyte subsets revealed that effective protection against *M. tuberculosis* correlated with cytolytic responses of CD4 $\alpha\beta$ T cells, CD8 $\alpha\beta$ T cells, and $\gamma\delta$ T cells (264). In further experiments, naked-DNA vaccination was used. Mice which received plasmid DNA encoding mycobacterial hsp60 were partially protected against subsequent challenge with *M. tuberculosis* (28, 179, 284). Similar protection was achieved with plasmid DNA encoding mycobacterial hsp70 (178).

SELECTION AND ACTIVATION OF T CELLS

Self tolerance involves the elimination of T cells in the thymus with specificity for self antigens (128, 296). By removing such T cells, the T-cell repertoire is biased to react with foreign antigens. T-cell selection depends on TCR-mediated interactions with MHC-peptide complexes expressed on thymic epithelial cells. TCR-MHC interactions with low avidity result in positive selection, while those with high avidity lead to negative selection (12, 127). Generally, avidity is determined by three parameters: the intrinsic affinity between the TCR and the MHC-peptide complex; the density of TCR on the surface of T cells; and the density of MHC-peptide complexes on the sur-

TABLE 3. hsp-specific immune responses that confer protection against infection

Pathogen	Protective immune responses against hsp in microbial infection	Reference
<i>Helicobacter pylori</i>	Vaccination with GroES and GroEL homolog of <i>H. pylori</i> protects mice against infection and gastroduodenal disease	78
<i>Histoplasma capsulatum</i>	Vaccination with hsp70 of <i>H. capsulatum</i> enhances host resistance against infection	94
	Vaccination with hsp60 of <i>H. capsulatum</i> protects mice against pulmonary histoplasmosis	95
<i>Mycobacterium tuberculosis</i>	Vaccination with transgenic cell line expressing mycobacterial hsp60 protects mice against infection with <i>M. tuberculosis</i>	262
	Adoptive transfer of $\alpha\beta$ T cells or $\gamma\delta$ T cells specific for mycobacterial hsp60 confers protection against infection with <i>M. tuberculosis</i>	263
	Treatment of mice with DNA encoding the mycobacterial hsp60 protects mice against <i>M. tuberculosis</i> infection	179
	Treatment of mice with DNA encoding the mycobacterial hsp70 protects mice against <i>M. tuberculosis</i> infection	178
<i>Mycobacterium bovis</i> BCG	Adoptive transfer of CD8 T cells specific for mycobacterial hsp60 confers partial protection against <i>M. bovis</i> infection in mice	310
<i>Plasmodium yoelii</i>	Adoptive transfer of $\gamma\delta$ T cells specific for hsp60 of <i>P. yoelii</i> confers partial protection against infection with <i>P. yoelii</i> in mice	289
<i>Yersinia enterocolitica</i>	Adoptive transfer of <i>Yersinia</i> hsp60-reactive CD4 $\alpha\beta$ T cells mediates protection against lethal infection with <i>Y. enterocolitica</i> in mice	206
	Immunization of mice with <i>Yersinia</i> hsp60 induces protection against <i>Y. enterocolitica</i>	207

face of APC. It is further strengthened by interactions of accessory molecules, such as CD8 or CD4, with conserved regions of MHC class I or class II molecules, respectively. Depending on the avidity of the TCR with an MHC-peptide complex, a unique peptide can induce both positive and negative selection of the corresponding T cell. Both pathways of T-cell selection require a high diversity of self peptides present in the thymus (2). In contrast to selection of $\alpha\beta$ T cells, selection processes for $\gamma\delta$ T cells during maturation are less well understood. Although there is evidence for positive and negative thymic selection of $\gamma\delta$ T cells, extrathymic selection occurs for at least some $\gamma\delta$ T cells (98). Thymic selection deletes the majority of self-reactive T cells. Elimination of $\alpha\beta$ T cells with self-reactive TCR in the thymus is nevertheless incomplete. Poor display or absence of self peptides in the thymus as well as self-reactive T cells with low avidity may result in incomplete negative selection. Such T cells then leave the thymus and enter the periphery (90).

Under normal conditions, potentially self-reactive T cells in the periphery are effectively controlled by different mechanisms leading to peripheral tolerance (76, 136, 253). In some models, downregulation of TCR maintains tolerance among peripheral T cells in response to continuous challenge with self antigen (76, 253). The extent of downregulation, however, depends on the availability of antigen. Downregulation of both TCR and CD28, a costimulatory molecule involved in T-cell activation, has been shown to initiate a stage of unresponsiveness in T cells that is termed anergy (171, 176). Because CD28 interacts with surface ligands B7-1 (CD80) and B7-2 (CD86) on APC, the level of B7 expression participates in regulating the activation of T cells not only in the periphery but also in the thymus (97). Once T cells become activated, they express CTLA-4 (CD152), an additional receptor which resembles CD28. CTLA-4, which is known to bind B7 on APC more avidly than CD28 does, delivers inhibitory signals to the T cells. It is suggested that the inhibitory effects of CTLA-4 on T cells lead to termination of the immune response by curtailing IL-2 production and inducing cell cycle arrest (286). Apparently,

interactions between B7 on APC and CTLA-4 on lymphocytes limit the response of activated T cells to antigen and B7. Conversely, interactions between B7 on APC and CD28 on lymphocytes are involved in the reversal of the anergic state (171, 176). Therefore, the increased expression levels of B7 in the periphery found during microbial infection may play a critical role by supporting the activation of both the T cells controlling infection and the T cells with self-reactive potential.

In recent studies, correlation of hsp and B7 expression has been found on APC from patients with inflammatory disease, suggesting that coexpression of hsp and B7 participates in initiation and maintenance of autoimmune responses in inflammatory diseases (214). The observation that hsp is presented by B7-positive cells indicates that sensitization to hsp may contribute to the loss of immune tolerance and to inflammation in patients with autoimmune disorders.

