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# Cell surface expression of heat shock proteins and the immune response

**Gabriele Multhoff[1] and Lawrence E. Hightower[2]**

[1]GSF-Institut für Klinische Hämatologie, Marchioninistr. 25, 81377 Munich, Germany
[2]Department of Molecular & Cell Biology, The University of Connecticut, Storrs, CT 06269–3044, USA

## INTRODUCTION

Heat shock proteins (Hsp) inhabit intracellular locales where at least one of their functions, molecular chaperoning of other proteins, has been firmly established. However, there are also scattered reports that nucleocytoplasmic Hsp, which contain no obvious ER-Golgi targeting signal sequences, are sometimes found outside of the procaryotic and eucaryotic cells that produced them. These observations can be divided into those involving the release of intact Hsp from cells or their transfer from producer to recipient cells and those involving the display of intact Hsp, fragments derived from them and proteins with domains related to Hsp on cell surfaces. Cell biologists are primarily interested in the mechanisms by which such Hsp are released from cells. However, little is known about the cellular pathways used by these Hsp to escape from the nucleocytoplasmic compartment. Experiments indicating that inhibitors of the ER-Golgi secretory pathway do not block their release have been interpreted by some workers as evidence of a non-ER-Golgi secretory pathway for nucleocytoplasmic Hsp; however, purists are awaiting a molecular mechanism before giving up the alternative interpretation that Hsp are only released by lysis of dying cells. For immunologists as well as tumor and transplantation biologists, the more interesting aspects of the externalization of Hsp are the consequences for immune responses of the various forms of Hsp which by whatever mechanism end up bound to or displayed on the surfaces of certain cell types. In addition to Hsp, the known and potential interactions of the stress protein MT with the immune system have been reviewed recently (Borghesi and Lynes 1996). This is an exciting, nascent area of investigation with the potential for a major impact on molecular medicine and a large part of this review is devoted to describing the results of these initial studies.

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*Correspondence* to: Gabriele Multhoff, Tel: +49 089 7099 403; Fax: +49 089 7099 400; E-mail: multhoff@gsf.de

## CELL-TO-CELL TRANSFER OF HSP

Approximately 15 years ago, Fredric White showed that vinblastine and calcium depletion block the movement of Hsp70 away from sites of synthesis in cells associated with the microvasculature of incubated rat brain slices, suggesting a transfer from vascular endothelial cells to glial cells (White 1980). More recently, Tytell and co-workers (1986) have described a set of proteins known as glia-axon transfer proteins which are synthesized in adjacent glial cells and transferred to the squid giant axon. The transfer process is $Ca^{++}$-dependent and appears not to involve the ER-Golgi secretory pathway of glial cells or pinocytosis by axons. Axonal phagocytosis of glial cell processes has been proposed as a mechanism by which these proteins, which include actin, Hsp100, Hsp70 and Hsc70, may be transferred (Tytell et al 1986). What purpose might such transfers of molecular chaperones serve? In their pioneering studies of stress proteins in brain tissue, Brown and co-workers used in situ hybridization to reveal large regional differences in the synthesis of Hsp70 and Hsc70 in rabbit brain tissue; glial cells accumulate heat shock mRNA whereas it is undetectable in neuronal cells in certain areas of the brain (Sprang and Brown 1987). Since Hsp synthesized by some neuronal cell bodies are distributed by slow axonal transport, it has been proposed that axons may obtain

molecular chaperones needed for repair processes from adjacent glial cells by cell-to-cell transfer. In this issue, Tytell and co-workers (Houenou et al 1996) provide evidence that the addition of purified Hsc70 to spinal sensory neurons prevents axotomy-induced death.

### Release of Hsp and other proteins via non-ER-Golgi pathways

Stimulated by White's findings and previous work by Lindquist (1986) and others showing Hsp located in cortical regions of cells including cellular processes, Hightower and Guidon (1989) searched for Hsp released from cultured rat embryo cells. We found that medium changes stimulated these cells to release a small set of proteins that included Hsc70 and non-muscle actin. Heat shocked cells released these proteins plus Hsp70 and Hsp110. The release of these proteins was not blocked by monensin and colchicine, inhibitors of ER-Golgi secretory pathways. Near the end of our study, we read the reports of Tytell and colleagues and were delighted by the similarity between the set of proteins released from rat embryo cells and glia-axon transfer proteins. This analogy was very helpful to us in making the case that our observations were not likely to be an artifact of cell culture, and in fact suggested that the transfers observed by Tytell and co-workers may not be unique to neuronal tissue. Of course the most troublesome concern in the cell culture studies is that the release of Hsp may be due to lysis of a small number of dying cells and not due to a selective release mechanism. We showed that Hsp synthesized in the presence of the lysine analogue aminoethyl cysteine were not released, presumeably because they were improperly folded or non-functional and could not engage the release mechanism. Also, control experiments in which cells were exposed to low concentrations of non-ionic detergents showed that Hsp were not readily released from damaged cells, in contrast to tubulin, for example.

