CHAPTER 13

Heat Shock Proteins in Inflammation and Immunity

M. Bachelet, G. Multhoff, M. Vignola, K. Himeno, and B.S. Polla

A. Introduction: Multiple Roles of Heat Shock Proteins in Inflammation and Immunity

Inflammation represents a localized or systemic response against tissue or cell injury, which, on the one hand, is essential among cell defense mechanisms and, on the other, is involved in a broad spectrum of diseases. According to the initiating event, the inflammatory response may involve, or not, an antigen-specific immune response. In the first case, the initiating agent is generally a microorganism or an antigen of unknown origin, while in the latter, cells may respond to injurious physical agents (foreign bodies, burns, radiations, trauma) or toxic chemicals. Lymphocytes are classically involved in the specific immune response, and phagocytes in its non-specific counterpart.

The mechanisms resulting in these different types of inflammation all include a highly conserved common inducible response that involves the synthesis of heat shock/stress proteins (Hsps). Hsps may play various roles in multiple steps of the inflammatory response. Here we will present the current status of knowledge on the inflammation-related heat shock/stress responses and their anti-inflammatory or pro-inflammatory functions, respectively. Several members of the Hsp family function as molecular chaperones, each of them in discrete cellular compartments; along with these intracellular chaperoning tasks, cytosolic/lysosomal members of the most conserved and abundant Hsp70 family also contribute to antigen processing (Vanbuskirk et al. 1991; Mariethoz et al. 1994; Jacquier-Sarlin and Polla, submitted).

Hsp gene polymorphism, in particular of the genes coding the 70-kDa Hsp that are localized within the major histocompatibility complex (MHC), is a likely contributor to disease susceptibility or stress resistance (Favatier et al. 1997). Hsp can act as antigens, either self or non-self, and, as mentioned above, contribute to antigen processing and presentation, and thus to the efficiency of an immune response. Furthermore, they have the ability to protect cells and tissues from the deleterious effects of numerous mediators of inflammation, such as reactive oxygen species (ROS) or tumor necrosis factor (TNF)α (Jättelä et al. 1989; Kantengwa et al. 1991; Villar et al. 1993). Particular attention will be paid here to the role of the Hsp70 family, which is endowed with critical protective properties. It should, however, be mentioned that the

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protective (anti-inflammatory) effects of the stress response might relate more directly to the inhibition of activation of the transcription factor NFκ-B (ROSSI et al. 1997; WONG et al. 1997) – NF-κB being central to the inflammatory process (BAEUERLE et al. 1996; BALDWIN et al. 1996) – than to the Hsps themselves.

With respect to the direct protective effects of Hsp70 against ROS, mitochondria have been proposed as selective targets for these effects (POLLA et al. 1996). While mitochondria are considered as the cellular switchboard for cell survival or cell death, and the type of cell death, whether apoptosis or necrosis (RICHTER et al.1996), numerous publications indicate that Hsp70 has the ability to protect cells from apoptosis (SAMALI and COTTER 1996; DIX et al. 1996; MEHLEN et al. 1996a). Although at first sight this anti-apoptotic effect might appear protective, it actually can promote persistence rather than resolution of acute inflammation, by preventing the physiological removal of inflammatory cells by apoptosis. Thus, the possibility that the anti-apoptotic effects of Hsp70 contribute to the amplification and the chronicity of the inflammatory process will be considered here, in particular in the light of recent results obtained in asthma, a paradigm for chronic eosinophilic inflammation of the upper airways, where overexpression of Hsps could contribute both to antigen processing and presentation, and to chronic inflammation.

While the precise regulation and functions of Hsps in inflammation are not yet fully understood, future research and new experimental approaches in this field appear of great potential.

B. Role of Hsp Localization in the Induction of an Immune Response

By the end of the 1980s, it was found that stress proteins were among the dominant antigens recognized by the immune system in a number of different diseases. They are important players in host-parasite interactions (ZUGEL et al. 1995; JACQUIER-SARLIN et al. 1994), in autoimmune diseases (ANDERTON et al. 1995; HEUFELDER et al. 1992), in neurodegenerative diseases (CHOPP 1993), in virus infections (DICESARE et al. 1992; SANTORO et al. 1989) and in transplant rejection (MOLITERNO et al. 1995). Although at first sight, a specific immune response induced by evolutionary conserved proteins appears paradoxical, stress proteins are indeed major targets for the cellular and humoral immune responses (YOUNG and ELLIOTT 1989). The involvement of Hsps in autoimmune diseases and in anti-cancer immune responses has been described by several groups (WINFIELD and JARJOUR 1991; HEUFELDER et al. 1992; KAUFMANN 1994; SRIVASTAVA 1994).

In order to be recognized as antigens, the Hsps have to be somehow expressed at the cell membrane of antigen-presenting cells (CHOUCHANE et al. 1994), e.g., monocyte-macrophages or B cells, and thus be accessible to cells of the immune system. This is particularly intriguing in the case of self Hsps.

Although indeed members of the Hsp70 family are found on the plasma membrane of certain cell types, the mechanism(s) underlying transport and anchorage to the plasma membrane has not been identified yet. Since all isoforms of Hsp70 lack a hydrophobic leader sequence, it has been assumed that Hsps may be transported to the plasma membrane following cell death and disruption of the plasma membrane integrity (MUTHUKRISHNAN et al. 1991). A number of recent observations, however, challenge this view and suggest specific, though yet unraveled, mechanisms for Hsp70 membrane expression:

1. Hsp70 is found immunohistochemically and by selective cell surface biotinylation on the surface of certain tumor cells but not of normal cells (MULTHOFF et al. 1995a, 1997).
2. Soluble Hsp70 is not detectable in the culture medium of viable tumor cells expressing membrane Hsp70 as well as non-expressing normal cells (MULTHOFF et al. 1995a).
3. Hsp homologs to rat Hsp70 are among a selected group of proteins that are transferred from glial cells to giant axons, thus indicating that Hsps may be transported through the plasma membrane without disruption of membrane integrity.
4. Recent data suggest that the transport of Hsp70 is clearly distinct from the classical ER to Golgi pathway. Brefeldin A, monensin or colchicine that block ER-to-Golgi trafficking, block the secretion of polypeptides containing hydrophobic signal sequences while they have no influence on the membrane expression of Hsp70 (MISUMI et al. 1986; MULTHOFF et al. 1997). Therefore, one might speculate that transport of Hsp70 to the cell surface occurs post-translationally through a non-Golgi-dependent pathway. Rapidly released proteins such as interleukin-1 (IL-1) or acidic and basic fibroblast growth factor (FGF) also leave cells via non-classical non-Golgi pathways.
5. Data derived from electron microscopy indicate that tumor cells expressing Hsp70 on their plasma membrane also express Hsps in vesicles that are co-stained with cathepsin D, suggesting that lysosomal vesicles are involved in the transport of Hsp70 to the plasma membrane (MULTHOFF, unpublished observation).