SURFACE EXPRESSION OF SELF HEAT SHOCK PROTEINS

During infection, potentially self-reactive T cells can be activated by dominant determinants of microbial antigens. Molecular mimicry between self and foreign molecules has been proposed to represent one possibility for activation of such cells in the periphery (275, 285). Among microbial antigens implicated in autoimmunity induced by molecular mimicry, hsp may play an exclusive role. Homology between hsp from the pathogen and the host confronts the immune system with the dilemma of distinguishing self from foreign. Poor expression of self-hsp peptides in the thymus could allow T cells specific for self hsp to evade selection. In the periphery, elevated expression of conserved epitopes from pathogen-derived hsp could break tolerance and activate immune reactions against self-hsp determinants.

Cell Surface Expression of Self Heat Shock Proteins

During microbial infections, hsp determinants expressed on the cell surface can be recognized by antibodies with specificity for self-hsp epitopes. The mechanisms involved in translocating hsp to the cell surface are still not fully understood, since hsp are typical cytosolic proteins that lack the specific leader sequences normally required for cell surface expression. Nevertheless, numerous studies have reported hsp presentation on the cell surface (198). So far, the consequences of hsp expression and recognition by self-reactive antibodies for autoimmunity remain to be elucidated. hsp-specific monoclonal antibodies were used to detect hsp expression on a number of tumor cells, e.g., hsp60 on human B-lymphoma (Daudi) cells (80, 149), hsp70 on Daudi and T-lymphoma (H9) cells (61, 219), and hsp90 on human tumor cells (77). Other studies identified cell surface-expressed hsp on nontransformed cells. Surface-expressed hsp60 was identified on macrophages (297), oligodendrocytes (86), and endothelial cells (3, 304). Moreover, hsp70 or hsp90 products were identified on B cells (290) and monocytes (72). Surface-expressed hsp70 may also function as a target structure for natural killer (NK) cells (199). Although these studies strongly favor cell surface expression of hsp, cross-reactivity of antibodies with other cell surface proteins cannot be excluded. Therefore, the identity of these proteins awaits precipitation of the surface molecules by monoclonal antibodies followed by amino acid sequencing. Accordingly, the question remains whether self-hsp expression is associated with autoimmune disease.

In a number of autoimmune disorders in human and animal models, hsp expression in affected cells has been observed, supporting the idea that hsp expression contributes to immunopathologic changes. For example, a significant proportion of patients with systemic lupus erythematosus (SLE) expressed hsp90 on lymphocytes and monocytes (72). Similarly, in MRL/lpr mice, a model for SLE, increased surface localization of hsp90 and antibodies against hsp90 have been observed (74, 166). In these two systems, overexpression of hsp90 and the presence of antibodies provide tentative evidence that this molecule serves as an autoantigen, possibly causing autoimmune alterations in SLE and MRL/lpr mice. hsp70 expression on reticular fibroblasts derived from patients with severe Graves' disease symptoms, but not from healthy donors, was significantly increased (115). Another example of hsp cell surface localization has been described for lesions in chronic experimental autoimmune encephalomyelitis; increased expression of hsp60 correlated with the accumulation of $\gamma\delta$ T cells (91). Moreover, in autoimmune plaques of multiple sclerosis (MS), the proportions of $\gamma\delta$ T cells were increased compared to those in the peripheral blood. Again, $\gamma\delta$ T cells colocalized with hsp-expressing oligodendrocytes (86). Because such $\gamma\delta$ T cells induce the lysis of oligodendrocytes *in vitro*, it is possible that oligodendrocytes represent targets for hsp-reactive $\gamma\delta$ T cells caused by increased hsp expression.

Recently, another hsp member, which is recognized by T cells specific for myelin, was identified in MS (14, 294). In these studies, increased expression of alpha B-crystallin, a member of the small hsp family, was found in astrocytes and oligodendrocytes in MS lesions. With respect to recognition of hsp determinants by $\gamma\delta$ T cells, it has been suggested that some $\gamma\delta$ T cells directly interact with surface-expressed self hsp60, because recognition could be significantly inhibited by treatment of target cells with hsp60-specific antibodies (80, 149).

Other studies suggest that a member of the hsp70 family (gp75) serves as a presenting molecule for a tumor antigen to a defined population of tumor-specific $\gamma\delta$ T cells (153). A

beneficial role of hsp60 in inflamed synovium of patients with rheumatoid arthritis (RA) has been proposed recently. Expression of self hsp60 at sites of autoaggregation may actively ameliorate disease by stimulating lymphocytes of the Th2 type (295).

The recent finding that intestinal epithelial $\gamma\delta$ T cells recognize unconventional MHC molecules, whose expression is controlled by heat shock elements similar to those of hsp70 genes, suggests that recognition of stressed cells involves molecules other than hsp themselves, which are, however, regulated in a similar way to hsp (96).

There are, therefore, several examples that provide evidence for hsp presentation on the cell surface. Self epitopes that are surface expressed enhance the opportunity for cells to become targets of self-reactive antibodies with specificity for hsp. Frequently, hsp surface expression is increased in affected tissue in autoimmune diseases, emphasizing a role of hsp determinants in autoimmune disease.

Presentation of Self Heat Shock Protein Peptides by Major Histocompatibility Complex Molecules

T cells specific for self-hsp epitopes have been found in both human and animal studies. An important prerequisite for stimulation of hsp-reactive T cells is the presentation of the corresponding peptides by MHC molecules. Since stressful conditions raise hsp synthesis in cells (124, 139, 145, 271, 273), it can be assumed that increased levels of hsp in cells correlates with intensified degradation of these proteins and subsequent generation of hsp peptides in the cytosol. Under these circumstances, hsp peptides, like other peptides, gain access to the loading compartments of MHC class I molecules.

