Review

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The HSP60 immune system network

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Heat shock proteins (HSPs) were initially discovered as participants in the cellular response to stress. It is now clear, however, that self and microbial HSPs also play an important role in the control of the immune response. Here, we focus on HSP60 and its interactions with both the innate and adaptive immune system in mammals. We also consider that circulating HSP60 and the quantities and specificities of serum antibodies to HSP60 provide a biomarker to monitor the immune status of the individual. Thus, the dual role of HSP60 as an immune modulator and a biomarker, provides an opportunity to modulate immunity for therapeutic purposes, and to monitor the immune response in health and disease.

HSP60 immune networks

Nodes and links in complex networks

Living systems, like other complex systems, can be characterized by their networks of interactions [1]. The immune system is a telling example of a network: the interactions that occur between subsets of innate and adaptive immune cells are necessary to initiate a functional and specific immune response. A standard approach for mapping these interactions is to formulate the architecture of interaction networks using a terminology of nodes and links. The nodes are the entities that interact, and the links are the interactions between the entities. This network analysis of living systems – that can be applied on many levels – is at the heart of systems biology. Network thinking enhances our understanding of living systems by combining web architecture (i.e. who is connected to whom) with web dynamics (i.e. the effects of changes in concentration over time).

The chaperone protein, heat shock protein (HSP)60, is a node in intracellular molecular networks, and a linking molecule in intercellular immune networks. Inside all cells – both prokaryotic and eukaryotic – HSP60 functions as a highly connected chaperone with links to most cellular proteins [2]. Although HSP60 is a major node in the intracellular chaperone network, evidence is lacking for the immune-specific function of HSP60 inside the cell. The HSP60 sister chaperones, HSP70 and HSP90, in contrast, participate in the cell biology of antigen processing and presentation [3,4], and in T cell polarization, through their interactions with transcription factors [5,6]. HSP90, for example, controls the activity of the ligand-activated transcription factor aryl hydrocarbon receptor [7], which has been shown recently to play an important role in the differentiation of FoxP3+ Treg cells [8,9], Th17 cells [8–10], and Tr1 cells [11,12]. In the extracellular environment, HSP60 alone or in combination with self or microbial proteins, acts as a link between immune cells, and can coordinate the activity of the immune system. This ability to behave as a link between components of the immune system is shared by other chaperones, such as HSP70 and HSP90 [13–15].

The immune response to HSP in general, and the role of other self-molecules in immune regulation has been discussed elsewhere [16,17]. This review focuses on the ability of extracellular HSP60 to link immune cells to infectious agents and to provide communication between immune cells and other cells of the body. The capacity of HSP60 to act as a self-antigen, a foreign antigen, a carrier of other functional molecules, and a ligand for innate toll-like receptor (TLR) signaling is considered.

Intercellular immune functions of HSP60

The presence of soluble HSP60 in the blood coincides with various inflammatory conditions (reviewed in [13,14]). It has been suggested that HSP60 is secreted from cells in detergent-soluble lipid vesicles [18] or exosomes [19,20]. The release of intact or fragmented HSP60 from damaged or dead cells is also a possibility [21]. Although the exact mechanism(s) by which HSP60 is secreted into the extracellular medium is not understood, it is clear that extracellular HSP60 is a link between body tissues and the immune system. Here we discuss the effects of extracellular HSP60 on various components of the immune system (summarized in Table 1).

Macrophages and dendritic cells (DCs)

HSP60 is an innate signal for macrophages and DCs, cells that are innate nodes in the immune system network. It was postulated that the effects of HSP60 on the innate immune system results from the presence of bacterial contaminants in preparations of recombinant mammalian HSP60 preparations [22]. It is now clear, however, that HSP60 on its own can activate innate immune receptors [23]. In macrophages and DCs, TLR4 signaling is activated in response to at least four sources of HSP60: (i) bacterial HSP60 [24], (ii) bacterial or self-HSP60 molecules that bear LPS or other bacterial ligands bound to them [25,26], (iii) self-HSP60 molecules produced by infected, transformed, damaged or stressed cells [13,14,26] and (iv) peptides of HSP60 [27]. Monocytes undergo activation and maturation in response to HSP60, resulting in the production of pro-inflammatory molecules and factors such as TNFα, IL-12, IL-15, IL-6, IL-1β and NO (reviewed in [13–15]).