Anchorage of Hsp70 within the plasma membrane might either be due to direct interaction of Hsp70 with fatty acids (HIGHTOWER and GUIDON 1989) or to the formation of a larger protein complex. Proteins of the Hsp70 family bind to a variety of other cellular proteins, including clathrin baskets and coated vesicles, the transformation related protein p53 (PINHASI-KIMHI et al. 1986) and cytoskeletal elements (OHTSUKA et al. 1986). The association of Hsp70 with the transferrin receptor during reticulocyte maturation could contribute to Hsp70 plasma membrane anchorage via the formation of a protein-receptor complex. The receptor-mediated endocytosis of *Chlamydia trachomatis* into host endometrial cells is another example of outer membrane association

mediated by an Hsp70-related protein (RAULSTON et al. 1993). Preliminary data from our group indicate that Hsp70 is able to form complexes with other molecular chaperones that contain a classical transmembrane domain; these complexes could be detected on the plasma membrane and in lysosomes of certain tumor cells. Thus, interactions between members of the Hsp70 family and normal or aberrant proteins are emerging as a unifying theme for membrane anchorage.

C. Hsps and Cell Adhesion in the Initiation of Inflammation

In order to create an inflammatory site, cells – mainly leukocytes – have to escape circulation and migrate into the tissues. The migration of leukocytes from the blood and their accumulation in a given tissue or organ is fundamental to the inflammatory process, and characterizes numerous inflammatory diseases such as bronchial asthma, rheumatoid arthritis or the adult respiratory distress syndrome (ARDS). Several steps of the migration process can be distinguished, including various adhesion molecules differentially affected by inflammatory mediators.

The first step of this process involves leukocyte adhesion. Indeed, leukocytes initially interact with the endothelium via selectins, causing cells to deviate from the normal flow and marginate on the endothelium. Next, chemoattractants induce the activation of leukocytes' integrins that mediate leukocyte migration into the inflamed site (ROSALES et al. 1995). Different types of leukocytes leave the bloodstream in an orderly fashion. Polymorphonuclear leukocytes (PMN) are first recruited and generally account for organ or tissue damage by an acute inflammation whereas monocytes and lymphocytes arrive later and may be recruited for long periods leading thereby to chronic inflammation (SPRINGER 1994; WARD and MARKD 1989). So far, one study reported that adherence to plastic surfaces was associated with an increase in Hsp70 expression in myelomonocytic cells (FINCATO el al. 1991). However, additional observations suggest a correlation between the expression of Hsps and the activation of adhesion molecules. Cells with a higher capacity to spontaneously adhere on plastic surfaces such as monocytes-macrophages (mφ) show higher levels of Hsp as compared to other cells (POLLA et al. 1995b), and differentiation of the myelomonocytic line U937 with 1,25-$(OH)_2$ vitamin D_3 increases the cell adhesion capacity in parallel with the expression of Hsp70 (POLLA et al. 1987). Moreover, we observed that adherent human monocytes are more resistant to elevated temperatures (44.5°C) as compared to the same cells cultured under non-adherent conditions (Bachelet, unpublished). This latter observation may reflect overexpression of Hsps upon adherence, thus resulting in thermotolerance. Leukocyte migration during inflammation involves morphological changes that allow leukocytes to cross the endothelium through cell junctions by ameboid movements or diapedesis.

Hsps may protect cells against mechanical stresses caused by shape changes during diapedesis.

The physiological importance of cell adhesion proteins has emerged, among others, from studies on patients with leukocyte adhesion deficiency (LAD), a clinical syndrome secondary to a genetic deficiency in adhesion proteins (ARNAOUT 1990). Patients with LAD are characterized by recurrent bacterial infections and abnormalities in a wide spectrum of adherence-dependent functions of leukocytes, mainly attributable to deficiency (or absence) of cell surface expression of $\beta 2$ integrins. We have examined the capacity of monocytes from one patient with LAD to synthesize Hsps upon thermal stress, using biometabolic labeling and sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). As observed in Fig. 1, monocytes from this patient appeared to synthesize similar levels of Hsps as compared to monocytes from a normal control, suggesting that decreased expression of $\beta 2$ integrins does not modulate Hsp synthesis.