Among the many natural peptides that have been identified so far, self-hsp peptides were also isolated from MHC molecules (129, 203, 204, 231). Although the cytosolic localization of hsp would suggest MHC class I loading only, self-hsp peptides have also been isolated from MHC class II molecules, which normally present endosomal peptides derived from exogenous proteins. These findings provide evidence that self-hsp peptides have access to both MHC class I and class II molecules. This emphasizes that the division between loading of MHC class I and class II molecules is not as rigid as was originally assumed.

In several instances, hsp were found to serve as target antigens for T cells in autoimmune processes. In healthy individuals, T cells specific for self hsp that escaped thymic selection are effectively controlled. Tolerance of T cells to self hsp may be additionally maintained by the permanent encounter of cross-reactive hsp epitopes derived from food and commensal organisms (197, 254). Induction of tolerance by oral administration of antigens has been demonstrated in other systems (298). In these studies, introduction of self antigens via the gut mucosa effectively suppressed several experimental autoimmune diseases in an antigen-specific fashion. Comparison of the amino acid sequence of naturally processed self-hsp peptides eluted from MHC molecules with the database of known proteins revealed similarity to antigens from food and microbes (143). Particularly in pathologic situations, e.g., in infections with microbial pathogens, expression of conserved hsp epitopes has a detrimental potential. If the immune system fails to ignore these cross-reactive regions, a protective immune responses may be converted to a pathological one.

PATHOGENIC IMMUNE RESPONSE TO HEAT SHOCK PROTEINS

In healthy individuals, there exists a well balanced network of potentially self-reactive antibodies and T cells that have evaded deletion processes. In this situation, the immune system responds to its own hsp in a manner that could promote the recognition and elimination of aberrant cells. However, when hsp expression and hsp-specific immune responses are regulated inappropriately, autoimmune reactions may follow. Tolerance to self antigens in particular may become distorted by the frequent encounter of the immune system with foreign (e.g., microbial) antigens with high similarity to self (196). In fact, molecular mimicry is widely discussed as one mechanism responsible for the induction of autoimmune disease (285).

Immune responses to conserved regions shared by pathogen and self hsp in individuals during active infection were not unexpected. Hence, in several infectious diseases, increased titers of antibodies reactive for conserved regions of hsp shared by the pathogen and host have been identified. For example, increased levels of antibodies against self hsp60 have been found in sera of patients with Lyme disease, suggesting an association between self-reactive antibodies and infection with *Borrelia burgdorferi* (257). Another example of harmful hsp effects is found in chlamydial infections, where hsp frequently represent strong antigens. Immune responses to chlamydial hsp60 significantly correlate with disease sequelae in humans, and hsp-specific antibodies cause marked inflammatory reactions in animal models of experimental *Chlamydia trachomatis* infection (38). A direct contribution of antibodies specific for chlamydial hsp60 to disease pathogenesis has been described (306). It is assumed that in chlamydial infection, bacterial hsp60 induces cross-reactive immune responses to self hsp60 and thus is in the focus of immune damage during chronic infection. Similarly, in sera from patients with malaria, antibodies responding to hsp70 of *Plasmodium falciparum* and self can be found (185). Moreover, a detrimental role of hsp-specific antibodies was discussed as part of the infection-induced autoimmune response in onchocerciasis (190). Together, these findings suggest that autoantibodies directed against host hsp can be induced by the homologous microbial protein.

Sequence comparison between the mycobacterial hsp60 and the mammalian hsp60 revealed a homology of about 60% (132), and, not surprisingly, conserved regions of hsp60 have been found to be the target of immune responses in mycobacterial infections. For example, in leprosy patients, antibodies directed to the mycobacterial hsp60 cross-react with self hsp60 expressed by sciatic nerves (167). Moreover, T cells specific for self hsp60 have been found in leprosy patients. Activation of self-hsp60-reactive T cells seems to occur preferentially during inflammatory responses (7). This indicates that some epitopes recognized by patient antibodies or T cells are shared by the mycobacterial hsp60 and that cross-reactivity may contribute to autoimmune processes found in patients with leprosy. Similarly, cross-reactive CD8 T cells recognizing regions of the mycobacterial hsp70 and the human hsp70 have been identified in patients with tuberculosis (233). In other studies, CD4 T cells that were isolated from leprosy patients and had specificity for mycobacterial hsp70 also recognized epitopes of the human hsp70 cognate (85). Although conclusive evidence is missing, it has been proposed that cross-recognition by hsp-reactive T cells plays a unique role in autoimmune processes in chronic inflammation, such as during leprosy and tuberculosis.

Further support for T-cell cross-recognition of hsp regions shared by the host and pathogen comes from epitope-mapping studies with a T-cell clone derived from mice primed with

mycobacterial hsp60. Interestingly, two cross-reactive peptides of the mycobacterial hsp60 and self hsp60 that have only intermediate homology were identified (252, 311). Importantly, both peptides are recognized by cross-reactive CD8 $\alpha\beta$ T cells at the low concentrations typical of CD8 T-cell epitopes (73, 232). This strongly suggests that during mycobacterial infection, an increased expression of such hsp60 epitopes by MHC class I molecules allows the stimulation of CD8 T cells with the aforementioned cross-reactivity. Because these CD8 T cells also respond to stressed host cells in vitro, these findings provide further support that foreign hsp epitopes with homology to self hsp can trigger autoimmune responses. Moreover, in vivo transfer of these CD8 T cells to $\alpha\beta$ -T-cell-deficient knockout mice resulted in fatal autoimmune disease due to gut epithelial-cell damage (274).