Hsp are not unique in their release from cells by non-ER-Golgi pathways. Non-muscle isoforms of actin are selectively released from cultures of developing quail myoblasts (Rubenstein et al 1982). These investigators used lactic dehydrogenase assays of medium and cells and Triton permeabilization of cells to control for non-selective release of actin and concluded that the release could not be explained by cell lysis. Basic fibroblast growth factor (bFGF) provides another example. bFGF has no recognizable secretory signal sequence and yet cells have high affinity receptors for bFGF on their surfaces, indicating that it has an extracellular function. Cell death or damage is often proposed as the mode of release of bFGF; however, using a clever single cell migration assay which ruled out release of bFGF from a few damaged cells in mass culture, Mignatti and co-workers (1992) have provided evidence of release by an exocytosis pathway separate from the ER-Golgi pathway. Monensin and brefeldin A, which block this latter pathway, and inhibitors of the multidrug resistance proteins did not inhibit cell motility which depended on the release of bFGF. Migration was stimulated by a calcium ionophore that enhances exocytosis and it was inhibited by methylamine and other conditions that block exocytosis. Release of interleukin 1β (IL-1β), a mediator of inflammation that also lacks a signal sequence from activated monocytes, goes by a non-ER-Golgi route that is blocked by inhibitors of exocytosis and stimulated by heat shock and a calcium ionophore (Rubartelli et al 1990). Some of the Intracellular IL-1β is associated with vesicles in cells that release the cytokine but not in another cell type that does not release it. Likewise, a small lectin (L-14) is released from differentiating myoblasts (Cooper and Barondes 1990) and transferrin receptors are released from maturing reticulocytes by exocytosis. Interestingly, Hsc70 is non-covalently bound to transferrin receptors in these exosomes (Mathew et al 1995). Exosomes are 50 nm vesicles which are held in multivesicular sacs inside reticulocytes prior to release. Hsc70 can be cross-linked to transferrin receptors using a cross-linker that reacts with ε-amino groups of lysine residues and it may be more than coincidental that a lysine analog blocked the release of Hsc70 from rat embryo cells in Hightower and Guidon's (1989) study. It was suggested that Hsc 70 may target transferrin receptors and other proteins for externalization via the exosome route (Mathew et al 1995). A DnaJ-like protein called cysteine string protein (Csp) because it contains a cysteine-rich 'string' region is associated with secretory vesicles. It is located in nerve terminals in *Drosophila* (Zinsmaier et al 1990), synaptic vesicles of the marine ray *Torpedo california* (Mastrogiacomo et al 1994), rat brain synaptic vesicles and pancreatic zymogen granule membranes (Braun and Scheller 1995). Csp may stabilize or chaperone the docking of proteins which are part of the exocytotic machinery, thereby regulating the process (Braun and Scheller 1995). Csp might also attract Hsc 70 to exosomes; however, there is no evidence yet for such an interaction. Why might such an alternative pathway exist? It has been suggested that some proteins, that cells must release, have sensitive sulfhydryls that may not survive the oxidizing environment of the ER and that such a pathway would keep ligands separated from their receptors inside the cell (Mignatti et al 1992).

The release of the acidic member of the FGF family aFGF, a potent inducer of angiogenesis, is particularly relevant because heat shock induces its release from NIH 3T3 cells (Jackson et al 1992). Heat-induced release

assayed by immunoblotting is reduced in the presence of actinomycin D and cycloheximide, suggesting that cell lysis is not the source. aFGF is thought to be an important contributor to tissue repair and inflammatory responses. Recently, Brown and co-workers showed that Hsp70 synthesis is rapidly induced in cells bordering surgical incisions and suggested a role in wound responses (reviewed in Brown 1994). Taken together, the proteins involved in non-ER-Golgi release, bFGF, aFGF, Hsc70, Hsp70 and IL-1β, have all been implicated in wound responses and inflammation, suggesting that these processes may be the in vivo venues for these unusual release mechanisms. It is interesting that elevated temperature and culture medium changes both stimulate release in vitro since these conditions may mimic fever responses, temperature increases associated with inflammation and breaks in tissue homeostasis.