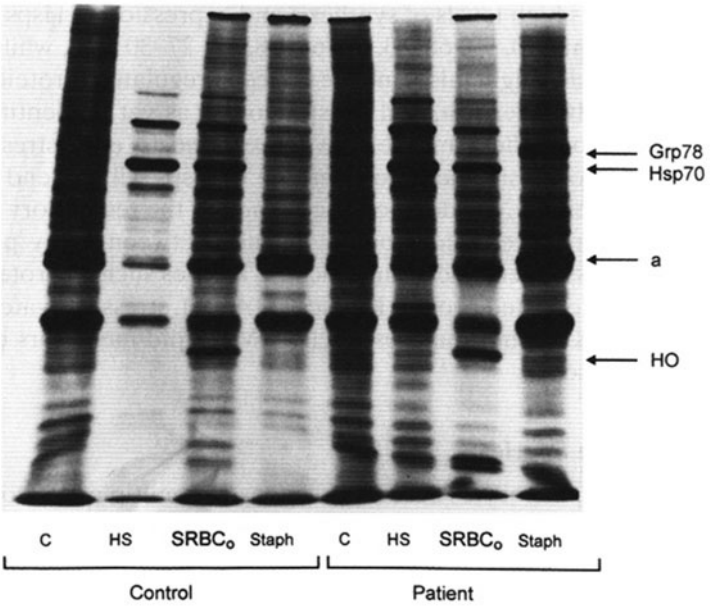

Fig. 1. Deficiency in adhesion molecules does not inhibit the heat shock response. Monocytes from a normal donor (control) or from a patient with LAD (patient) were exposed to heat shock, or allowed to phagocytose opsonized erythrocytes (SRBCo) or *Staphylococcus aureus* (*Staph*). Cells were labeled and processed for SDS-PAGE; aliquots corresponding to equal cell numbers were loaded onto each lane. Heat shock induced Hsp70; SRBCo, Hsp70 and heme oxygenase (HO); and *Staph*, predominantly glucose regulated protein (Grp)78. These proteins were induced both in control and in LAD cells, and rather more so in LAD than in control cells

D. Non-specific Immunity: Cells and Mediators Involved in the Induction of a Heat Shock/Stress Response

Non-specific inflammatory cells include mφ, PMN, eosinophils, and platelets, as well as many other cell types. Here we will concentrate on the first three, which are professional phagocytes involved in defense mechanisms. Activation of these phagocytes results in the release of a large repertoire of inflammatory mediators including ROS, lipid mediators and cytokines, that in turn participate in the inflammatory-related heat shock/stress response. We will focus in particular on mφ, as essential players in chronic inflammation, and as the highest producers of Hsps among the human cells examined. We will also examine specific differences between mφ and PMN. Many of the functions of mφ somehow relate to the heat shock/stress response, and we will consider both Hsp induction/regulation, and their functions in inflammation.

I. Monocytes-Macrophages

Monocytes-macrophages (mφ) display, among the circulating cells, a particularly interesting, most complex and diversified stress response. Heat shock induces in these cells high levels of synthesis and expression of Hsps of apparent molecular weight 110, 90, 68–73, 60–65, 58, 47, 27–30 kDa, while stresses such as phagocytosis might also induce glucose-regulated proteins (Grp), heme oxygenase (HO), ferritin, and possibly other as yet unidentified stress proteins. Furthermore, these mφ selectively respond to each stress: for example, during phagocytosis, the precise profile of Hsps will depend upon the type of phagocytic stimuli, the degree of activation of the respiratory burst and the type of ROS produced, and upon a balance between many potentially relevant second messengers, including calcium, kinases such as protein kinase C (PKC), mitogen activated protein kinases (MAPK), stress-activated protein kinases (SAPK), PKC, phosphatases, cyclic AMP, lipid mediators of inflammation, and proteases.

1. Reactive Oxygen Species

ROS generation by phagocytes occurs in response to multiple stressors including receptor-mediated phagocytosis, activation of PKC or the release of arachidonic acid metabolites, through the activation of the membrane associated NADPH-oxidase (TAUBER 1987; WATSON et al. 1990). Reduction of molecular oxygen to H_2O via NADPH-oxidase proceeds through a sequential one-electron transfer yielding superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (•OH) (ROSEN et al. 1995). ROS play important roles in non-specific defense mechanisms such as killing of pathogenic microorganisms. At high levels, ROS can, however, be deleterious for the host as well, exerting proinflammatory effects and inducing cell or tissue injury. In contrast, at low levels, ROS are important signaling molecules. Selective ROS

(H_2O_2, $ONOO^-$, •OH, but not O_2^- or nitric oxide [•NO]), induce Hsps as a protective mechanism against oxidative injury (JACQUIER-SARLIN et al. 1994; JACQUIER-SARLIN and POLLA 1996; POLLA et al. 1996). •OH in particular has been suggested to be involved in Hsp70 expression upon phagocytosis of xenogenic erythrocytes, or, in the presence of exogenous iron, of *Staphylococcus aureus* and *Pseudomonas aeruginosa* (KANTENGWA et al. 1993; BARAZZONE et al. 1996). Rodent peritoneal mφ activated with phagocytic stimuli also generate reactive nitrogen species such as •NO, that may be toxic to invading bacteria (MARLETTA 1989), while •NO does not by itself induce Hsp synthesis. •NO may interact with O_2^- to form peroxynitrite anions ($ONOO^-$) that are strong pro-oxidants and induce Hsp70 in human monocytes (Richard et al., unpublished). Thus only specific ROS (H_2O_2, •OH and $ONOO^-$) are inducers of Hsps, while O_2^- and •NO are not.

In terms of functions, Hsps clearly exert protective functions against the toxic effects of ROS. Mitochondria are selective targets of these protective effects (POLLA et al. 1996). Interestingly, mitochondria might also be central to the induction of Hsps by ROS, the difference between Hsp-inducing and non-inducing ROS being that they induce, or not, mitochondrial membrane depolarization (Polla et al., unpublished).

2. Lipid Mediators of Inflammation

Membrane phospholipids from leukocytes contain large amounts of arachidonic acid leading to the generation of numerous lipid mediators such as arachidonic acid (AA) derivatives and PAF-acether, upon activation of phospholipase (PL)A_2 and PLC activities (HOLTZMAN 1991). In human mφ, thromboxane (Tx)A_2 is the major AA metabolite produced, followed by leukotriene (LT)B_4, 5-hydroxyeicosatetraenoic acid (HETE), and prostaglandin (PG)E_2 (HOLTZMAN 1991). AA metabolism is associated with an influx of inflammatory cells to the site of inflammation: LTB_4, and to a lesser extent 5-HETE, have potent chemotactic activity for human PMN and eosinophils but not for mφ, while TxA_2 acts as a potent constrictor of vascular and airway smooth muscle. So far, major lipid mediators generated by mφ have not been demonstrated to interfere with the expression of Hsps in the same cells. This is not unexpected, since, on the one hand, mφ lack receptors for TxA_2 (BACHELET et al. 1992), and on the other, the synthesis of AA derivatives depends mainly upon PLA_2 activation in the presence of high extracellular Ca^{2+}, two factors shown not to affect Hsp expression in general (POLLA et al. 1995a). However, leukotrienes from the 5-lipoxygenase pathway exert several receptor-mediated biological activities on mφ that may account for the LTB_4-induced release of factors stimulating fibroblast proliferation (POLLA et al. 1985). Additional data from the group of KÖLLER describing 12-HETE-induced Hsp synthesis in human leukocytes (KÖLLER and KÖNIG 1990; KÖLLER et al. 1993) indicate that leukotrienes might indeed modulate Hsp expression in inflammation.

