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Review

Heat shock protein 60: regulatory role on innate immune cells

C. Habich* and V. Burkart

German Diabetes Clinic, German Diabetes Center, Leibniz Institute at the Heinrich-Heine-University of Düsseldorf, 40225 Düsseldorf (Germany), Fax: +49 211 3382 603, e-mail: christiane.habich@ddz.uni-duesseldorf.de, burkart@ddz.uni-duesseldorf.de

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Abstract. Human heat shock protein 60 (Hsp60) exhibits immunoregulatory properties, primarily by inducing pro-inflammatory responses in innate immune cells. Extensive analyses identified specific receptor structures for the interaction of Hsp60 with these cells. The existence of distinct receptor structures responsible for Hsp60 binding and for Hsp60-induced release of pro-inflammatory mediators has been demonstrated, implying that the interaction of Hsp60 with innate immune cells is a multifaceted process. Distinct Hsp60 epitopes responsible for binding to innate immune cells and for the activation of these cells have been identified. Depending on the cell-type, the amino acid (aa) region 481–500 or the regions aa241–260, aa391–410 and aa461–480 are involved in Hsp60-binding to innate immune cells. An entirely different Hsp60-region, aa354–365 was found to bind lipopolysaccharide, thereby mediating the pro-inflammatory effects of Hsp60. Because of its immunoregulatory properties, Hsp60 has been proposed to act as intercellular danger signal, controlling innate and adaptive immune reactions.

Keywords. Hsp60, innate immune cells, receptor structures, Hsp60 epitopes, lipopolysaccharide.

Introduction

Heat shock proteins (HSPs) belong to the superfamily of stress proteins, which represent a group of ubiquitously expressed and evolutionary highly conserved proteins. Based on their relative molecular mass, HSPs are classified into six major families, comprising small HSPs, HSP40, HSP60, HSP70, HSP90 and HSP110, which are localized in different cellular compartments, e.g. in the mitochondria or in the endoplasmic reticulum (Table 1). HSPs serve as molecular chaperones with important functions in the correct folding of nascent proteins, in the transport of newly synthesized proteins to their intracellular target compartments and in the degradation of aged or damaged proteins via the proteasome. HSPs are expressed constitutively, and in situations of cellular stress (e.g. high temperature, radiation, inflammation) the synthesis of HSP is markedly upregulated [1, 2]. Changes in the intracellular distribution of HSPs and expression on the cell surface have been observed in response to a variety of cellular stress conditions [3–6]. Moreover, HSPs, initially considered as intracellular proteins, have been found to be released into the extracellular space by stressed cells [7–10]. These observations point to a potential role of extracellular HSPs as intercellular signalling molecules. It had been assumed that HSPs present a link between infection and autoimmunity because of the high amino acid sequence homology of 50% between mammalian and microbial HSP, which might lead to immunological cross-reactivity [11, 12]. Meanwhile, it has been realized that the association of HSPs and inflamma-

* Corresponding author.

tory conditions cannot be explained by cross-reactive immune responses alone [13]. It has become evident that extracellular HSPs act as potent intercellular signalling molecules that serve as danger signals to the innate immune system when expressed on or released by autologous cells, influencing a wide range of immune reactions [13, 14]. In particular, immune responses directed against members of the HSP60, HSP70 and HSP90 families are implicated to contribute to the pathogenesis of a variety of tissue-specific autoimmune disorders, vascular diseases and inflammatory skin disorders [12, 15–18]. For members of the HSP60 family, which have been described as immunodominant molecules in various diseases, a potential immunoregulatory role in the development of tissue-specific autoimmune disorders such as rheumatoid arthritis and type 1 diabetes and in vascular diseases like arteriosclerosis has been suggested [19–23].

Table 1. Major mammalian HSP families.

Families	Prominent members	Localization
Small HSP (12—43 kDa)	αB-crystallin Hsp27	cytoplasm cytoplasm, nucleus
HSP40	Hsp40	cytoplasm, nucleus
HSP60	Hsp60 TCP-1	mitochondria cytoplasm
HSP70	Hsp70 Hsc70 Grp78/BiP	cytoplasm, nucleus cytoplasm ER
HSP90	Hsp90 (α and β) gp96	Cytoplasm ER
HSP110	Hsp110	cytoplasm

ER, endoplasmic reticulum.

Therefore, elucidation of the regulatory role of Hsp60 on innate immune cells has been a focus of recent research interest.

Immunoregulatory potential of Hsp60

In the circulation of healthy human individuals Hsp60 and Hsp70 are present in readily detectable amounts [23–25]. Under various pathological conditions like hypertension [26], arteriosclerosis [23, 27] and renal vascular diseases [28, 29] strong increases in HSP serum levels are observed. This observation points to an enhanced release of HSP into the extracellular compartment when cells or tissues experience situations of stress, e.g. during inflammation. From several studies it became obvious that extracellular HSPs exhibit broad immunoregulatory properties by acting as danger signals to the innate immune system, thereby mediating and modulating a number of inflammatory reactions [13, 14]. Numerous studies demonstrate that HSPs are potent activators of cells of the innate immune system, i.e. macrophages, dendritic cells (DCs) and endothelial cells (ECs) [13, 30].