### *Cortical and membrane localization of Hsp in cells and possible mechanisms of release*

In a series of autoradiographic and immunohistochemical studies, Lindquist and colleagues showed that a portion of Hsp70 is located at or near the plasma membrane of *Drosophila* cells (see Hightower and Guidon 1989 for original references to this section). Hsc70 was initially called clathrin-uncoating ATPase and there is evidence for the association of Hsc70 with clathrin-coated pits in membranes. Additional evidence of a cortical location for Hsp include the localization of an Hsp70 family protein in the ruffling membranes of fibroblasts by LaThangue and Latchman (1988), the immunoprecipitation of Hsp70 and Hsc70 in a complex with a cell surface glycoprotein gp90 by Hughes et al (1983), and the association of Hsc70 with synaptosomal membranes in rat brain (L Lim et al 1984, H Lin et al 1993). Welch and Suhan (1986) showed that Hsp70 colocalizes with ribosomes at the periphery of rat fibroblasts recovering from stress. It may be more than coincidental that cortical areas of the cell are also regions of active assembly and disassembly of F-actin and G-actin, another readily releasable cytoplasmic protein. A number of investigators have found Hsc70 associated with microfilaments and other cytoskeletal elements and have suggested a chaperoning role for Hsc70 in microfilament dynamics.

Given this background, it is now possible to consider three plausible mechanisms by which Hsp may be externalized by cells. First, Hsp may chaperone proteins into exosomes and may be incorporated into these vesicles in the process. Dice and co-workers (Terlecky 1994) have shown that Hsc70 chaperones proteins to lysosomes and they have recently obtained evidence of an Hsp70 family member located inside lysosomal vesicles (Terlecky 1994). Exocytosis in maturing reticulocytes and synaptosomal vesicle formation in neurons may be exaggerations of processes that occur in many differentiated cell types in vivo. Independent evidence of a vesicular location for bFGF and IL-1β and their non-ER-Golgi release suggests a broader role for cytoplasmic protein exocytosis. Second, Hsp may be associated with microfilaments in cell surface projections, such as microspikes, which may be either shed or captured by adjacent phagocytic cells. This may become a major transfer process during apoptosis and in certain differentiated tissues such as glia-axon transfer in brain. Third, release of Hsp by lysis from cells damaged during tissue trauma followed by wound and inflammatory responses may be an important rapid release mechanism in vivo. This may implicate Hsp in molecular chaperoning of proteins with extracellular roles in these responses, perhaps involving interactions with extracellular matrix, binding to cell surface receptors, and as mediators of immune responses.

There are several additional properties of Hsp70 family proteins that can be variously viewed as unpleasant complications, exciting enhancements and 'wild cards' that may be thrown into the three mechanisms discussed above. Hsc70 and Hsp70 can bind fatty acids non-covalently (Guidon and Hightower 1986) and family members can also bind bacterial lipopolysaccharide. Hsp70 can form ion-conducting pores in unilamellar lipid vesicles (Alder et al 1990). These observations raise the possibility that Hsc70 and Hsp70 may interact directly with the lipid components of membranes. Hsp70 family proteins, including bovine Hsc70, bind sulfoglycolipids (Boulanger et al 1995). Remarkably, a sulfoglycolipid binding protein (SLIP 1) on the surface of rat germinal cells is immunologically related to Hsp70 family proteins. In addition, the sperm receptor on sea urchin eggs has at least one domain related to the Hsp70 family, as does an outer membrane protein of *Chlamydia trachomatis*, and several mycoplasma species have surface receptors for the same sulfoglycolipids (see Boulanger et al 1995 for references). The *C. trachomatis* protein has been implicated in receptor-mediated endocytosis into host endometrial epithelial cells (Raulston et al 1993). Thus, the possibility must be considered that anti Hsp70 antisera may cross-react with structurally related extracellular domains of receptors on some cell-types.

Hsp70 and Cpn60 family members of both bacterial pathogens and vertebrate host cells are immunodominant antigens, and in some cases, the antigenic peptides have been identified. Therefore, it is necessary to consider cell surface presentation of peptide fragments derived from Hsp in the context of MHC class II and class I pathways and receptors, as shown for the latter in the Figure. In addition to serving as potent antigens, the chaperoning functions of Hsp suggests roles in the actual pathways of antigen processing and presentation (Cristau et al 1994; Jacquier-Sarlin et al 1994). Processing involves