Moreover, mφ as well as PMN and eosinophils, bear receptors for PG. PGE_2 markedly increases intracellular cAMP levels in mφ and exert dual functions in inflammation, acting either as pro-inflammatory or anti-inflammatory factor (BONTA and PARNHAM 1982). PG of the type A and J (cyclopentenone PG) have been shown to exert antiproliferative and antiviral activities through a mechanism involving Hsp70, in several mammalian cell types (for review, SANTORO 1997). The antiviral activity of cyclopentenone PG, observed against a wide variety of DNA and RNA viruses, is always associated with their capacity to induce Hsp70.

3. Cytokines

Pro-inflammatory cytokines modulate Hsp synthesis via their pyrogenic activity as well as distinct mechanisms. IL-1 induces an ROS-dependent increase in Hsp70 in β cells of pancreas (HELQVIST et al. 1991), and IL-2 an IL-2 receptor-dependent accumulation of Hsp70 mRNA in lymphocytes (HAIRE et al. 1988). TNFα has been reported to induce Hsp70 in myelomonocytic cells (FINCATO et al. 1991), although other groups have been unable to reproduce these data (Polla et al., unpublished). In cultured chicken embryo cells, transforming growth factor (TGF)β increases the expression of Hsps, secondary to the stimulation of general protein synthesis and a subsequent increase in chaperone requirements (TAKENAKA and HIGHTOWER 1992). However, in many cells, cytokines do not appear to induce the synthesis of Hsps and whether or not cytokines induce a stress response seems to be tissue specific and to depend largely on the cytokine effects on the oxidant/anti-oxidant (im)balance.

Numerous studies on the interactions between Hsps and cytokines have highlighted the striking protection Hsps may provide against the toxic effects of cytokines, in particular, TNFα and IL-1 (for review, JACQUIER-SARLIN et al. 1994 and references therein). TNFα and IL-1 are cytokines with pleiotropic activities. These include the activation of the respiratory burst enzyme NADPH-oxidase, leading to a rapid rise in intracellular ROS originating in the mitochondria (HENNET et al. 1993), and provide a target for the protective effect of Hsps. Preexposure of the highly TNFα-sensitive mouse fibrosarcoma cell line WEHI 164, to temperatures ranging from 39°C to 42°C, prevents the cytotoxic effects of TNFα; the involvement of Hsp70 in this protection was confirmed in WEHI-transfected cells that overexpress Hsp70 (JÄÄTTELÄ et al. 1989; JÄÄTTELÄ 1993). In pancreatic β cells, Hsp70 also protects cells against a selective oxidative stress induced by IL-1 (MARGULIS et al. 1991).

In addition, TNFα and IL-1 induce phosphorylation of the low molecular weight Hsp27 resulting in its activation. Hsp27 also exerts specific protective functions: the protein is involved in the stabilization of the actin microfilament network (ARRIGO 1990), and counteracts TNFα-induced apoptosis (MEHLEN et al. 1996a), probably because of its ability to replenish intracellular reduced

glutathione, thereby leading to increased levels of intracellular ROS (MEHLEN et al. 1996b), while oxidative stress has been proposed as a common final signal where several pathways associated with apoptosis converge (BUTTKE and SANDSTROM 1994).

4. Nuclear Factor κB (NF-κB)

Recent studies have suggested that an important mechanism by which the heat shock/stress response exerts protective effects during inflammation involves the inhibition of NF-κB nuclear translocation. NF-κB is found in the cytoplasm of cells in an inactive form associated with the inhibitor IκBα. Upon activation, IκBα is phosphorylated and undergoes proteolytic degradation to allow active NF-κB to translocate to the nucleus and stimulate transcription (BALDWIN 1996; BAEUERLE 1996). NF-κB plays a crucial role in regulating the transcription of several pro-inflammatory cytokines and chemokines including TNFα, IL-1 and IL-8, a potent chemoattractant for PMN (LEONARD and YOSHIMURA 1990). ROSSI et al. (1997) first reported that activation of HSF was associated with inhibition of NF-κB, through a mechanism involving inhibition of IκBα phosphorylation and degradation, an observation that has been confirmed since (WONG et al. 1997). Thus, inhibition of NF-κB activation represents a novel, Hsp-independent, anti-inflammatory effect of all HSF-activating compounds or factors, including heat shock and cyclopentenone PG (ROSSI et al. 1997).

II. Granulocytic Phagocytes

1. Polymorphonuclear Leukocytes (PMN)

PMN generally express Hsps to a lesser extent than mφ and their stress response appears to be differentially regulated (POLLA et al. 1995b). Phagocytic stimuli activate the generation of ROS in PMN as well as in mφ; however, Hsp synthesis is induced only in the latter. In addition, Hsp synthesis is known to occur in PMN, but not in mφ, stimulated with formyl methionyl leucyl phenylalanine (fMLP), the synthetic bacterial peptide analog that increases intracellular calcium and stimulates NADPH-oxidase, PLC, and PKC (TAUBER 1987).

These observed differences have been related to the inability of PMN to produce •OH, the putative key oxygen metabolite for the induction of Hsp. Indeed, lactoferrin, a typical PMN secretory product, is considered to prevent the generation of •OH through its ability to bind metal iron, a major promoter of •OH formation via the Fenton reaction (LIOCHEV and FRIDOVICH 1997). In the absence of •OH, the fMLP-induced Hsp expression may be mediated by the activation of PKC, via PLC activity (JACQUIER-SARLIN et al. 1995). Another hypothesis to explain these differences is that PMN are short-lived phagocytes that may not require significant protective mechanisms such as Hsp synthesis.