We and others have shown that human Hsp60 activates pro-inflammatory reactivity in innate immune cells [14, 31–33] [unpublished observations]. As shown in Figure 1, incubation of DCs with Hsp60 resulted in a significant release of tumor necrosis factor α (TNF-α), and interleukin-6 (IL-6) and the CC chemokines RANTES and MIP-2. Hsp60 also induces the formation and release of the pro-inflammatory mediators IL-1β, MCP-1, MIP-1α and the short-lived radical nitric oxide (NO). Hsp60 further stimulates production of the T helper 1 cell promoting cytokines IL-12 and IL-15 in innate immune cells, and it provokes the maturation of DCs as revealed by the upregulation of major histocompatibility complex molecules and of the co-stimulatory surface molecules CD40, CD54 and CD86. Recent investigations focussed on Hsp60-induced signalling pathways leading to the production and release of pro-inflammatory mediators from innate immune cells. We and others found that Hsp60 activates the stress-activated protein kinases c-Jun N-terminal kinase 1/2 (JNK1/2) and p38, the mitogen-activated protein kinases/extracellular signal-regulated kinases 1/2 (ERK1/2) and the transcription factor NFκB [33] [unpublished observations]. The use of specific inhibitory compounds allowed us to assess the role of distinct signalling proteins in Hsp60-induced cytokine production. As shown in Figure 2, preincubation of macrophages with the NFκB inhibitor SN50 or the ERK1/2 inhibitor PD98059 resulted in a significant and dose-dependent inhibition of Hsp60-stimulated TNF-α and IL-6 production, indicating a prominent role for NFκB and ERK1/2 in this process.

Taken together, these findings demonstrate that Hsp60 represents an important danger signal for the innate immune system.

Receptor structures for Hsp60 on innate immune cells

Aiming at the development of strategies to interfere with the immunoregulatory functions of HSPs, current investigations focus on the identification of specific receptor structures for stress proteins on innate immune cells. To characterize the receptor structure(s) for Hsp60, we established a flow cytometry-based binding assay with human Hsp60, labelled with

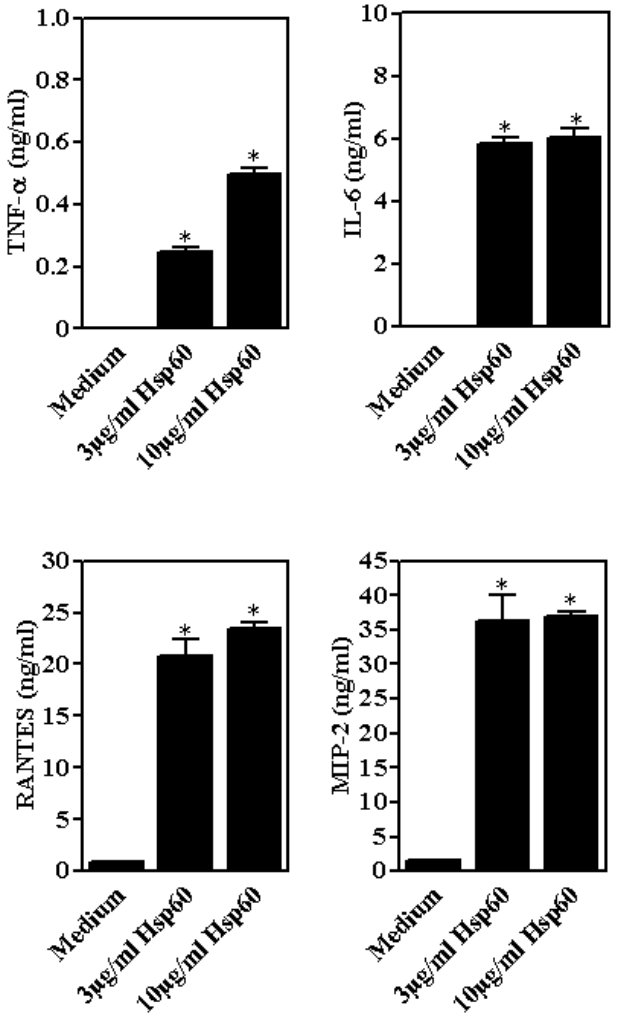

Figure 1. Hsp60 induces the production of pro-inflammatory mediators in dendritic cells. Bone marrow-derived dendritic cells from BALB/c mice were incubated with medium alone or medium supplemented with different concentrations of human Hsp60. TNF-α, IL-6, RANTES and MIP-2 concentrations in cell supernatants were determined by enzyme-linked immunosorbant assay (ELISA). The data represent the mean values of the different cytokine and chemokine concentrations + SD. Significant differences from medium control alone are indicated: *, $p < 0.05$.