Especially in chronic inflammatory diseases, structural homology between microbial hsp and self hsp was originally postulated to provide a basis for autoimmunity (196, 285). Indeed, immune responses to conserved regions shared by self hsp and microbial hsp are found frequently in chronic inflammatory diseases. This cross-reactivity prompted the idea that at least in some autoimmune disorders, the trigger for autoaggression may lie in a microbial infection which activated immunity against self hsp. Increased antibody reactivities to hsp60 have been detected in the sera of patients with inflammatory bowel diseases, e.g., Crohn's disease and ulcerative colitis (69, 276). The hsp60 antibodies from patients with Crohn's disease reacted with mycobacterial hsp60 only, whereas sera from patients with ulcerative colitis were directed against the human hsp60 but not the mycobacterial hsp60. Other studies have provided substantial evidence for association of mycobacterial hsp60 with Crohn's disease (214). The presence of antibodies reactive with the mycobacterial hsp60 in patients with Crohn's disease suggests that mycobacterial infections represent an inducing factor for this disease. Similarly, the humoral response against hsp60 and hsp70 of *M. bovis* was augmented in patients with rheumatoid arthritis (RA) and with SLE (212). In patients with recurrent oral ulcers, cross-reactive lymphocytes which respond to a shared epitope of the mycobacterial hsp60 and human hsp60 have been isolated (108). It has been proposed that the high load of microorganisms that colonize the oral mucosa, combined with the molecular mimicry between microbial and human hsp epitopes, elicits autoimmune responses in the oral mucosa.

While these studies favor a role for microbial hsp in inducing cross-reactive hsp responses in chronic inflammatory diseases, the role of autoimmune antibodies or T cells in disease pathogenesis still remains to be elucidated. The fact that antibodies as well as T cells cross-reactive for epitopes shared between pathogen hsp and mammalian hsp have been identified in healthy individuals (164, 200), probably because of the abundant presence of commensal organisms, argues against a disease-provoking role for hsp antibodies in autoimmune disease.

Role of Sequence Homology for Cross-Reactivity of T-Cell Epitopes

Cross-reactive T-cell responses against hsp from the microbe and host have been found in a number of studies (7, 164, 200, 224). However, most of these studies were based on the use of synthetic peptides and hence fail to directly demonstrate natural processing of cross-reactive determinants. Experiments have been performed to evaluate whether physiologic processing generates self-epitopes presented by host cells to cross-reactive T cells specific for microbial hsp. In initial studies, an in vitro stimulation system allowed the generation of murine

CD8 $\alpha\beta$ T cells, which respond to stressed host cells by stimulation with mycobacterial hsp60 peptides (160). Since cells augment hsp synthesis under stressful conditions, these results favored cross-recognition of mycobacterial hsp60 peptides and peptides derived from self hsp60. A T-cell epitope of the mycobacterial hsp60 which specifically stimulates CD8 $\alpha\beta$ T cells derived from mice immunized with mycobacterial hsp60 (311) or infected with *M. bovis* BCG (310) has been identified. These cells recognized host cells previously exposed to stress-inducing agents, thus demonstrating cross-reactivity with naturally processed determinants. Experiments in which target cell lysis by CD8 T cells was inhibited by treatment of stressed target cells with hsp60-specific antisense oligonucleotides further emphasize that autoimmune lysis by hsp60-reactive CD8 T cells is based on recognition of self-hsp peptides (273). In these studies, although recognition by T cells was restricted exclusively to peptide presentation by the murine MHC class I molecule H-2D^b, the mycobacterial hsp60 peptide did not fully correspond to the characteristic motif of naturally eluted H-2D^b peptides. The CD8 T cells failed to respond to a peptide from a conserved region of the mammalian hsp60 representing highest homology in the self-hsp60 sequence. Rather, T cells responded to a self-hsp60 peptide with intermediate homology but encompassing the characteristic anchor residue essential for binding to H-2D^b. Apparently, the lack of reaction with the homologous peptide from the mammalian hsp60 was caused by the lack of a single residue required for anchoring the peptide in the H-2D^b groove.

In sum, these findings suggest that T-cell cross-reactivity is influenced primarily by two features. First, the requirements for binding of peptides to the MHC molecule have to be fulfilled; and second, homology of the MHC-bound peptide to a stimulatory peptide decides whether cross-reactive T cells are activated. Significant homology between regions shared by host hsp and microbial hsp therefore does not necessarily imply cross-reactivity. Additionally, different origins of both proteins—exogenous or endogenous—may result in distinct MHC-processing pathways. Further, it has been shown that flanking sequences of epitopes in proteins strongly influence the enzymatic cleavage site, leading to different epitopes (33, 152). In conclusion, cross-reactive determinants of microbial hsp can prime self-hsp-reactive T cells. However, cross-reactivity between hsp peptides is more complex and influenced not only by highest homology but also by other factors.

Anti-Heat Shock Protein Responses in Animal Models of Autoimmune Disease

For a better understanding of the mechanisms underlying human autoimmune diseases, several experimental models have been exploited. Although the pathogenic mechanisms vary, the contribution of hsp-specific immune responses to disease has been demonstrated in a number of models (Table 4). Data from several arthritis models such as adjuvant arthritis (AA), pristane-induced arthritis, streptococcal cell wall-induced arthritis, and collagen type II-induced arthritis favor a role for hsp60 autoimmune T cells in disease (22, 287, 291, 293). Perhaps the most striking evidence for the role of hsp60 as a critical autoantigen in the development of autoimmune disease has been obtained in two animal models, AA in rats and insulin-dependent diabetes mellitus (IDDM) in NOD mice. Certain rat strains immunized with heat-killed *M. tuberculosis* in incomplete Freund's adjuvant develop a severe polyarthritis which resembles RA in humans (42). T cells are critical, since this disease can be adoptively transferred to naive recipients by CD4 T cells from arthritic rats. Epitope analysis