Among the secretory products of PMN stored in azurophilic granules (acid hydrolase, myeloperoxidase, lysozyme, and neutral proteases such as elastase and cathepsin G), cathepsin G appears most interesting, inducing in mφ the synthesis of a member of the Hsp70 family, the 78-kDa calcium-dependent Grp78 (PINOT et al., unpublished). Whether such induction also occurs in PMN and whether it relates to intracellular protein degradation remains to be determined.

2. Eosinophils

Eosinophils may exert beneficial roles in modulating immunoglobulin (Ig)E-mediated injury and in controlling parasitic infections but can also be harmful to the host in numerous clinical situations associated with eosinophilia, such as asthma. Eosinophil toxicity is not unexpected, since these cells secrete lipid mediators of inflammation, cytokines, ROS, and the granule constituents, major basic proteins (MBP), eosinophil cationic protein (ECP), and eosinophil peroxidase (EPO), which are highly toxic for neighbor cells or parasites. While there is currently no available information about the capacity of eosinophils to generate their own Hsps, Hsps have been shown to be overexpressed in alveolar macrophages and in the bronchial epithelium from patients with severe asthma and persistent airway eosinophilia (VIGNOLA et al. 1995; CHRISTIE et al. 1995). The in vitro exposure of human alveolar macrophages to purified eosinophil-derived proteins, however, does not induce Hsp synthesis, indicating that the mechanism by which activated eosinophils may induce Hsp synthesis in neighboring cells requires alternative events than eosinophilic toxic proteins (CHRISTIE et al. 1995). Among cytokines produced by eosinophils, the Hsp70-inducing TGFβ (TAKENAKA and HIGHTOWER 1992) represents an interesting candidate. Another such candidate is LTC4, the major arachidonic acid metabolite released by eosinophils, which exerts biological functions in mφ.

Figure 2 summarizes the interactions between inflammatory mediators in phagocytes, their own Hsps and the resulting proinflammatory/anti-inflammatory potential.

E. Cellular Immunity

Hsps are both extremely conserved and extremely immunogenic. These two characteristics appear quite divergent at first glance – and in order to reconcile them, Young and Cohen proposed the immunological homunculus theory, based on selection, classification and overrepresentation of certain conserved self-antigens, i.e., Hsps (COHEN and YOUNG 1991). These authors also make a clear distinction between autoimmunity and autoimmune disease, the former being actually protective against the latter. Thus, the fear for molecular mimicry of Hsps leading to autoimmune diseases is progressively vanishing, although Hsps have the ability to induce a broad immune response,

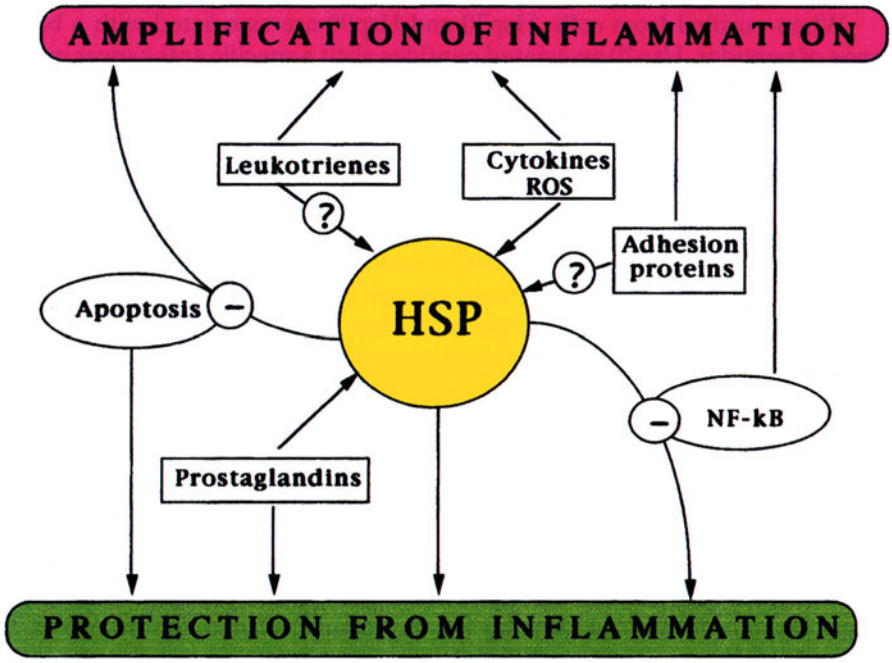

Fig. 2. Dual effects of Hsps and different inflammation-related pathways in phagocytes. Hsps can be induced in inflammation by various mediators, and might exert both protective and potentially deleterious effects

with Hsp-reactive T cells, $\gamma\delta$ T cells, natural killer (NK) cells . . . and multiple anti-Hsp antibodies.

I. T Cells

T cell mediated immune responses have been described predominantly for members of the Hsp60–65, Hsp70 and Hsp90 families. Hsp-reactive T cells are found in normal individuals and in cord-blood, indicating that these cells have the ability to escape clonal deletion, which likely relates to the immunological homunculus theory (Munk et al. 1989; Cohen and Young 1991). In terms of pathology, however, emerging fields in which Hsps appear to play important roles are, on the one hand, rheumatoid arthritis (discussed later in this book by van Eden) and the immune response against cancer. Indeed, human tumor-infiltrating CD4+ 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 locally in certain tumor tissues and support the local anti-tumor T cell response in tumors.

When purified from cells, Hsp70 and Hsp90 are associated with a broad range of peptides. Hsp70 associated peptides elicit a specific anti-cancer immunity in methylcholanthrene-induced sarcomas in mice (UDONO et al. 1994). Vaccination of mice with tumor-derived Hsp70 preparation renders the mice immune to substantial challenge with the autologous tumor cells, while the Hsp90-related glycoprotein gp96 is able to prime CD8+ cells in vivo and purified tumor Gp96 fractions elicit a potent T cell response (UDONO et al. 1994). Furthermore, vaccination with autologous tumor-derived Hsp-peptide complexes reduces the growth of the primary tumor as well as the metastatic burden, effects that can be abrogated by the depletion of CD4+ CD8+ T cells or NK cells, thus indicating the involvement of all three cell types in the protective immunity.