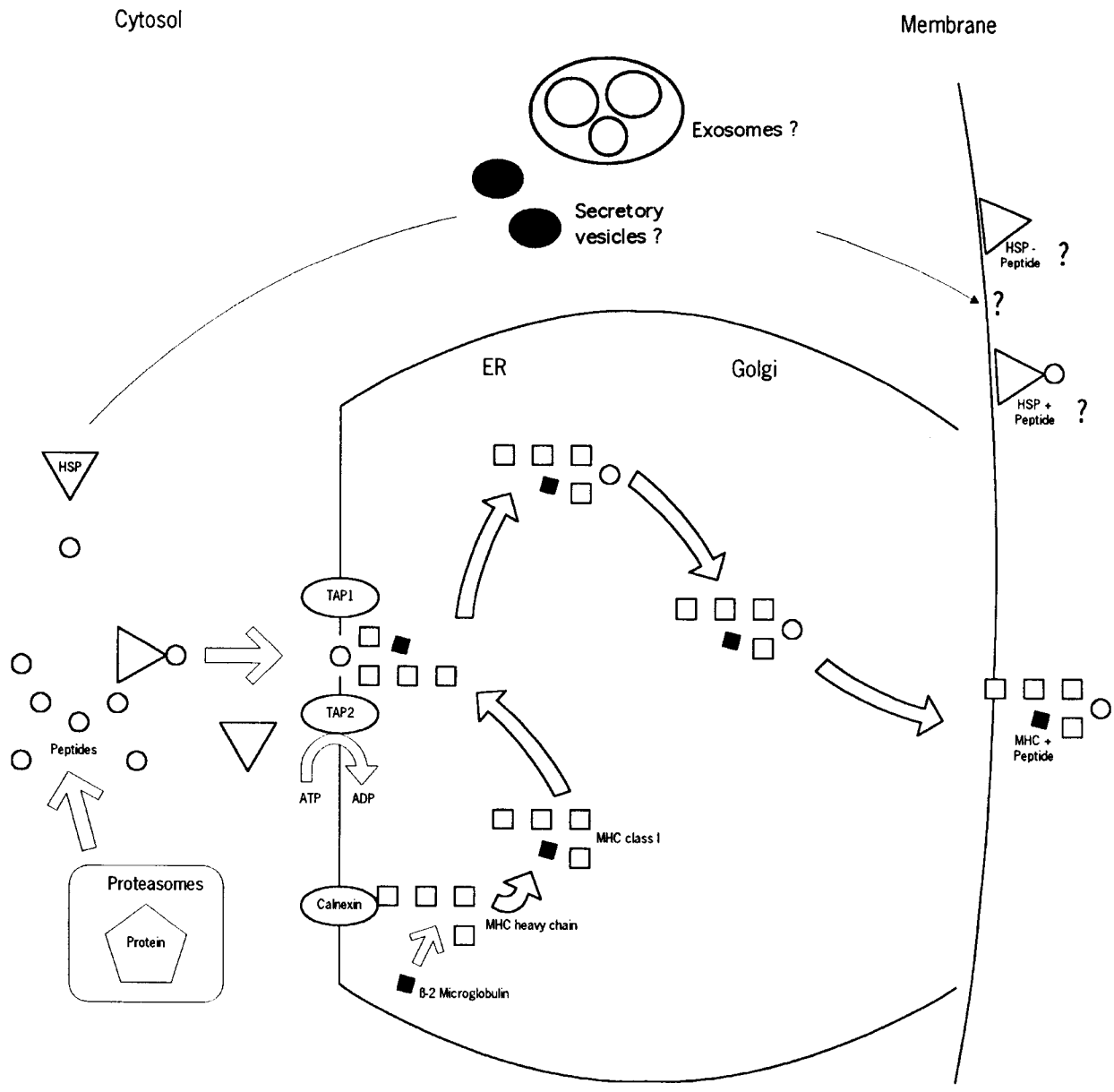


**Figure** Model for the transport and cell surface localization of Hsp70. Possible transport mechanisms of Hsp70 to the cell surface and anchorage to the plasma membrane (? indicates largely unknown pathway). Immunological consequences of cell surface expressed Hsp (? indicates largely unknown consequences).

the internalization of antigen into acidic compartments, proteolysis and binding of peptides to the presenting molecule. This complex assembly procedure may require the participation of molecular chaperones of the Hsp70 family (DeNagel and Pierce 1992). For example, if Hsc70 transported peptides from proteasomes to Tap transporters in the ER membrane, it would strongly influence the kinds of peptides presented by MHC class I receptors.