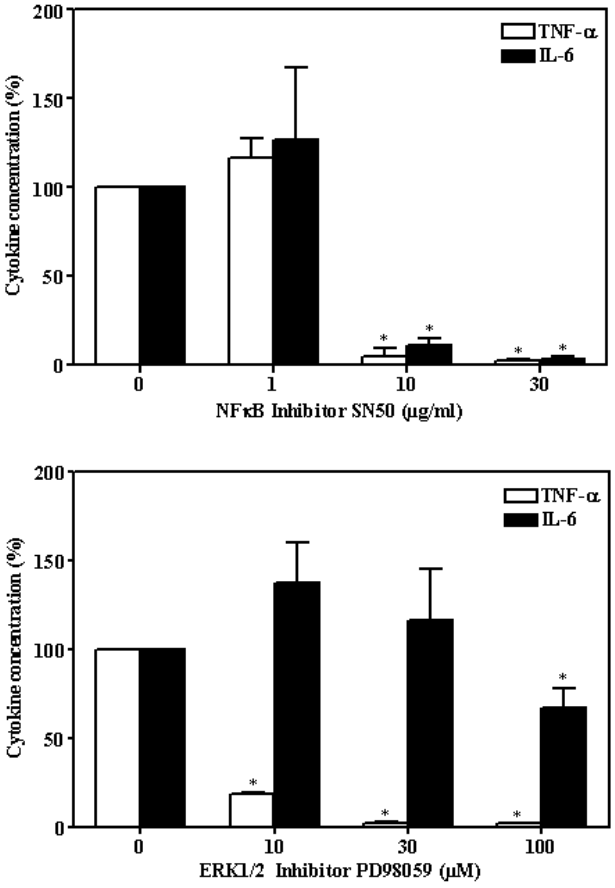

Figure 2. Signalling proteins involved in Hsp60-induced cytokine release from innate immune cells. Bone marrow-derived macrophages from C57BL/6J mice were preincubated with medium alone or medium supplemented with NFκB inhibitor SN50 or the ERK1/2 inhibitor PD98059 at the indicated concentrations. Subsequently, Hsp60 (10 µg/ml) was added. After cultivation periods of 6 or 24 h, TNF-α and IL-6 concentrations in cell supernatants were determined by ELISA. The data represent the mean values of the TNF-α and IL-6 concentrations in percentage of the cytokine concentrations determined in the supernatants of the cells treated with Hsp60 alone (mean + SD). Significant differences to samples treated with Hsp60 alone are indicated: *, $p < 0.05$.

the fluorescent dye Alexa Fluor 488. This assay allows the analysis and quantification of the binding of Hsp60 to the surface of innate immune cells. Using this approach we could demonstrate specific Hsp60 binding to macrophages [34]. Binding of human Hsp60 to macrophages showed the typical characteristics of specific ligand-receptor interactions, i.e. Hsp60 binding occurred at submicromolar concentrations, was saturable and could be competed only with unlabelled Hsp60 (K_d ~ 300 nM), but not with unrelated control proteins. Incubation at physiological temperatures, at which endocytosis occurs, resulted in an apparent specific uptake of Hsp60. Specific Hsp60 binding was further confirmed by the use of confocal microscopy, clearly showing surface binding of Hsp60, but not of control proteins [34]. Meanwhile, we could also demonstrate specific binding of Hsp60 to EC (Fig. 3) and DC [unpublished observations]. As shown in Figure 3, binding of fluorescent-labelled Hsp60 to ECs was demonstrable by the increasing fluorescence intensity of the cells. Hsp60-binding was specific because it could almost completely be inhibited by preincubation of EC with unlabelled Hsp60. In context with our previous findings (data not shown), these observations demonstrate that the binding of Hsp60 to ECs and DCs shows similar characteristics as Hsp60 binding to macrophages.

Approaches to characterize the receptor structures for human Hsp60 on innate immune cells revealed that toll-like receptor 2 (TLR2), TLR4 and CD14, origi-

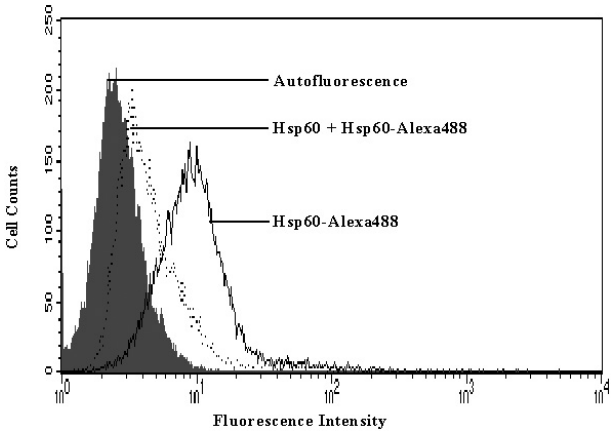

Figure 3. Specific Hsp60 binding to endothelial cells. Endothelial cells of the line MHEC5-T were incubated in the absence (autofluorescence) or presence of 1.75 μM unlabelled human Hsp60, followed by the addition of 350 nM Alexa Fluor 488-labelled human Hsp60. Fluorescence intensities were plotted against cell counts.

nally described as receptors for the recognition of microbial products such as lipopolysaccharide (LPS) [35–39], are responsible for the pro-inflammatory effects of Hsp60 [33, 40–42]. More detailed analyses were performed with TLR-deficient mice and with antibodies directed against TLR and CD14. As shown in Figure 4, antibodies against TLR4 or CD14 did not interfere with the binding of fluorescent-labelled Hsp60 to macrophages. Together with our previous findings [unpublished observations], these results show that binding of Hsp60 to innate immune cells is independent of TLR2, TLR4 and CD14 [34].