II. $\gamma\delta$ T Cells

Evidence is accumulating that non-MHC restricted $\gamma\delta$ TcR positive cells 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 $\gamma\delta$ T cells are involved in the recognition pathway of members of the Hsp60–65 families. Among the mycobacterial antigens Hsp65 is an immunodominant target for $\gamma\delta$ T cells.

Several lines of evidence suggest that $\gamma\delta$ T cells which recognize Hsp65 function in host defenses against pathogens (BORN et al. 1990; O'BRIEN et al. 1992). Recently, however, NAGASAWA et al. (1994) reported that $\gamma\delta$ T cells play an essential role rather in the expression of Hsp65, in particular in host mφ of mice which acquired resistance against infection with *Toxoplasma gondii*. Hsp65 overexpressed in host mφ appears to play an essential role in host defenses by preventing apoptotic death of infected cells.

The proposed mechanisms for Hsp65 and its biological functions are illustrated in Fig. 3. In a first step, $\gamma\delta$ T cells (HISAEDA et al. 1995), especially extrathymic $\gamma\delta$ T cells (HISAEDA et al. 1996a), recognize *Toxoplasma*-associated antigens, a prime candidate of which is *Toxoplasma*-derived Hsp65, presenting on the surface of mφ as well as cytoplasma and mitochondria (NAGASAWA et al. 1992). $\gamma\delta$ T cells then secrete cytokines such as IFNγ and TNFα, which in turn activate mφ (HISAEDA et al. 1996b). The activated mφ exhibit an enhanced respiratory burst, releasing high levels of ROS and NO intermediates, which contribute to the killing of intracellular pathogens. The mφ then synthesize self Hsp, which is effective in protecting infected mφ from apoptotic cell death (HISAEDA et al. 1997). This programmed cell death appears to be caused by apoptosis-inducing factor(s) produced by *Toxoplasma protozoan*, especially high-virulent *Toxoplasma*. Although the biological role and biochemical characteristics of this product still are under investigation, its targets appear to be mφ and not $\gamma\delta$ T cells. In any case, a synergistic effect between Hsp65 expression preventing apoptotic death of host cells and NO production, both mediated by IFNγ and TNFα which are generated by

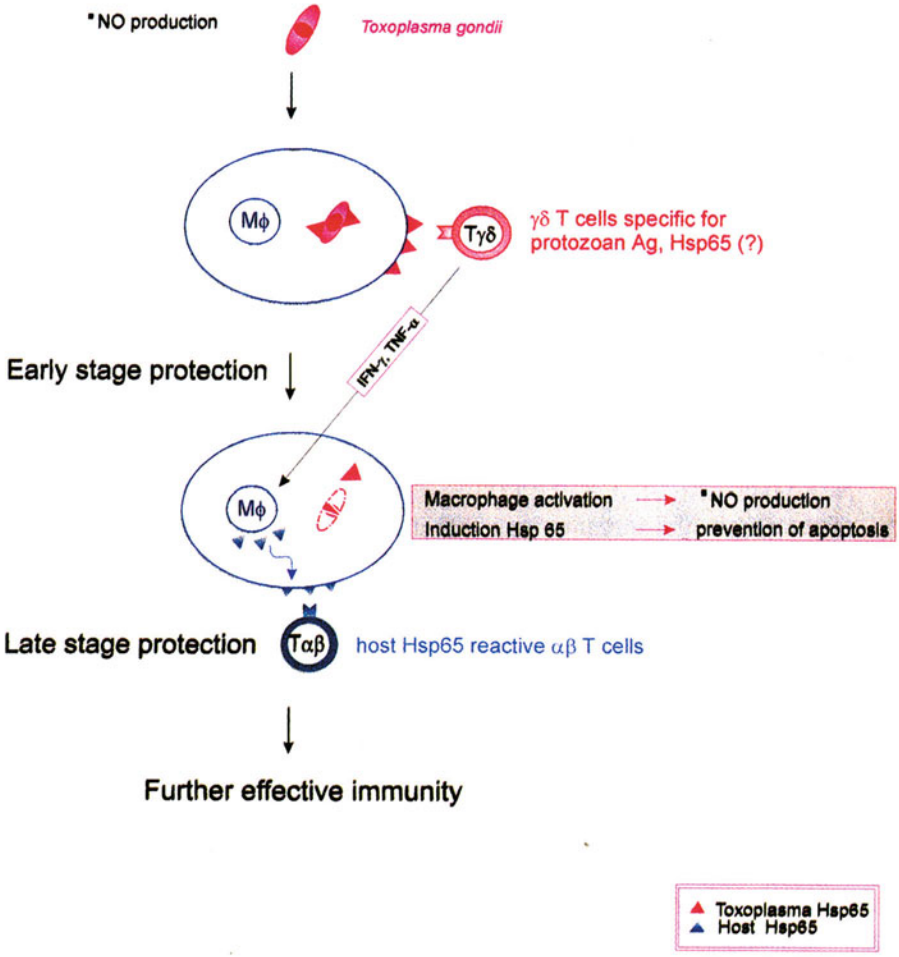

Fig. 3. Induction of an Hsp-dependent immune response by *Toxoplasma gondii*. *Toxoplasma*-derived Hsp65 is expressed on the surface of mφ, activating host $\gamma\delta$ T cells. These cells then secrete IFNγ and TNFα, thus activating mφ and leading to host Hsp65 induction and membrane expression, thereby amplifying the immune response

$\gamma\delta$ T cells, is required for the host defense. Furthermore, it should be noted that signals provided via receptors for cytokines toward Hsp65 expression and NO production are different from each other (Hisaeda and Himeno 1997).

This contribution of Hsp65 to host defenses against *Toxoplasma* may actually apply broadly to host-pathogen interactions (Ishikawa et al. 1997). For example, in infections with *Leishmania major* and *Trypanosoma cruzi*, which like *Toxoplasma gondi*, are obligate intracellular parasites, the role of Hsp65 in protective immunity is quite similar (Himeno and Hisaeda 1996).