of an arthritogenic T-cell clone revealed specificity for a non-conserved mycobacterial hsp60 peptide aa 180 to 188, with only 3 of 9 residues identical to those of the mammalian hsp60 (293). Paradoxically, the same antigen specificity was described for CD4 T cells which conferred protection against this disease (292). Further characterization of the T-cell clones revealed that the arthritogenic and protective clones produced different amounts of IFN- γ , which is responsible for modulation of the immune responses in either a beneficial or a detrimental fashion (6). Attempts to induce AA by immunization with mycobacterial hsp60 alone failed; instead, immunization with mycobacterial hsp60 induced a state of resistance to AA (22, 293). Moreover, complete protection from AA was induced by treatment with the immunodominant peptide aa 180 to 188 of the mycobacterial hsp60, and adoptive transfer of T cells from immunized donors to naive recipients conferred protection (305). Similarly, a therapeutic effect against AA was achieved by administering a recombinant vaccinia virus expressing the hsp60 (177). Further, suppression of AA was induced by oral administration of mycobacterial hsp60 (101) or nasal tolerization with the hsp60 peptide aa 180 to 188 (222). In other studies, immunization with a mycobacterial hsp60 peptide, which primes for cross-reactive T-cell responses to the corresponding region of the self hsp60, protected against AA (8). This runs counter to the accepted theory that cross-reactive T-cell responses are responsible for autoimmunity. It has been suggested that in AA, cross-reactivity between bacterial hsp60 and self hsp60 maintains a regulatory protective T-cell population which becomes fully activated by immunization with the cross-reactive mycobacterial hsp60 epitope. These data provide evidence that recognition of self-hsp60 can have beneficial effects in arthritis and may offer new strategies for improved control measures in inflammatory processes by administration of peptides cross-reactive to self determinants.

Another example of hsp involvement in an autoimmune-disease model is IDDM in NOD mice. The incidence of IDDM in NOD mice is drastically reduced by immunization with mycobacterial hsp60 (66). Other studies extended these findings by showing that the development of diabetes in such mice is prevented by infection with *M. avium* (32). Here, mycobacteria, and probably their hsp60, may be responsible for modulating the immune response in NOD mice and preventing diabetes. Although circulating antibodies to the self-hsp60 are increased in NOD mice, the T-cell response is mainly in charge of the onset of diabetes. In other studies, adoptive transfer of a CD4 T-cell clone specific for the mycobacterial hsp60 accelerated the onset of IDDM, suggesting that β -cell destruction in the pancreas results from cross-recognition of a self protein (68). The finding that β -cells of NOD mice show increased expression of hsp60 supports the idea that self hsp is a candidate for the autoantigen (36, 37). An epitope of the human hsp60 (p277) with the amino acid sequence VLGGGCALLR-CIPALDSLTPANED which was recognized by CD4 T-cell clones isolated from NOD mice was identified (68). Interestingly, this peptide differs only by 1 aa from mouse hsp60. After adoptive transfer, T-cell clones produced profound insulinitis in mice. However, when attenuated by gamma irradiation, the same T cells protected NOD mice against IDDM. Similarly, immunization of NOD mice with the hsp60 peptide p277 conferred significant protection (65, 68) whereas immunization of standard strains of mice induced diabetes (67). Recent data suggest that p277 treatment of NOD mice induces a Th2 cytokine burst which downregulates the Th1-mediated autoimmune response to hsp60 (64).

Although these results provide strong evidence that one of the antigens in diabetes is related to hsp60, recent observations

TABLE 4. hsp involvement in experimentally induced autoimmune diseases

Disease	Strain	Causative agent	Evidence for hsp60 involvement in disease	Major reference(s)
IDDM	NOD mice	Spontaneous	Transfer of CD4 T cells specific for mycobacterial hsp60 accelerates the onset of IDDM	66
			Epitope shared by mycobacterial and mammalian hsp60 is recognized by CD4 T cells causing insulinitis	68
			Immunization with an hsp60 peptide confers protection	65, 67, 68
			Immune response to hsp60 correlates with insulinitis	258
			Tolerance induced to a self-hsp60 epitope in NOD mice carrying a self-hsp60 transgene	23
AA	Lewis rats	Heat-killed <i>M. tuberculosis</i>	Isolation of an arthritogenic T-cell clone with specificity for a nonconserved mycobacterial hsp60 peptide	293
			Protection is induced by immunization with an hsp60 peptide	305
			Protection against AA is induced by vaccination with hsp60-expressing vaccinia virus	177
			Protection against AA is induced by immunization with a mycobacterial hsp60 peptide cross-reactive to self-hsp60	8
			Administration of the mycobacterial hsp10 delays onset and severity of disease	226
			Protection against AA is induced by immunization with naked DNA encoding mycobacterial hsp60	225
			Intranasal tolerization to an hsp60 peptide suppresses the onset of AA	222
			Protection is induced by immunization with mycobacterial hsp70	157
			Pristane arthritis	Mice
Induction of disease depends on CD4 T cells specific for hsp60	270			
Self hsp60 is a target for serum antibodies and T cells	16			
Self-hsp60 expression is found in inflamed joints	15			
Streptococcal cell wall-induced arthritis	Lewis rats	Cell walls of streptococci	Immunization with mycobacterial hsp60 confers protection against disease	291
Collagen-induced arthritis	Lewis rats	Collagen type II	Immunization with mycobacterial hsp60 slightly suppresses disease	22
Experimental autoimmune encephalomyelitis	Lewis rats	Myelin basic protein	Immunization with a peptide of the myelin protein cross-reactive with a mycobacterial hsp60 peptide protects against disease	26

favor a 64-kDa protein, glutamic acid decarboxylase (GAD), as a major self-antigen of IDDM in both NOD mice and humans (13). When administered to NOD mice, GAD induced tolerance and protected mice against IDDM development (140, 288). The potential of GAD to induce protection against IDDM could be explained by assuming that autoimmunity to GAD induces a cascade of reactivities to other β -cell self antigens, including hsp60, and that vaccination with GAD influences reactivity not only to self but also to other antigens. Interestingly, hsp60 and GAD share a conserved region, and a T-cell epitope of GAD is within this shared region (140).