At least one peptide fragment of mammalian HSP60 appears to participate in the activation of innate immune

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Table 1. Effects of HSP60 on monocytes, T cells and B cells.

Cell type	Effects	References
Macrophages and DC	Production of TNFα, NO, IFNα and IL-6	[25,99–103]
	DC maturation	[75,104]
Effector T cells	Regulation of T cell adhesion and migration	[73,74]
	Promotion of Th2 polarization	[75,76]
Regulatory T cells	Increase in suppressive activity of FOXP3+ Treg	[77]
	Increase in TGFβ1 and IL-10	[77]
	Activation of anti-ergotypic Treg	[71]
B cells	Production of IgG3	[41]
	Production of IL-10 and IL-6	[41]
	Increased antigen presentation	[41]
	Inhibition of apoptosis	[42]

cells: a 17 amino acid peptide from the 458-474 sequence of human HSP60 (p458) can activate mouse macrophages via TLR4, provided that the HSP60 peptide is conjugated to another molecule to create a polyvalent ligand [27].

B cells

HSP60, both foreign and self, is an antigen for B cells. HSP60 was first noted to be a common and dominant bacterial antigen; immune responses to bacterial infection or vaccination are marked by the production of relatively large amounts of antibodies to bacterial HSP60 [28]. Subsequently, autoantibodies to self-HSP60 were found to characterize a number of different autoimmune and inflammatory diseases, including type 1 diabetes [29–32], rheumatoid arthritis [33], multiple sclerosis [34], lupus [35], atherosclerosis [36], Behcet's disease [37], and inflammatory bowel disease [38]. It seems that tissue damage, which results in upregulated HSP60 expression and HSP60 release from dying cells, activates specific B cells to produce anti-HSP60 autoantibodies. We [39] and others [40] have shown that simultaneous activation of TLR and B cell receptor (BCR) signaling by antigens that are themselves TLR ligands can trigger the induction of antibodies in a T cell-independent manner. HSP60 is a TLR4 ligand that activates B cells [41,42]; thus it is possible that the simultaneous activation of TLR4- and BCR-dependent signaling in HSP60-specific B cells facilitates the induction of HSP60 antibodies following the release of HSP60 by damaged tissues. The ability to mount HSP60-specific antibody responses is wired into the immune network, based on the role of HSP60 as a link for nodes in both the innate and the adaptive immune response.

Strikingly, autoantibodies to HSP60 are also prevalent in healthy subjects; infants are born with a significant amount of HSP60-binding autoantibodies of the IgM isotype in their cord blood [43,44]. Healthy human adults manifest both IgM and IgG autoantibodies to HSP60 [31,43,44]. Using antigen arrays, however, clear differences were found in the fine specificity of anti-HSP60 antibodies that were detected in the sera of multiple sclerosis patients versus healthy controls [34]. Thus, during the course of autoimmune disease, the inflammatory environment provides additional signals that influence the processing and/or immune recognition of HSP60. This might be achieved through a modified architecture of the cytokine network [45] in the context of autoimmunity. The interaction of cytokine signaling cascades is known to have dramatic effects on the immune response: T cell activation in the presence of TGFβ1 results in the differentiation of FoxP3+ Treg cells [46], whereas T cell activation in the presence of TGFβ1 and IL-6 [47,48] or IL-21 [49] promotes the differentiation of pro-inflammatory Th17 cells.