III. Hsp, NK Cells and Cancer Immunity

Although a large number of Hsp-related tumor antigens have been identified so far (LURQUIN et al. 1989; VAN-DEN EYNDE et al. 1991), the direct interaction of tumor antigens with effector cells is not fully understood, especially in the case of non-MHC restricted effector mechanisms. NK cells were functionally defined in mediating the host's antitumor immune response for a long period of time.

More recently, several groups (MORETTA et al. 1994; LONG et al. 1996) provided direct evidence for the existence of distinct NK subclones with defined specificities against certain HLA alloantigens. TAMURA and colleagues (1993) investigated the role of the 70-kDa constitutive/cognate Hsp (Hsc70) as a possible tumor antigen, and suggested that Hsc70 expressed on the surface of tumor cells acts as recognition structure for non-MHC restricted CD4/CD8-double negative T cells.

Non MHC-restricted, TcR/CD3 negative NK-like effector cells are also to be considered in the immune response against Hsps. Indeed, the inducible Hsp70 is expressed on the surface of certain tumor cells, where it acts as a positive recognition signal for TcR/CD3 negative NK cells. In sarcoma cells, the cell surface expression of Hsp70 is induced by non-lethal heat shock (MULTHOFF et al. 1995a,b; MULTHOFF and HIGHTOWER 1996) or by treatment with the membrane-reactive alkyl-lysophospholipid derivative ET18-OCH3 (BOTZLER et al. 1996). This stress-inducible Hsp70 expression correlates with an enhanced sensitivity to lysis mediated by non-MHC restricted NK cells (MULTHOFF et al. 1995b).

Certain carcinoma cell lines exhibit Hsp70 cell surface expression under physiological conditions: the colon carcinoma line CX2 is known to stably express Hsp70 on about 60% of cells. Following cell sorting by Hsp70 expression, two sublines, CX+ and CX-, were generated. CX+ shows a stable high expression level of Hsp70 whereas CX- shows Hsp70 cell surface expression only on a minor population. Interestingly, HLA antigens and adhesion molecules were not different among CX+ and CX- cells. These two sublines provide an autologous tumor cell system for studying the role of Hsp70 as a recognition site on tumor cells.

Stress-independent plasma membrane expression of Hsp70 occurs in parallel with an increased sensitivity of NK-mediated tumor cells lysis (MULTHOFF et al. 1997). Hsp70 might act as one possible recognition structure for a distinct TcR/CD3 negative NK subpopulation. Although the complete Hsp70 protein could be immunoprecipitated from the membrane fraction of Hsp70-expressing tumor cells, the C-terminal part of Hsp70 is particularly immunogenic for NK cells, as determined by antibody binding studies using different Hsp70 specific antibodies with mapped recognition epitopes (Multhoff et al., unpublished). The positive signal for Hsp70-mediated lysis appears to dominate the negative regulatory signal for inhibition of lysis mediated by MHC alleles. Indeed, MHC class I expression is identical in both tumor cell types

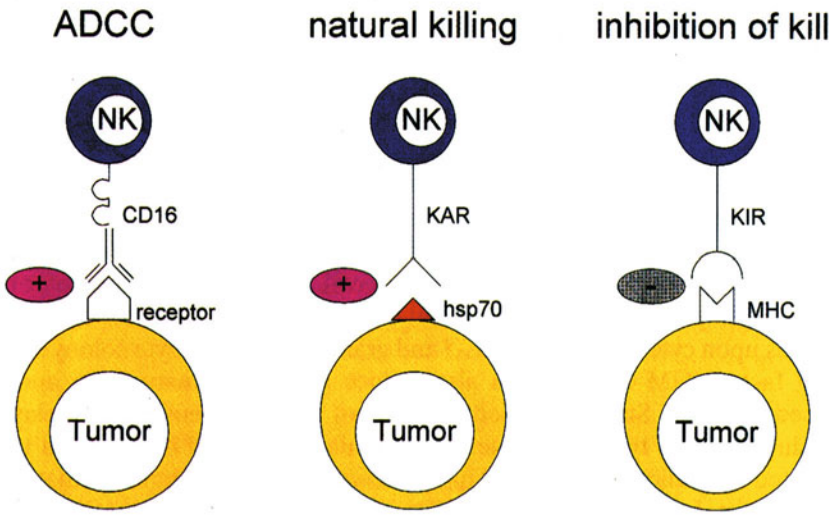

Fig. 4. Stress-independent plasma membrane expression of Hsp70 as an effector mechanism for the NK-mediated tumor cell lysis. NK cells contribute to antitumor immunity by either ADCC, or recognition of tumor cell membrane expressed Hsp70, while killer cell inhibitory/activatory receptors (KIR/KAR) modulate killing

(MULTHOFF et al. 1997), and even following treatment with IFNγ that enhances MHC expression on both tumor lines, the Hsp70 expressing CX+ is lysed better by NK cells. Furthermore, MHC class I-specific antibodies are unable to increase the lysis of Hsp70 expressing tumor cells and NK cells, the specificity of which against Hsp70 expressing tumor cells can be generated from MHC divergent donors.

A schematic illustration of different NK effector mechanisms is shown in Fig. 4. Besides CD16-antibody-mediated antibody-dependent cellular cytotoxicity (ADCC), Hsp70 might be considered as an immunogenic target structure on tumor cells for a specific TcR/CD3 negative NK subpopulation. Blocking studies, using purified recombinant Hsp70, revealed that these NK cells might express an Hsp70 receptor, the molecular characterization of which is currently under investigation. In this context the role of killer cell inhibitory/activatory receptors (KIR/KAR) with specificity against distinct MHC alleles is also being analyzed.