Cpn10, the cochaperonin of Cpn60, has also been spotted outside of cells. For example, it has been recovered from the culture medium *of M. tuberculosis,* either released from dead cells or secreted by living bacteria (Verbon et al 1990; Orme et al 1992). Cpn10 has been detected in human maternal serum and it has been suggested that it is identical to early pregnancy factor (Morton et al 1974; Cavanagh and Morton 1994).

Can exogenous Hsp bind to cell surfaces? There has been surprising little exploration of this possibility. Radio-iodinated Hsc70 binds to the surface of serum-deprived arterial smooth muscle cells in culture (Johnson

and Tytell 1993). However, bovine serum albumin in addition to unlabeled Hsc70 competed with radioiodinated Hsc70 for binding and there was no evidence of internalization of the radiolabeled material, raising the possibility that the binding of Hsc70 to the cell surface in this particular culture system may be relatively low affinity or non-specific. More work needs to be done using a variety of different cell types to search for Hsp receptors on cell surfaces.

The surface display of Hsp immunoreactive material on the surface of tumor cells and pathogen-infected cells, as well as the detection of Hsp antigens in association with a variety of chronic disease states and in tissue grafts, have been demonstrated. The immunological consequences of Hsp on cell surfaces will be discussed below.

*Overview of the interaction of Hsp with immune responses*

Beside their intracellular chaperoning tasks, members of the Cpn60, Hsp70 and Hsp90 families are regarded as highly immunogenic for the host's cellular and humoral immune system. At first sight a specific immune response appears paradoxical, since most Hsp are highly conserved between nearly all species and are ubiquitously distributed. However, a number of Hsp are expressed in a relatively tissue-specific manner. Investigations of antigens that are involved in inflammatory processes, in bacterial, viral and fungal infections led to the observation that stress proteins are major targets for the cellular and humoral immune response (Young and Elliott 1989; Shinnick 1991). Furthermore, an involvement of Hsp in several autoimmune diseases and in anti-cancer immune responses have been described by several groups (Ullrich et al 1986; Vanbuskirk et al 1989; Winfield and Jarjour 1991; Ferrarini et al 1992; Heufelder et al 1992; Chouchane et al 1994). Also, increased Hsp synthesis has been found following T lymphocyte activation with mitogens or cytokines (Lanks 1986; Lindquist 1986; Craig 1993). In order to formally distinguish the different roles of Hsp in eliciting an immune response, Srivastava (1994) has proposed the following four paradigms:

1. Hsp as classical species-specific foreign antigens: Despite high sequence homology, all Hsp contain regions that act as foreign epitopes for the host's immune system.
2. Hsp as self-antigens that are expressed in a tissue-specific manner.
3. Hsp as examples of molecular mimicry between Hsp and self proteins.
4. Hsp as presenting molecules for foreign antigens in a non-covalent complex.

In all of the above mentioned paradigms, Hsp elicit an immune response because they are located on the cell surface.

**Expression of Hsp on the surface of tumor cells**

The following observations regarding the surface display of Hsp on tumor cells have been made:

- Soluble Hsp70 can be detected in the culture medium of tumor and normal cells only after disruption of the membrane integrity.
- Brefeldin A does not block the membrane localization of Hsp70 (G. Multhoff, personal communication).
- Hsp70 can be found immunohistochemically on the cell surface of certain vital tumor cell types but not on the cell surface of several vital normal cell types (Multhoff 1995a).
- Hsp70 cell surface expression can be detected by immunoprecipitation experiments selectively in the membrane fractions of tumor cells and after selective cell surface radioiodination of intact tumor cells (G. Multhoff, personal communication).

The finding that most Hsp are expressed selectively on the cell surface of virally or bacterial-infected cells or on tumor cells, but not on the cell surface of normal cells, might be of importance to understand the transport mechanism of Hsp. Probably the often profoundly altered metabolism in tumor cells may result in perturbations in the secretory pathway. Alterations in the level of intracellular calcium levels lead to the secretion of reticuloplasmins in fibroblasts (Booth and Koch 1989). It is known that the environment of some tumor cells is acidic, hypoxic and nutrient-depleted (Vaupel et al 1989), thus leading to lower intracellular pH compared to normal cells. In this context, one might speculate that a lower pH could be responsible for conformational changes of Hsp that result in cell surface localization. In order to analyze this hypothesis in more detail the primary amino acid sequences of soluble proteins that are bound to the cell membrane at low pH were compared to that of Hsp70. As an example, diphteria toxin, a water soluble globular protein under physiological conditions, interacts with the plasma membrane at low pH. The transmembrane domain of diphteria toxin consists of nine alpha helices that are bundled together. Two of the helices are quite hydrophobic, but are shielded away from water by the other seven which are fairly amphiphilic. Another family of proteins that undergo large conformational changes upon membrane association are the lipoproteins which are soluble bundles of amphiphilic alpha helices. In the case of HIV glycoproteins gp120 and gp41, cell surface localization is achieved by the fact that these proteins form oligomers (Earl et al 1990) and undergo cleavages (Willey et al 1988). Analysis of the primary amino acid sequence of gp41 reveals several segments that are hydrophobic or highly amphiphilic (Eisenberg and Wesson 1990). These regions are known to play an essential role in membrane