These studies hence provide evidence for a critical role of hsp60 in the development of autoimmune diseases such as IDDM in NOD mice and AA in rats. The fact that hsp60, like other stress proteins, is expressed in every cell raises the question of how T-cell immunity to hsp60 causes organ-specific

diseases such as AA or IDDM. It can be assumed that hsp, together with other organ-specific self antigens, serve as targets for the autoimmune response.

IMMUNE RESPONSE TO HEAT SHOCK PROTEINS IN HUMAN AUTOIMMUNE DISEASES

Several studies do point to a role of hsp in human autoimmune diseases. However, in contrast to animal models, the evidence is less convincing (Tables 5 and 6). Involvement of hsp in autoimmune responses depends on two criteria: first, hsp need to be expressed by cells of the target organ in a different way from at other tissue sites to allow organ-specific recognition by T cells and antibodies; and second, control of natural regulatory mechanisms for organ-specific inflammation must be disturbed.

TABLE 5. hsp involvement in human autoimmune diseases

Disease	hsp-specific responses	Reference(s)
RA	Antibodies specific for mycobacterial hsp60	188
	Binding of hsp60-specific antibodies to synovial tissue of patients with RA	55
	Raised expression of self-hsp60 in inflamed synovium in juvenile chronic RA	30
	Response of cross-reactive $\alpha\beta$ T cells from inflamed sites to mycobacterial hsp60 and human hsp60	173
	Cross-reactive $\alpha\beta$ T cells from synovial fluid of patients with juvenile chronic RA specific for conserved regions of the mycobacterial and human hsp60	56, 112
	T- and B-cell responses to mycobacterial hsp60 in juvenile RA	50
	$\gamma\delta$ T cells in synovial fluid of RA reactive for mycobacterial hsp60	121
	Response of cross-reactive $\gamma\delta$ T cell clone from synovial fluid to mycobacterial and human hsp60	104
	Overexpression of hsp60 on synovial fluid lymphocytes from RA patients	245
	Localization of mycobacterial hsp60-reactive B cells within affected joints	240
	Correlation of T-cell responses to human hsp60 in early course of oligoarticular RA with disease remission	223
T cells of synovial fluid and peripheral blood reactive for mycobacterial hsp60	40	
SLE	Overexpression of hsp90 in B and T cells of SLE patients	72
	Raised antibody levels to hsp90 in a significant group of SLE patients	44
	Antibodies reactive with hsp90 in childhood-onset SLE	45
MS	hsp70-specific antibodies in serum and cerebrospinal fluid of MS patients	24
	Detection of hsp70-reactive and hsp60-reactive $\alpha\beta$ T cells in spinal fluid of MS patients	25, 242
	Correlation of overexpression of hsp60 by immature oligodendrocytes with localization of $\gamma\delta$ T cells	256
	Accumulation of $\gamma\delta$ T cells in areas of demyelination	256
	Detection of $\gamma\delta$ T cells in acute lesions of MS	303
	Induction of hsp expression in oligodendrocytes in vitro	86
	T cells specific for the small hsp, alpha B-crystallin, in MS patients	294
	Increased expression of alpha B-crystallin in astrocytes and oligodendrocytes in MS patients	294
	Increased expression of alpha B-crystallin in early stage of lesional development in a subpopulation of oligodendrocytes	14
Reactive arthritis	Response of $\alpha\beta$ T cells from synovial fluid to human hsp60, to stressed host cells, and to mononuclear cells isolated from inflamed joints	112
	Selective stimulation of $\gamma\delta$ T cells by in vitro stimulation with hsp60	113
	Recognition of HLA-B27-restricted chlamydial hsp60 peptide by CD8 T cells derived from synovial fluid of patient with chlamydia-induced reactive arthritis	184
	Identification of a T-cell epitope within the chlamydial hsp60 recognized by a T-cell clone isolated from a patient with reactive arthritis	52
Kawasaki disease	Antibodies to mycobacterial hsp60 and autoantibodies to epitopes of human hsp60-specific antibodies	307
Behcet's disease	hsp60-specific antibodies in serum from patients	170
	T-cell response to self-hsp60	217
	Stimulation of T cells from patients with Behcet's disease by self hsp60 peptides	138
	$\gamma\delta$ T cells of patients reactive to mycobacterial hsp60 peptides	109
Psoriasis	Antibodies specific for mycobacterial hsp60	229
Atherosclerosis	hsp60-specific antibodies	304
	Correlation of intensity of hsp60 expression with recruitment of hsp-reactive T cells	159
	Lysis of macrophages expressing hsp60 from patients by antibodies against self-hsp60	247
Systemic sclerosis	Antibodies specific for mycobacterial hsp60	50
Chronic gastritis	Colocalization of $\gamma\delta$ T cells with hsp60 expression of inflammatory gastritis epithelium	71
Hashimoto's thyroiditis	High expression of self-hsp60 in follicular cells from patients	162

Antibodies to Heat Shock Proteins

Humoral immune responses to hsp have been found in a number of human autoimmune diseases (143). However, because titers against hsp varied from patient to patient and because hsp-specific antibodies were occasionally found in healthy individuals, the role of these proteins in autoimmune

diseases is incompletely understood. Despite these inconsistencies, a correlation between anti-hsp antibodies and the severity of disease holds true for certain autoimmune or chronic inflammatory diseases. Increased levels of hsp60-specific antibodies in serum have been found in atherosclerosis (304), systemic sclerosis (50), psoriasis (229), Kawasaki disease (307),