F. The Paradigm of Asthma

Airway inflammation is considered the main cause of asthma. Acute asthma attacks induced by allergen challenge in allergic patients lead to an early inflammatory response characterized by a specific IgE-mediated activation of

mast cells, alveolar macrophages and bronchial epithelial cells. IgE-mediated activation involves the release of proinflammatory mediators such as histamine, AA metabolites, PAF-acether, ROS, neuropeptides and cytokines that altogether lead to the constriction of airway and smooth muscle, mucus secretion and vasodilation. This acute phase response is often followed by a late response that contributes to the chronicity and to the exacerbation of inflammation which are typical features of the illness. It is suggested that the recruitment of peripheral blood cells, particularly eosinophils, plays a central role in the late phase asthmatic response. Besides cell-cell and cell-extracellular matrix adhesion processes, the recruitment of eosinophils into the airways clearly depends upon cytokines such as IL-5 and granulocyte-monocyte colony stimulating factor (GM-CSF), which also induce eosinophil maturation and increased survival. Such increased survival of activated eosinophils plays a prominent role in the development of chronic asthma and is suggested to be mediated by reduced apoptosis. This reduced apoptosis might be mediated, at least in part, by Hsp70 overexpression in the airways of asthmatic patients (SAMALI and COTTER 1996; VIGNOLA et al. 1995). Indeed, Hsp70 expression in asthma significantly correlates with clinical severity (VIGNOLA et al. 1995; FAJAC et al. 1997), indicating that Hsp parallels the development of chronic airway inflammation (VIGNOLA et al. 1995).

There are several possible biological consequences of the increased expression of Hsp70 in asthma. Firstly, Hsp70 may provide an effective protective mechanism against ROS and proinflammatory cytokines, widely released in the inflamed airways. The theoretical protective role of Hsp70 in asthma is supported by the evidence that anti-Hsp70 immunoreactivity of epithelial cells or alveolar macrophages significantly correlates with the number of eosinophils recovered in the bronchoalveolar lavage of asthmatics (VIGNOLA et al. 1995). The toxicity of eosinophils mainly results in epithelium shedding caused by extracellular ECP; overexpression of Hsp70 by bronchial epithelial cells may protect these cells against the deleterious effects of the latter mediator.

Secondly, Hsp70 may contribute to the amplification of the inflammatory response. Indeed, Hsp70 may participate to antigen processing and/or presentation (CORRIGAN and KAY 1992) as well as in class II expression, or act itself as an antigen. Since the airway epithelium of patients with severe asthma is infiltrated by an increased number of T lymphocytes bearing both the $\alpha\beta$ and the $\gamma\delta$ receptors, it is conceivable that Hsp expressed by bronchial epithelial cells may activate Hsp-specific T cells, contributing to the perpetuation of the airway's inflammation.

Thirdly, the increased expression of Hsp70 (and Hsp27) in asthma may play a role in the regulation of cell apoptosis in epithelial cells and in inflammatory cells. With regard to the bronchial epithelium, recent evidence shows that very few epithelial cells are apoptotic in asthmatic patients (Vignola, unpublished). When apoptosis is detected, it localizes to the superficial layer of metaplastic lesions or to desquamated ciliated bronchial epithelial cells. This

localization suggests that apoptosis contributes to tissue turnover by elimination of the epithelial cells after terminal differentiation or cellular damage (MOUNTZ et al. 1994), as well as to the maintenance of a balance between the rate of cell proliferation and death (SAVILL 1994; SCHULER et al. 1994). Thus, in asthma, despite the release of a wide range of cytotoxic or pro-apoptotic mediators, a low number of bronchial epithelial cells are apoptotic: these cells may efficiently protect themselves against noxious stimuli. Interestingly, the increased expression of Hsp70 in the epithelium of asthmatics is paralleled by an increased expression of bcl-2, and a low level of p53 and proliferating cell nuclear antigen (PCNA) expression, suggesting that the regeneration of the epithelial layer in asthma may be related more to the survival of basal epithelial cells than to their replication. By contrast, the increased expression of Hsp70 in inflammatory cells infiltrating the bronchial mucosa of asthmatic patients may have deleterious consequences. By their ability to reduce cell apoptosis and increase cell survival, the overexpression of Hsp70 by inflammatory cells may play a crucial role in the persistence of these cells in the inflamed tissues and in the pathogenesis of the chronicity of airway inflammation. Hence, the potential biological consequences of the expression of Hsp70 seem to be different according to the different cell types expressing these molecules.

In contrast, the low expression of Hsp70 observed in the airways of chronic bronchitis patients suggests that the mechanisms underlying the airway inflammation in this distinct disease differ from those involved in asthma. The presence of non-degranulated eosinophils observed in biopsies of patients with chronic bronchitis suggests a lower "aggressivity" of eosinophils in this clinical situation as compared to asthma. Airway inflammation in asthma and chronic bronchitis may differ in terms of cell recruitment, activation, and mediator release. These differences may be relevant to the regulation of Hsp70 expression, which may be specific for asthma rather than a general feature of chronic inflammation of the airways.

Finally, Hsps have been described to associate with cytosolic steroid receptors, suggesting a fundamental anti-inflammatory role for these proteins, particularly relevant to glucocorticoid therapy. Glucocorticoids, which suppress the release of arachidonic acid derivatives by inhibiting PLA_2, possess cytosolic receptors which bind Hsp90 molecules and form an inactive complex unable to bind DNA. This inactive form of the receptor is a multiprotein complex that also includes Hsp70. In the absence of steroids, it appears that the Hsp90/Hsp70 chaperoning system is required to maintain a proper receptor conformation for high steroid binding affinity. Upon steroid binding, Hsp90, but not Hsp70, dissociates, triggering receptor transformation from the inactive form to a steroid-activated state that binds to the appropriate response element in the promoters of glucocorticoid responsive genes (GRE), to bring about the final response in target cells (PRATT 1993), including the anti-inflammatory effects.

G. Conclusions and Perspectives

Though the primary function of Hsps is to rescue other proteins from denaturation, the fields of application of these proteins have expanded during recent years to broader areas, and particularly to the biomedical field, which includes infection, cancer and inflammatory diseases, as illustrated in several chapters of this book. Besides the evidence that Hsps exert protective, anti-inflammatory effects – though eventually, pro-inflammatory effects as well – a novel role for these proteins has been suggested, as a promising prognostic/diagnostic marker in inflammatory and (auto)immune diseases. To promote this specific research area, we recently developed a new test that allows the rapid evaluation Hsp70 in human peripheral blood monocytes with an increased sensitivity and accuracy (BACHELET et al., 1998). The use of such new tools may allow in the near future the definition of clues to a better understanding of the influence of Hsps in immune and inflammatory-related diseases.

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