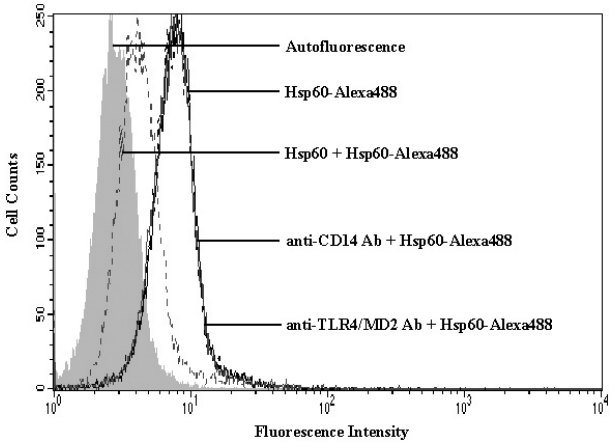

Figure 4. CD14 and TLR4 are not the binding receptor structures for HSP60 on innate immune cells. J774A.1 macrophages were incubated in the absence (autofluorescence) or presence of 1.75 μM unlabelled human Hsp60 or 20 μg/ml of the appropriate antibodies (Ab), followed by the addition of 350 nM Alexa Fluor 488-labelled human Hsp60. Fluorescence intensities were plotted against cell counts.

In parallel studies by Binder et al. [43] and Basu et al. [44], the α_2-macroglobulin receptor, also known as CD91, was identified as a common binding receptor for gp96, Hsp90 and Hsp70 on innate immune cells. To investigate the ability of CD91 to act as binding receptor for Hsp60, the potential inhibitory effect of gp96, Hsp90, Hsp70 and α_2-macroglobulin on Hsp60 binding to macrophages was analysed. Our results demonstrated that CD91 is not involved in Hsp60 binding, because no inhibitory effect could be observed [34]. This finding was confirmed by studies analysing the effect of different antibody clones directed against CD91 on Hsp60 binding (Fig. 5). Preincubation of macrophages with anti-CD91 antibodies did not result in inhibition of the binding of fluorescent-labelled Hsp60 to these cells. As independent proof, we investigated the influence of Hsp60 on fluorescein isothyocianate (FITC)-labelled gp96-binding to innate immune cells. Hsp60 did not impair gp96 binding to these cells [34]. These results demonstrate that the receptor structure for Hsp60 is not identical to the common receptor for gp96, Hsp90 and Hsp70.

Overall, these observations indicate that the interaction of Hsp60 with innate immune cells represents a highly complex process, including the contact of Hsp60 with cell surface structures involved in binding and in initiation of pro-inflammatory responses. Currently available data from other research groups allow extension of this conclusion to other HSPs. CD91 and the scavenger receptor-A have been identified as binding receptors for gp96 on innate immune cells, whereas gp96 signalling is TLR2/4-dependent [43–47]. CD91, CD40, LOX-1 and CD94 have been described as binding receptors for human Hsp70 on innate immune cells [43, 44, 48–50], whereas CD14 [51], TLR2, and TLR4 [52] have been found to be involved in Hsp70 signalling.

In order to approach the issue of potential cross-reactivity between prokaryotic and eukaryotic Hsp60 as a potential cause for the development of autoimmunity, parallel studies focussed on the question whether prokaryotic and eukaryotic Hsp60 species are recognized by the same binding receptor structures on innate immune cells. In comparative analyses the effects of different prokaryotic and eukaryotic Hsp60 preparations on human Hsp60 binding to macrophages were studied [42, 53]. We could show that none of the tested prokaryotic Hsp60 preparations (*Mycobacterium bovis* Hsp65, *Escherichia coli* GroEL, *Chlamydia pneumoniae* Hsp60) impaired binding of human Hsp60 to macrophages. In contrast, all eukaryotic Hsp60 proteins investigated (human, mouse, rat and hamster Hsp60 and Hsp60 from *Histoplasma capsulatum*) inhibited human Hsp60 binding in a