interactions (Hoekstra 1990). Hydrophobic segments near the N-terminus of many viral surface glycoproteins have been implicated as a general principle in the fusion process between a number of viruses and host membranes (Fujii et al 1992).

The possibility that Hsp70 family proteins interact directly with membranes should not be excluded, especially since a receptor for Hsp70 on cell surfaces has not been identified. By analysis of the primary sequence of Hsp70, several segments at positions 43–59, 64–86, 231–249, 300–333 and 507–523 were found that have large hydrophobic moments. They might adopt amphiphilic alpha helical conformations under certain conditions such as interaction with a lipid environment. In addition, there were also seven segments (133–151, 161–185, 194–212, 278–299, 333–356, 391–414, 459–476) that were sufficiently hydrophobic to be classified as multimeric transmembrane helices (G. Fujii, personal communication). Based on the similarities between Hsp70 and several other surface-expressed proteins it is reasonable to consider this mode of membrane anchorage to the plasma membrane as well.

### Immunological consequences of cell surface-expressed Hsp

Stress proteins are among the dominant antigens recognized by the immune system in a number of different diseases. They play an important role in parasite-host interactions (Young and Elliott 1989; Engman et al 1990; Shinnick 1991, Jacquier-Sarlin et al 1994; Gomez et al 1995; Nagasawa et al 1995; Zugel et al 1995), in autoimmune diseases (Lamb et al 1989; Heufelder et al 1992; Boehnke et al 1994; Xu et al 1994, Anderton et al 1995; de-Graeff-Meeder et al 1995; van Eden et al 1995), in inflammatory processes (Winfield and Jarjour 1991; Weiss et al 1994), in neurodegenerative diseases (Chopp 1993; Gao et al 1995) and in virus infections (DiCesare et al 1992; Bartz et al 1994; Silva et al 1994). Furthermore, it was demonstrated that Hsp are involved in transplant rejection (Moliterno et al 1995) and can also act as specific antigens in the anti-cancer immune response (Ferrarini et al 1992; Fisch et al 1992; Kaur et al 1993; Tamura et al 1993; Chouchane et al 1994; Srivastava 1994; Udono and Srivastava 1994; Udono et al 1994; Yoshino et al 1994; Multhoff et al 1995b).

In order to clarify this complex situation of immune responses against stress proteins the antigenicity of Hsp was analyzed in relationship to the humoral and cellular immune response.

### Hsp and the humoral immune response

Previously it was shown that mycobacterial Hsp of the Cpn60, Hsp70 and Hsp90 families are able to elicit an antibody-mediated immune response. After immunization of mice with mycobacterial Cpn60-conjugated peptides in the absence of adjuvants, the production of antimycobacterial Cpn60 antibodies was induced. These antibodies were found to be highly cross-reactive with Hsp homologs of other prokaryotes and are weakly cross-reactive to human Cpn60 (Barrios et al 1994).

Hsp synthesis is also inducible after in vitro infection by a variety of different viruses such as adenovirus, SV40 virus, polyoma virus, Herpes simplex virus (La Thangue and Latchman 1988) and HTLV type I virus (Chouchane et al 1994). Furthermore, the induction of Hsp70 antibodies that was observed in HTLV-infected rabbits could be positively correlated with resistance to lethal doses of HTLV viruses. This indicates that the production of Hsp70-specific antibodies affects the outcome of disease. Chronically HIV-infected lymphomas show an increased expression of Hsp70 and, moreover, an Hsp70-specific monoclonal antibody was able to mediate a strong antibody-dependent cellular cytotoxicity (ADCC). Therefore, infected target cells that express Hsp on their cell surface were eliminated efficiently by the host's immune system (DiCesare et al 1992). Taken together, these data indicate that mycobacterial as well as several virals infections are able to induce the expression of Cpn60, Hsp70 and Hsp90 antibodies that might support the host's immune system to deal with the disease. However, compared to the cellular immune response, the humoral response against Hsp plays only a minor role.