TABLE 6. Findings militating against a role of hsp in autoimmune diseases

Disease	Finding	Reference(s)
IDDM in NOD mice	GAD is the major disease-inducing antigen	140, 288
	Levels of anti-self-hsp60 antibodies do not exceed levels in control mice	183
Reactive arthritis	The frequency of hsp60-reactive T cells is low in patients with reactive arthritis	175
	In inflammatory synovitis, no response to self-hsp60 of synovial fluid cells is observed	220
RA	Synovial-fluid-derived T cells do not respond to hsp60 in adults with chronic RA	56
	Inconsistent levels of self-hsp-reactive antibodies are found in patients with RA	130
	Similar T-cell responses of synovial fluid and peripheral samples from patients and normal individuals are found with respect to hsp60 reactivity	81
SLE	No evidence is found for increased anti-self-hsp antibody levels	156
	An inconsistent pattern of elevated IgM antibody levels to hsp60 is found in SLE patients	212
MS	hsp60 expression is absent in MS lesions	14

and Behcet's disease (170). In many cases, antibody titers exceeded the levels found in healthy individuals. Other studies have provided direct evidence that antibodies against hsp specifically bind to target tissues of the autoaggressive response. Thus, hsp60-specific antibodies show reactivity for synovial tissue in AA in rats and in patients with RA (55). Similarly, in juvenile chronic arthritis, such hsp antibodies react with synovial membranes and expression of self-hsp60 in inflamed synovium is raised significantly (30). Antibodies to hsp60 have also been detected in patients with cystic fibrosis, SLE, or juvenile chronic arthritis, who apparently have significantly elevated titers compared to healthy individuals (57). However, with respect to anti-hsp60 antibody levels in SLE patients, evidence to the contrary also exists (130, 156) (Table 6). It has been claimed that antibodies to the mycobacterial hsp60 play a role in ankylosing spondylitis and RA, and cross-reactivity to self-hsp may play a role in these diseases to some extent (188). Antibodies against human hsp60 cross-reacting with *E. coli* hsp60, which significantly exceeded the titers found in controls, have also been detected in patients with RA (118). Anti-self-hsp60 antibodies could be induced by commensal organisms such as *E. coli* via molecular mimicry. Similarly, elevated levels of hsp90-reactive antibodies have been detected in some autoimmune diseases, implying that these antibodies participate in autoimmune processes. For example, overexpression of hsp90 was found in B and T cells in 20% of SLE patients, and this correlated with active central nervous system and cardiorespiratory disorders (72). Consequently, increased antibody levels to hsp90 have been described for a group of SLE patients (44). hsp-reactive antibodies have been found in MS patients as well. High titers of antibodies against hsp70 were identified in serum and cerebrospinal fluid of these patients (43). Despite these reports of autoantibodies to hsp in human autoimmune diseases, the general significance of the humoral anti-hsp response with respect to pathogenesis remains to be determined. The low prevalence of hsp in patients indicates that expression of hsp and formation of antibodies plays a pathogenic role in a subset of patients only.

Response of $\alpha\beta$ T Cells to Heat Shock Proteins

As with autoantibodies against hsp, hsp-reactive T cells seem to be less prominent in human autoimmune diseases than in experimental models. The concept of overexpression of self hsp either on the cell surface proper or as peptides presented by MHC products has been central to the hypothesis that

hsp-specific antibodies and T cells play a role in the pathogenesis of human autoimmune diseases.

Many events, such as bacterial or viral infections or ischemic processes which cause inflammation, may trigger the expression of self hsp. In fact, evidence for increased hsp expression has been presented for various inflammatory diseases in humans (143). In atherosclerosis, the intensity of hsp60 expression correlates with recruitment of hsp-specific T cells (159). Whenever hsp overexpression includes conserved hsp determinants, activation of cross-reactive T cells may occur. Such cross-reactivity has been demonstrated for $\alpha\beta$ T cells isolated from sites of inflammation in patients with RA, which specifically respond to the mycobacterial hsp60 and the human hsp60 (173). Likewise, $\alpha\beta$ T cells specific for conserved regions of the mycobacterial hsp60 and the human hsp60 have been detected in the synovial fluid of patients with juvenile chronic arthritis (56, 112). Interestingly, children with juvenile RA showed T-cell responses to hsp60 and to aa 180 to 188 of mycobacterial hsp60, which also serves as a dominant antigen in AA in rats (50). Since cross-reactivity to hsp60 was not observed in adult patients with chronic arthritis, hsp seem to play a role in juvenile forms of RA only (56) (Table 6).

T-cell responses to hsp60 have also been found in patients with Behcet's disease (169, 217). Two mycobacterial hsp60 peptides and homologous peptides from the self hsp60 stimulated $\alpha\beta$ T cells and caused uveitis when administered to Lewis rats (269). These studies suggest that different peptide determinants within the hsp60 are involved in the pathogenesis of Behcet's disease. Additionally, $\alpha\beta$ T cells with hsp reactivity may play a role in the pathogenesis of MS, as indicated by the results of studies showing that hsp70-reactive and hsp60-reactive $\alpha\beta$ T cells were more frequently found in patients than in healthy controls (25, 242). Epitope mapping revealed that the response of hsp60-specific T cells is directed to conserved epitopes (243).

In reactive arthritis, which represents an inflammatory arthritis that follows microbial infection, $\alpha\beta$ -T-cell responses to hsp60 epitopes shared by the mycobacterial hsp60 and the self-hsp60 cognate proteins seem to play a prominent role (112, 175). This notion is supported by the isolation from synovial fluid of *Y. enterocolitica*-reactive CD4 $\alpha\beta$ T cells which respond to human hsp60. These cells also react with heat-stressed APC and with mononuclear cells from the synovial fluid of inflamed joints (112). These findings provide further support for induction of hsp-directed autoimmune T-cell responses by natural infection. However, the prevalence of

hsp60-reactive T cells in lesions of patients with reactive arthritis is low (175), and reactivity to human hsp60 of synovial-fluid cells from patients with inflammatory synovitis was absent in other studies (220) (Table 6). Obviously, the relative importance of T-cell responses to hsp60 in reactive arthritis needs to be further evaluated. At present, it appears likely that T cells specific for conserved regions of hsp60 in reactive arthritis play a role in the maintenance of disease rather than causing pathogenesis.