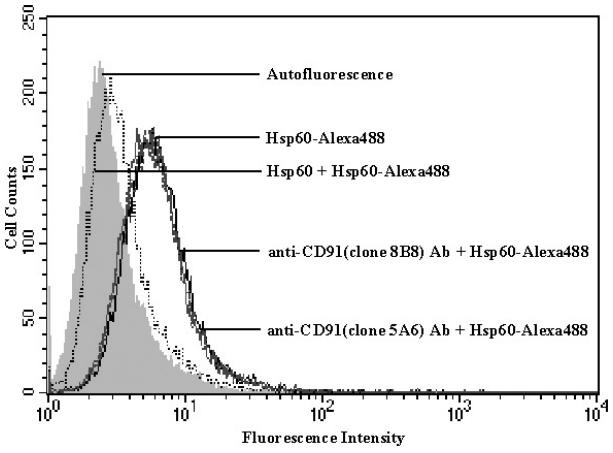

Figure 5. CD91 is not the Hsp60-binding receptor. J774A.1 macrophages were incubated in the absence (autofluorescence) or presence of 1.75 μM unlabelled human Hsp60 or 20 μg/ml of the appropriate Ab, followed by the addition of 350 nM Alexa Fluor 488-labelled human Hsp60. Fluorescence intensities were plotted against cell counts.

similar range [53]. The results of this study indicate that eukaryotic Hsp60 species are recognized by the same binding structures on macrophages, while Hsp60 species of prokaryotic origin interact with different receptor structures. Support for this suggestion comes from other reports, describing how human Hsp70 and mycobacterial Hsp70 bind CD40, but use different binding sites of this cell surface molecule [48, 54]. Furthermore, a recent study shows an elevated release of Hsp70 from peripheral blood mononuclear cells stimulated with the bacterial homologue of human Hsp60, GroEL, but not with human Hsp60. The authors propose the existence of different receptor structures for human and bacterial HSPs [10]. The discrimination of endogenous (self) and infectious (non-self) HSPs by innate immune cells on the receptor level is of critical importance for the maintenance of immunological homeostasis, because HSPs are abundant in eukaryotic and prokaryotic cells and both possess strong immunoregulatory potential.

Epitopes of the Hsp60 molecules involved in the interaction with innate immune cells

Recent studies are focussing on the identification of epitope regions of the human Hsp60 molecule involved in communication with cell surface structures on innate immune cells. As outlined before, the contact of Hsp60 with these cells is a highly complex process, involving separate receptor structures for Hsp60 binding and for Hsp60-induced cell activation. Based on these observations, it can be assumed that different regions of the Hsp60 molecule might be involved in interactions with appropriate receptor structures.

Hsp60 epitopes involved in binding to receptor structures on innate immune cells

Extensive analyses were performed to define the epitope regions of the human Hsp60 molecule responsible for binding to receptor structures on innate immune cells. In these studies we investigated the potential of distinct sets of overlapping oligopeptides of different length, covering the human Hsp60 sequence and of selected Hsp60 deletion mutants to interfere with the binding of Alexa Fluor 488-labelled human Hsp60 to macrophages. An epitope in the C-terminal region of the Hsp60 molecule, i.e. aa481–500, was found to account for Hsp60 binding to cells of the macrophage line J774A.1 [55]. Unexpectedly, parallel analyses using primary bone marrow-derived macrophages from C57BL/6J mice resulted in identification of three completely different epitope regions of the Hsp60 molecule, i.e. aa241–260, aa391–410 and aa461–480 [53] (Fig. 6). Based on these findings we conclude that, depending on the cell type, different regions of the Hsp60 molecule are engaged in interactions with adequate receptor structures on innate immune cells. In view of these observations, it is conceivable that different cell-type-specific receptors might be in the contact with the relevant epitope regions of Hsp60.

Our results are in agreement with reports describing different, cell-type-specific receptors for other HSPs. For Hsp70 several binding receptors have been described on different immune cell populations, i.e. CD91 on macrophages, CD40 on macrophages and DCs, the class E scavenger receptor LOX-1 on DCs and CD94 on NK cells [44, 48–50, 56].

Our results also support our assumption that eukaryotic and prokaryotic Hsp60 species use different binding receptor structures on innate immune cells. Sequence alignment of the identified epitope regions of the human Hsp60 molecule with Hsp60 from other species revealed strong sequence homology among mammalian Hsp60 species, but not with prokaryotic Hsp60 species (Table 2).

Hsp60 epitopes involved in the activation of innate immune cells

Receptor structures including CD14, TLR4 and TLR2 have been shown to mediate the pro-inflammatory effects of LPS and bacterial lipoprotein as well as of HSPs on innate immune cells [31, 33, 35, 41, 46, 52].