### Hsp and the T cell receptor (TcR) $\alpha/\beta$-mediated T cell response

T cell-mediated immune responses have been described for members of the Cpn60, Hsp70 and Hsp90 families. First, members of the Cpn60 family have been found to be highly immunogenic in the case of mycobacterial infections. Although the murine and mycobacterial Cpn60 peptides recognized by cytotoxic T cell clones showed only about 50% sequence homology, the data suggest that endogenous Cpn60 itself could act as the source of peptides presented by stressed host cells to CD8-positive cytotoxic T lymphocyte clones (Zugel et al 1995).

In contrast to the hypothesis that cross-reactive T cell recognition might induce an aggressive autoimmune disease, more recent data suggest that the opposite is true. Anderton's group (1995) showed that cross-reactivity between bacterial and self-Cpn60 might also provide an effective protection against self-reactive T cells in Lewis rats. These findings are in line with data received from patients with juvenile arthritis. Nearly all patients with remitting course of disease exhibited a T lymphocyte reactivity to human Cpn60 thus indicating that this T cell reactivity might be part of the T cell regulatory

mechanism that is involved in the remission of arthritis (de-Graeff-Meeder et al 1995). In the case of the pathogenic fungus *Histoplasma capsulatum* that induces pulmonary histoplasmosis in mice, HIS-62, a member of the Cpn60 family, as well as the recombinant Cpn60, protects mice against the disease (Gomez et al 1995).

Cell surface localization of Hsp70-related proteins is thought to be involved in chronic inflammatory lesions of several experimental autoimmune diseases, i.e. Graves opthalmopathy (Heufelder et al 1992) or toxin-induced interstitial nephritis (Weiss et al 1994). The location of Hsp70 on the cell surface of affected retrooccular fibroblasts from patients with Graves ophthalmopathy might have implications in the immune process of the disease. The induction of Hsp70-reactive CD4-positive TcR α/β T cell lines might initiate or facilitate inflammatory damage in the kidney in interstitial nephritis (Weiss et al 1994). In contradiction to these findings it has been demonstrated that members of the Hsp70 family possess the ability to protect tissues from deleterious effects of inflammation (Jacquier-Sarlin et al 1994). Similar to Cpn60-related proteins, members of the Hsp70 family may also exert protective effects in the immune system by contributing to the processing and presentation of bacterial and tumoral antigens. The mechanisms by which such protection occurs include prevention of DNA strand breaks induced by reactive oxygen species and lipid peroxidation as well as protection of mitochondrial structures and functions.

Despite these potential protective effects of members of the Cpn60 family in dealing with autoimmune diseases, fungal, bacterial and viral infections, Cpn60 and Hsp70-related proteins are thought to play a role in the immune cascade of inflammatory processes of allograft rejections (Moliterno et al 1995). After addition of Cpn60 and Hsp70 proteins, graft-infiltrating lymphocytes showed augmentation in the proliferative responses.

Another emerging field in which members of the Hsp70 family play an important role is the immune response against cancer. Human tumor-infiltrating CD4-positive T cells (TIL) derived from melanomas, ovarian lung, renal cell and breast cancer have been shown to react specifically to Hsp70-expressing cell lines (Yoshino et al 1994). From these results it was concluded that Hsp70-reactive T cells have to exist in certain tumor tissues and, furthermore, these lymphocytes might support an anti-tumor T cell response at local tumor sites. In addition, peptides bound to Hsc70 were shown to elicit a specific anti-cancer immunity in methylcholanthrene-induced (Meth A) sarcomas in mice (Srivastava et al 1994 Udono and Srivastava 1994). Therefore, vaccination of mice with tumor-derived Hsp70 preparations renders the mice immune to substantial challenge with the autologous tumor cells (Udono and Srivastava 1994).

In the case of Hsp90, the same group (Udono et al 1994) could demonstrate that the Hsp90-related glycoprotein gp96 was able to prime CD8-positive T cells in vivo. These purified tumor gp96 fractions have been shown to elicit an autologous tumor-specific immunity. In summary, members of the Hsp70 and Hsp90 family are predominantly involved in the antitumor immune response. However, more recently, they also appear to fulfill a protective function against inflammatory diseases.

### Hsp and non-MHC-restricted immune responses mediated by TcR γ/δ and NK cells

Evidence is accumulating that non-MHC-restricted γ/δ TcR-positive T lymphocytes participate in the immune response to parasitic infections, autoimmune diseases, virus-induced diseases and also in the anti-cancer immune responses. In all these cases, γ/δ T cells are involved in the recognition pathway of members of the Cpn60 families. Among the mycobacterial antigens, Cpn60 is an immunodominant target for γ/δ T cells. Mice that were vaccinated with a tumor cell line that expresses mycobacterial Cpn60 showed a remarkably high degree of protection against challenge with lethal doses of virulent *Mycobacterial tuberculosis*. TcR γ/δ and TcR α/β cells interact synergistically in the lysis of mycobacteria-infected macrophages that express Cpn60 (Silva et al 1994).