Self-HSP60 is a ligand for innate TLR4 on B cells

HSP60, by activating TLR4 and MyD88 signaling, can induce naïve mouse B cells (B2), to proliferate, to secrete IL-10 and IL-6, and to upregulate the expression of MHCII and other activation molecules [41]. B cells that are activated by HSP60 present antigens to allogeneic T cells to enhance T cell secretion of both IL-10 and IFNγ [41]. HSP60 induces production of polyclonal IgG3 antibodies [41], and is able to serve as a second signal to activate specific IgG3 antibodies to LPS. Thus, extracellular HSP60 impacts B cell function in terms of cytokine expression, antigen presentation and antibody secretion. These innate, polyclonal effects of HSP60 on B cells represent a self-ligand response and cannot be attributed easily to bacterial contaminants; B cells respond innately to mammalian HSP60, but not to bacterial HSP60 derived from *Escherichia coli* or from *Mycobacterium tuberculosis* [41].

HSP60 induces the resistance of B cells to both spontaneous and antigen-induced apoptosis [42]. Unlike the effects of HSP60 on B cell activation [41], the effects of HSP60 on B cell survival are not mediated via TLR4, but do depend on innate MyD88 signaling [42]. These effects of HSP60 are associated with enhanced survival of B cells *in vivo* [42]. Thus, mammalian HSP60 influences many aspects of B cell physiology.

T cells

T cells recognize epitopes of self-HSP60 as specific antigens both in health and in autoimmune disease. The effect of HSP60 on T cells is varied and unexpected. T cell responses to bacterial infections include T cells that are reactive to bacterial HSP60 [50], particularly TCRγδ+ T cells [51,52], and foreign HSP60 is a dominant T cell antigen just as it is a dominant B cell antigen. Healthy humans manifest significant cytolytic T cell reactivities to peptide epitopes of mycobacterial HSP65 that are identical to peptide sequences of human HSP60 [53]. Indeed, the cord blood of healthy newborns contains a high frequency of T cells responsive to these HSP60 self-peptides [54]. Thus, effector T cell autoimmunity to HSP60 is natural and present from birth. Healthy mice also manifest T cells reactive to various peptides of self-HSP60 [55].

At about the same time that T cell immunity to self-HSP60 was discovered in healthy individuals, it was reported that pre-diabetic NOD mice manifest T cells reactive to a peptide epitope of human/mouse HSP60 [30,56], and that clones of these T cells mediate transient insulitis and hyperglycemia in otherwise healthy mice [30,56]. It was later discovered that patients with new-onset type 1 diabetes (T1D) also manifest peripheral blood T cells with specific reactivity to the same self-HSP60 peptide epitope [29,57].

In summary, T cell reactivity to defined peptide epitopes of self-HSP60 is demonstrable both in autoimmune disease and in health. Indeed, T cell responses to self-HSP60 are associated with a good prognosis in spontaneous juvenile chronic arthritis in humans [58,59] and with resistance to experimental autoimmune arthritis in rats [60–64]. Thus, the cytokine profile of HSP60-reactive T cells might determine whether HSP60 autoreactivity is noxious or beneficial: anti-HSP60 T cells that produce pro-inflammatory cytokines could be detrimental in autoimmune disease and beneficial in protection against pathogens. In contrast, anti-HSP60 T cells that produce suppressor cytokines can be beneficial in autoimmune disease and detrimental in infections [63–66].

Anti-ergotypic regulatory T cells recognize HSP60 peptides presented by activated effector T cells

Anti-ergotypic T cells are a type of regulatory T cells that recognize, via their T cell receptor (TCR), peptide epitopes presented by the MHC molecules of activated effector T cells [67–69]. Only activated T cells present ergotopes (activation markers) to anti-ergotypic regulators; resting T cells do not simulate anti-ergotypic T cells [67–69]. Recently we discovered that HSP60 is an ergotope; peptides of HSP60, in addition to other ergotypic molecules [68–70], are presented by the MHC molecules of activated but not of resting T cells [71]. Anti-ergotypic T cells downregulate the proliferation and secretion of pro-inflammatory cytokines by effector T cells *in vitro* [71], and suppress the development of experimental autoimmune diseases [67,70,71]. Although anti-ergotypic T cells have been reported in multiple sclerosis patients following T cell vaccination [72], we do not yet know whether human ergotopes include HSP60.