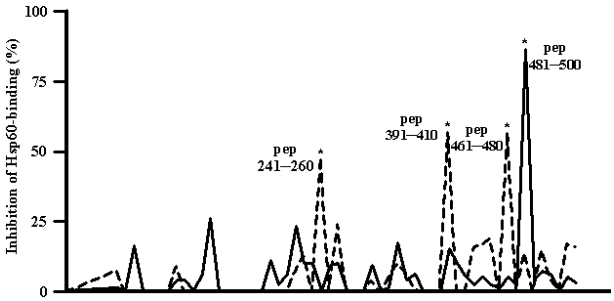

Figure 6. Effect of Hsp60 peptides on Hsp60 binding to permanent and primary macrophages. Macrophages of the line J774A.1 (straight line) or primary bone marrow-derived macrophages from C57BL/6J mice (dotted line) were incubated with overlapping Hsp60 peptides (pep, 125 μM) of 20aa, spanning the sequence of the unprocessed precursor of human Hsp60 (Swiss-Prot P10809) from aa1–560. Subsequently, Alexa Fluor 488-labelled human Hsp60 (350 and 500 nM, respectively) was added. The analysis was performed by flow cytometry. Inhibition of binding of Alexa Fluor 488-labelled Hsp60 is indicated as percentage. Inhibitory peptides are indicated and marked by asterisks.

Table 2. Sequence alignments between the binding-epitope regions of human Hsp60 and other Hsp60 species.

HSP60 species	Sequence alignments
Human Hsp60 epitope aa241–260:	
Human	$_{241}$DAYVLLSEKKISSIQSIVPA$_{260}$
Hamster	$_{241}$DAYVLLSEKKISSVQSIVPA$_{260}$
Mouse	$_{241}$DAYVLLSEKKISSVQSIVPA$_{260}$
Rat	$_{241}$DAYVLLSEKKISSVQSIVPA$_{260}$
Histoplasma capsulatum	$_{264}$KPLIVLSEKKISAVQDIIPA$_{283}$
Chlamydia pneumoniae	$_{217}$DALILIYDKKISGIKDFLPV$_{236}$
Mycobacterium bovis	$_{214}$DPYILLVSSKVSTVKDLLPL$_{233}$
Escherichia coli	$_{216}$SPFILLADKKISNIREMLPV$_{235}$
Human Hsp60 epitope aa391–410:	
Human	$_{391}$NERLAKLSDGVAVLKVGGTS$_{410}$
Hamster	$_{391}$NERLAKLSDGVAVLKVGGTS$_{410}$
Mouse	$_{391}$NERLAKLSDGVAVLKVGGTS$_{410}$
Rat	$_{391}$NERLAKLSDGVAVLKVGGTS$_{410}$
Histoplasma capsulatum	$_{404}$QERLAKLSGGVAVIKVGGAS$_{423}$
Chlamydia pneumoniae	$_{366}$QERLAKLSGGVAVIRVGAAT$_{385}$
Mycobacterium bovis	$_{363}$QERLAKLAGGVAVIKAGAAT$_{382}$
Escherichia coli	$_{365}$QERVAKLAGGVAVIKVGAAT$_{384}$
Human Hsp60 epitope aa461–480:	
Human	$_{461}$QKIGIEIIKRTLKIPAMTIA$_{480}$
Hamster	$_{461}$QKIGIEIIKRALKIPAMTIA$_{480}$
Mouse	$_{461}$QKIGIEIIKRALKIPAMTIA$_{480}$
Rat	$_{461}$QKIGIEIIKRALKIPAMTIA$_{480}$
Histoplasma capsulatum	$_{475}$QLRRISSLVSAITRPARTIV$_{494}$
Chlamydia pneumoniae	$_{438}$EAIGTRIILKALTAPLKQIA$_{457}$
Mycobacterium bovis	$_{432}$EATGANIVKVALEAPLKQIA$_{451}$
Escherichia coli	$_{435}$QNVGIKVALRAMEAPLRQIV$_{454}$
Human Hsp60 epitope aa481–500:	
Human	$_{481}$KNAGVEGSLIVEKIMQSSSE$_{500}$
Hamster	$_{481}$KNAGVEGSLIVEKILQSSSE$_{500}$
Mouse	$_{481}$KNAGVEGSLIVEKILQSSSE$_{500}$
Rat	$_{481}$KNAGVEGSLIVEKILQSSSE$_{500}$
Histoplasma capsulatum	$_{495}$ENAGLEGSVIVGKLTDEHAS$_{514}$
Chlamydia pneumoniae	$_{458}$SNAGKEGAIICQQVLARSAN$_{477}$
Mycobacterium bovis	$_{452}$FNSGLEPGVVAEKVRNLPAG$_{471}$
Escherichia coli	$_{455}$LNCGEEPSVVANTVKGGDGN$_{474}$

Swiss-Prot Acc. No. of the Hsp60 species: human (P10809), hamster (P18687), mouse (P63038), rat (P63039), *H. capsulatum* (P50142), *C. pneumoniae* (P31681), *M. bovis* (P06806), *E. coli* (P06139).