γ/δ TcR-positive cells also play an essential role in the protective immunity against infection with *Toxoplasma gondii* antigen. Cpn60 that was shown to confer the immunoprotection was detected on the cell surface of peritoneal macrophages of the host (Nagasawa et al 1994). In chronic experimental autoimmune encephalomyelitis (EAE) lesions, γ/δ T cells co-localize with areas of an increased immunoreactivity against Cpn60, thus indicating that development of inflammation in the central nervous system is associated with differences in the Cpn60 expression patterns (Gao et al 1995).

Also, proteins related to Cpn60 have been detected on the surface of macrophages from bone marrow (Wand-Wurttenberger et al 1991) and some monocyte-derived cell lines (Ferm et al 1992). Daudi Burkitt's lymphoma cells specifically express Cpn60 molecules on the cell surface. However, other lymphoma cell lines (i.e. Raji) or normal Epstein-Barr virus (EBV)-transformed B cells did not show any Cpn60 cell surface expression (Fisch et al 1992 Kaur et al 1993). A specific proliferative and cytotoxic response of a distinct γ/δ T cell subset (Vγ9/Vδ2 TcR V region chain) was found and could be correlated with the cell surface expression of a Cpn60-related epitope. This immunogenic Cpn60 molecule, that is selectively expressed on the cell surface of Daudi lymphoma cells, is also present in mycobacterial extracts.

Although a large number of different tumor antigens have been identified so far (Konno et al 1989; Lurquin et al 1989; Parham 1989; Van-den-Eynde et al 1991; the direct interaction of the tumor antigens with the effector cells is not fully understood, especially in the case of non-MHC-restricted effector mechanisms. Natural killer (NK) cells were functionally defined in mediating the host's anti-tumor immune response for a long period of time. However, more recently, Moretta's group (1994) provided direct evidence for the existence of distinct NK subclones with defined specificities against certain HLA alloantigens. Besides the protective role of γ/δ TcR cells in the anti-tumor immune response against members of the Cpn60 family, non-MHC-restricted TcR and CD3-negative NK-like effector cells have to be considered as well in the immune response against Hsp. Tamura and colleagues (1993) investigated the role of Hsc70 as a possible tumor antigen. They presented data that led to the conclusion that Hsc70 expressed on the cell surface of tumor cells might act as an antigen-presenting molecule for certain cellular proteins that interact with double negative T cells. These findings are in line with data of the group of Srivastava (1994) who postulated a non-MHC-restricted α/β TcR-mediated recognition of immunogenic peptides presented by Hsp of the 70 and 90 families.

The results of our group suggest that the inducible Hsp72 that is expressed on the cell surface of certain tumor cells acts as a recognition signal for CD3 and TcR-negative NK cells. Non-lethal heat shock (Multhoff et al 1995a) and treatment with the membrane-reactive alkyl-lysophospholipid derivative ET18-OCH3 (Botzler et al 1996) were able to increase cell surface expression of Hsp72 selectively in sarcoma and lymphoma cells that correlates with an enhanced sensitivity to lysis mediated by non-MHC-restricted NK cells (Multhoff et al 1995b). Carcinoma cell lines exhibited an Hsp72 cell surface expression already under physiological conditions that corresponds with an increased sensitivity to lysis by NK cells (manuscript in preparation). Therefore, it was concluded that Hsp72 might act as one possible recognition structure for a distinct TcR-negative NK subpopulation.

It appears that highly immunogenic Hsp belong to the Cpn60, Hsp70 and Hsp90 families. These Hsp predominantly fulfill protective roles in dealing with infections, inflammatory processes and in a number of autoimmune diseases. Furthermore, Hsp of the Cpn60 and Hsp70 families are also shown to play an important role as tumor-specific recognition structures. According to the four paradigms of Srivastava (1994)

1. they can directly act as immunogenic structures.
2. they represent tissue-specific self-antigens
3. they mimic other immunogenic molecules
4. they behave like antigen presenting molecules for tumor-specific peptides.

Further studies are necessary to solve the problem of whether Hsp themselves, or immunogenic peptides that are presented by Hsp, will provide powerful tools to design effective vaccines against a large panel of different diseases.

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