In summary, several findings support the involvement of $\alpha\beta$ T cells specific for hsp in autoimmune diseases, but their role in disease development is probably less dominant than was originally thought. It appears most likely that tissue destruction in autoimmune disease is initiated primarily by T cells specific for organ-specific antigens. Because these conditions promote the expression of self hsp, T cells specific for hsp may arise in a second wave and then may be attracted to sites of inflammation, where they contribute to autoaggression. The possibility also exists that tissue-specific antigens have sequence similarity between hsp and consequently allow the activation of hsp-reactive T cells. In fact, homology of hsp to a major autoantigen has been shown in IDDM (135). The $\alpha\beta$ T cells specific for GAD are probably the earliest cells in the autoimmune cascade. While the original proposal that hsp60 serves as an important target of the immune response in IDDM has not been confirmed, sequence similarities between hsp60 and GAD in IDDM may reflect a common relation between hsp and other autoantigens in a number of autoimmune disorders.

Response of $\gamma\delta$ T Cells to Heat Shock Proteins

Several lines of evidence indicate that hsp-reactive $\gamma\delta$ T cells are triggered in various pathological conditions (143, 250), whereas the mechanisms by which $\gamma\delta$ T cells contribute to the immune response remain elusive. One of the first studies which demonstrated hsp60 responsiveness of $\gamma\delta$ T cells in humans is based on their identification in synovial fluid of a patient with RA (121). In this study, the isolated mycobacterial hsp60-reactive $\gamma\delta$ T-cell clone responded to the human homolog, suggesting that cross-reactive hsp60-specific $\gamma\delta$ T cells are involved in the pathogenesis of RA (104). As with $\alpha\beta$ T cells, $\gamma\delta$ T cells specific for hsp60 are frequently found in healthy individuals, pointing to a regulatory role rather than an active effector role for $\gamma\delta$ T cells (105). In healthy individuals, the use of V γ V δ genes is highly restricted, with V γ 2 δ 2 (or V γ 9 δ 2) being the most frequent TCR combination for $\gamma\delta$ T cells in adults. In RA patients, V γ use seems to be significantly skewed. In these patients, V γ 2 was less dominant whereas a selective expansion of the V γ 3 subset of $\gamma\delta$ T cells was observed in synovial fluid (137). This difference may play a role in the autoaggression found in RA. In other studies, an elevated proportion of $\gamma\delta$ T cells expressing V δ 1 was found in the synovial fluid of patients with RA (150, 158, 265) whereas among peripheral lymphocytes from healthy individuals, the frequency of the V δ 1-expressing population was low (98).

Accumulation of $\gamma\delta$ T cells in areas of demyelination has been detected in MS patients (256, 303). These $\gamma\delta$ T cells colocalized with immature oligodendrocytes which overexpressed hsp60 (256). Moreover, in patients with a recent onset of MS, increased numbers of $\gamma\delta$ T cells have been observed, which may reflect the involvement of $\gamma\delta$ T cells in disease development (259). Lysis of oligodendrocytes by $\gamma\delta$ T cells has been demonstrated, supporting the notion that $\gamma\delta$ T cells are involved in the T-cell-induced damage in MS (87). Finally, colocalization of $\gamma\delta$ T cells and hsp60-expressing oligodendrocytes in chronic brain lesions and isolation of $\gamma\delta$ T cells from

synovial fluid of MS patients emphasizes that hsp-reactive $\gamma\delta$ T cells play a role in the pathogenesis of MS. $\gamma\delta$ T cells also colocalized with hsp60 expression in inflammatory gastric epithelium in patients with chronic gastritis (71) and in atherosclerotic lesions (159).

Several findings indicate a role for hsp60-reactive $\gamma\delta$ T cells in reactive arthritis. Stimulation of synovial-fluid lymphocytes with mycobacterial hsp60 or members of the family *Enterobacteriaceae* led to preferential expansion of the number of $\gamma\delta$ T cells (113). Similarly, the contribution of synovial-fluid $\gamma\delta$ T cells to antibacterial and self-directed aggression in the arthritic joints of patients with reactive arthritis is indicated by recent studies (114).

In conclusion, although many findings support a role for hsp-reactive $\gamma\delta$ T cells in autoimmune disorders in humans, their importance for pathogenesis awaits further verification. Recognition of self hsp by $\gamma\delta$ T cells is also considered a beneficial mechanism in infection and inflammation. It can be easily imagined that increased expression of self hsp at sites of inflammation induces the recruitment of $\gamma\delta$ T cells, which promotes rapid mobilization of host defense mechanisms (119).

CONCLUDING REMARKS

This review has attempted to summarize evidence for a functional role of hsp in antigen processing and recognition. It has also summarized data which corroborate the role of hsp as an antigen in infection and autoimmune disease, a role which is probably related to the high sequence homology of hsp cognates in different species. It was this homology which, more than a decade ago, led to the notion that hsp could be more important than other antigens in host defense and autoaggression. Since then, a vast number of data supporting this notion have accumulated. Indeed, it is safe to state that in certain infections and autoimmune diseases, hsp play a role in protection and pathogenesis, respectively. However, the evidence to support generalization of this conclusion is far from convincing, since after a wave of corroborative findings, data in support of the contrary viewpoint emerged. Thus, in several infections and especially autoimmune diseases, the implications of immune responses against hsp are still not fully understood. It is therefore important to collect more data emphasizing unique situations and to refrain from overgeneralization. For these reasons, this review has focused on assembling the available data, has tried to present the pros and cons for the role of hsp in immunity to infections and autoimmune diseases, and has abstained from far-fetched conclusions. However, an equal overinterpretation would be to negate any role of hsp in infection and autoimmune disease. As is often the case, the truth is midway between the extremes, with major deflections to either side in different situations. If there is any general conclusion to be drawn today, it is that hsp, rather than initiating anti-infectious or autoaggressive immune responses, chaperone the immune response induced by other antigens and thus both influence its strength and sustain it. In this way, the term "chaperone," originally used to describe the biological function of hsp, also fits well as a description of its role as an antigen in infection and autoaggression.

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