For this reason, HSPs have been considered to be endogenous ligands for TLR. The fact that various HSPs exhibit similar stimulatory effects on innate immune cells and the involvement of the same receptor systems raised the question whether endotoxin contaminations of recombinant HSP preparations might be responsible for the observed effects [13, 30, 57–59]. We re-examined this issue by performing extensive control experiments to elucidate the potential role of LPS in Hsp60-mediated activation of innate immune cells [60]. First, the effects of protease and heat treatment on the immunoregulatory properties of Hsp60 preparations and LPS were analysed. The macrophage-stimulatory capacity of Hsp60 was found to be protease- and heat-sensitive, while the stimulatory capacity of LPS was not. Adding a defined quantity of LPS to Hsp60 resulted in augmentation of the stimulatory effects of Hsp60 on innate immune cells. The increase in stimulatory activity became protease- and heat-resistant, reflecting the amount of LPS admixed. Complementary observations were made with the potent LPS inhibitory peptide polymyxin B (PmB). The macrophage-stimulatory activity of Hsp60 was PmB-resistant, whereas the increased stimulatory potential, observed after addition of a defined LPS amount, was PmB-sensitive. Another LPS-binding peptide, the defensin magainin II amide, was also able to impair the stimulatory activity of Hsp60 and of LPS [60–62]. These results indicated that it is not contamination with free LPS that is responsible for the immunoregulatory effects of Hsp60, but LPS or structurally related molecules tightly bound to Hsp60. Consequently, an experimental system with radioactive-labelled LPS was established to directly prove the potential of Hsp60 to bind LPS [60]. This approach allowed us for the first time to demonstrate specific LPS binding to Hsp60. Binding of LPS to Hsp60 was saturable (K_d ~300nM) and could be competed by unlabelled LPS. Consequently, we attempted to define the LPS binding site of the Hsp60 molecule. Intensive analyses, using defined antibodies directed against Hsp60 and selected oligopeptides of the human Hsp60 sequence, identified the Hsp60-epitope region aa354–365 involved in specific LPS binding [60]. This region includes the central amino acid sequence motif LKGK, which was found to be critical for LPS binding, as shown by sequence alignments with a highly efficient LPS-binding protein, i.e. factor C from *Limulus polyphemus* [60]. Sequence comparisons revealed that this amino acid motif exists in all mammalian Hsp60 species, but not in Hsp60 of prokaryotic origin. We conclude that the Hsp60-mediated activation of innate immune cells, i.e. the induction of a pro-inflammatory response, is mediated by specifically bound LPS. Support of our hypothesis

comes from other studies, where mammalian Hsp70 and Hsp90 have been found to act as receptors for LPS [63–65]. In normal healthy individuals serum levels of autologous Hsp60 and Hsp70 reach concentration ranges up to mg/ml [24, 25]. Furthermore, in humans LPS concentrations have been observed which are most likely high enough to allow loading of extracellular Hsp60 with LPS under physiological conditions, in the absence of a bacterial infection [66, 67]. Based on these facts, the concept that autologous, extracellular HSPs act as danger signals for the innate immune system must be extended. HSPs themselves not only represent danger signals, but also serve as sensors for hazardous signals, such as LPS. This is supported by other studies reporting the specific binding of LPS by Hsp70 and gp96 [68–70], while Hsp90 has been described as a primary receptor for immunostimulatory bacterial CpG DNA [71]. Thus, besides their role as molecular chaperones, HSPs exhibit an important immunoregulatory function. The presentation of microbial structures by mammalian HSPs to innate immune cells may result in effective recognition of these structures, thereby increasing the efficiency of immune responses.

Localization of Hsp60 epitopes involved in Hsp60 binding and Hsp60-mediated activation of innate immune cells

Under physiological conditions Hsp60 is an oligomer, composed of two stacked rings each built by seven identical subunits, the Hsp60 monomers [72]. This polymeric structure exists in a dynamic equilibrium between monomers, heptamers and tetradecamers [30]. In a 3D model of the human Hsp60 molecule, deduced from *Escherichia coli* GroEL (swissmodel.expasy.org/repository), the bacterial homologue of human Hsp60, the identified binding epitope regions can be located in the apical (aa241–260), small intermediate (aa391–410) and in large equatorial (aa461–480 and aa481–500) domains, (Fig. 7). The apical domain forms the specific LPS-binding site in the region aa354–365 (Fig. 7). The positions of the epitope regions indicate that the contributing amino acids are neither involved in intramolecular interactions nor in contacts to flanking monomers. Thus, these regions are accessible for Hsp60 receptor structures and for LPS in the monomeric as well as in the oligomeric conformation of the Hsp60 molecule. The identified epitopes involved in the interaction with innate immune cells might also be responsible for the interaction of Hsp60 with adaptive immune cells, although experimental evidence for this assumption is still missing.

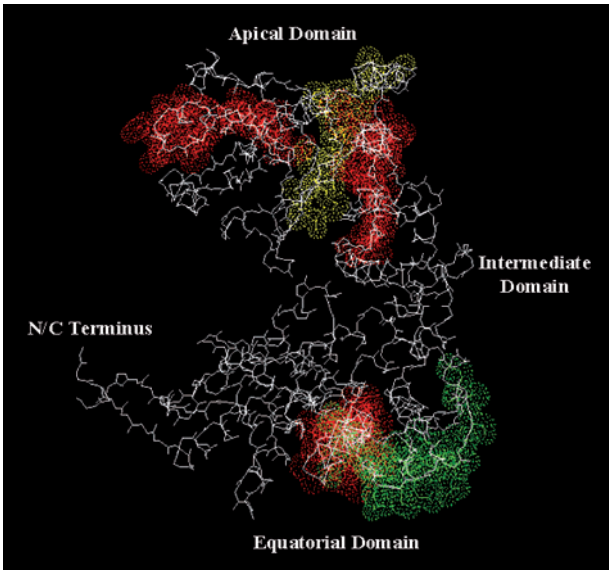

Figure 7. Human Hsp60 monomer. Localization of Hsp60 epitopes involved in receptor binding to permanent (green) and primary (red) macrophages, and in specific LPS binding (yellow) in a 3D model of the human Hsp60 monomer.

Conclusions

Increasing evidence shows that Hsp60 is a potent regulator of innate immune cell activity, thereby mediating strong immunoregulatory effects with potential impact on the development of various inflammatory and autoimmune diseases. To improve our understanding of the immunoregulatory potential of Hsp60, it is essential to characterize the processes involved in the initial interactions of the stress protein with cells of the innate immune system. Our current knowledge of these events indicates that the initial contact of Hsp60 with innate immune cells represents a multifaceted process with important consequences for the activation of innate immune responses with a potential impact on the development of adaptive immune responses. Accumulating data support our model where Hsp60 is thought to be released by damaged or stressed cells (Fig. 8). Specific, but so far unknown receptor structures for Hsp60 binding have been demonstrated on innate immune cells. The contribution of other cell surface structures (e.g. TLRs) to the Hsp60-mediated activation of innate immune cells has been shown. Different epitope regions of the Hsp60 molecule have been found to be involved in Hsp60-binding to receptor structures on innate immune cells in a cell-type-specific manner. Moreover, it could be demonstrated that Hsp60 mediates its immunoregulatory effects by specifically bound LPS. The interaction of Hsp60 with innate immune cells has been found to result in the release of pro-inflammatory mediators such as TNF-α, IL-1β,

IL-6 and NO. These mediators may in turn affect target cells, thereby contributing to the initiation and progression of inflammatory processes such as beta cell destruction in type 1 diabetes or vascular damage in arteriosclerosis. Because of its immunoregulatory properties, Hsp60 has been proposed to act as an intercellular danger signal, performing essential biological tasks, i.e. function as a sensor for microbial structures such as LPS, thereby improving the recognition of such structures by innate immune cells. Numerous investigations have identified danger signal qualities for other HSPs, and therefore it is conceivable that HSPs interact in a highly complex network in the regulation of inflammatory responses.

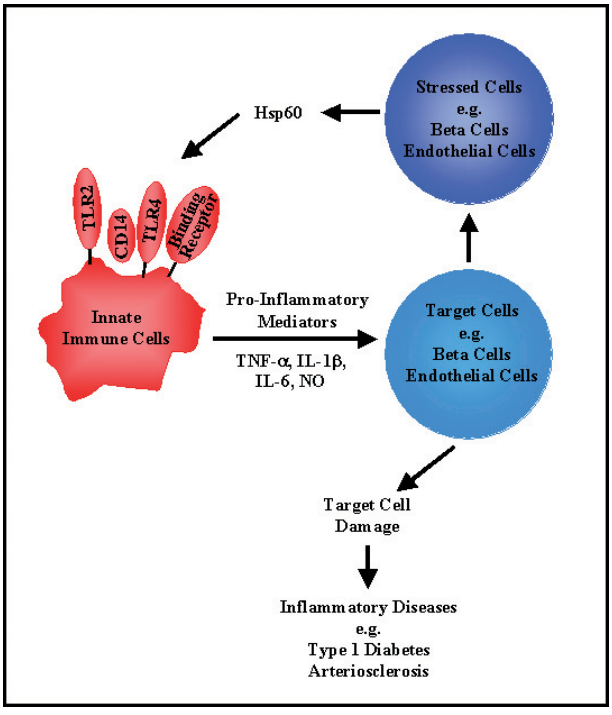

Figure 8. Immunoregulatory effects of Hsp60 on innate immune cells. During stressful conditions such as inflammation, Hsp60 is supposed to be released from stressed cells, e.g. beta cells or endothelial cells. Hsp60 has been shown to stimulate the formation and release of pro-inflammatory mediators from innate immune cells such as macrophages, dendritic cells and endothelial cells. These mediators then may affect target cells, e.g. beta cells or endothelial cells, leading to cell damage, thereby contributing to the initiation and/or progression of inflammatory disorders like type 1 diabetes or arteriosclerosis.

Further research efforts will be necessary to improve our understanding of the immunoregulatory capacity of Hsp60 and its potential cross-talk with other HSPs. These approaches could help to identify specific events involved in the interaction of Hsp60 with innate immune cells as potential targets for interventions to modulate the inflammatory activity of those cells.

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