HSP60 regulates the activity of effector T cells via innate TLR2 signaling

T cells purified from human peripheral blood as CD3+ and CD45RO+ or CD45RA+ cells were found to respond to human HSP60 or to its p277 peptide via TLR2 signaling, which triggered protein kinase C and Pyk-2 activation [73,74], and led to downregulation of chemokine receptors and activation of cell adhesion [73,74]. Subsequent studies have shown that HSP60 induces upregulation of SOCS3 [75], downregulation of T-bet, NFκB, and NFAT, and upregulation of GATA-3 in human T cells, leading to a shift towards Th2 cytokines [75,76]. HSP60 administered intraperitoneally inhibited immune inflammation in a mouse model of hepatitis induced by concanavalin A and mediated by Th1 T cells [76]. The HSP60 peptide, p277, induced similar anti-inflammatory effects in mouse type 1 diabetes via TLR2 signaling [74].

HSP60 is a co-stimulator of CD4+CD25+ T regulatory cells (Tregs) via innate TLR2 signaling

The description of the innate effects of HSP60 and its p277 peptide on human T cells were followed by the discovery that these ligands, via TLR2 signaling, enhance significantly the anti-inflammatory effects of Tregs [77]. Incubation of purified Tregs with HSP60 augmented their ability to suppress CD4+CD25- effector T cell proliferation and pro-inflammatory cytokine secretion in response to mitogenic anti-CD3. These effects were associated with specific changes in signaling events in both the Tregs (altered AKT, Pyk2, p38 and ERK signaling) and the T effector cells (altered ERK, T-bet and NFκB signaling). Note that T cell TLR2 activation requires approximately one thousand times less HSP60 than is required for the TLR4 response in monocytes and B cells. Thus low concentrations of free HSP60 primarily activate Tregs [77], whereas higher concentrations of HSP60 trigger a pro-inflammatory response in monocytes.

Concluding remarks

Is HSP60 pro-inflammatory or anti-inflammatory; a danger signal or a resolving signal?

Figure 1 summarizes our current knowledge of the HSP60 network: HSP60 is both an antigen, via the TCR and the BCR, and an innate link to adaptive immunity via activation of TLR4 and TLR2. HSP60 is both a pro-inflammatory signal, via monocytes, B cells and effector T cells, and an anti-inflammatory signal, via B cells, Tregs, and anti-ergotypic T cells. The integrated effects of HSP60 on the immune response depend on the concentration, the particular HSP60 epitope (bacterial or self), and the cohort of cells present at the site where HSP60 acts. One might expect that a low 'constitutive' concentration of HSP60, for which chaperone housekeeping activities are a possible source, contributes to maintain immune equilibrium by promoting Treg activity and downregulating effector T cell activity. By contrast, high concentrations of HSP60 liberated at sites of tissue damage, neoplasia or infection in the presence of macrophages and dendritic cells might enhance the pro-inflammatory responses of monocytes and effector T cells; B cells will be stimulated to proliferate, to resist apoptosis and to produce IgG3 antibodies. The B cells will also contribute to the resolution of inflammation by secreting increased amounts of IL-10. As the acute injury and/or cellular stress decrease, resolution sets in as the local concentration of HSP60 falls, Tregs become the prominent responders to HSP60 and anti-ergotypic T cells begin to downregulate activated effector T cells.

Moreover, HSP60 seems to be closely linked to other HSP networks. For example, administration of DNA vaccines encoding HSP70 or HSP90 downregulates experimental arthritis, and does so by activating Treg responses to peptides of HSP60 [64]. Thus, it would be an oversimplification to label HSP60 a pro-inflammatory danger signal or an anti-inflammatory signal only. HSP60 is a key link in complex networks in which the immune system communicates with itself and with the body; thus, HSP60 mediates immune physiology.

What might the immune system gain from multifaceted recognition of HSP60?

One might reason that there is an evolutionary advantage to having an immune system that recognizes and responds to HSP60 in different ways from birth [44]. Because upregulation of HSP60 is a dependable sign of cell stress or hyperactivity, HSP60 might allow the body to sense disruptions in tissue homeostasis [78,79]. Indeed, increased HSP60 levels in the circulation have been linked to several

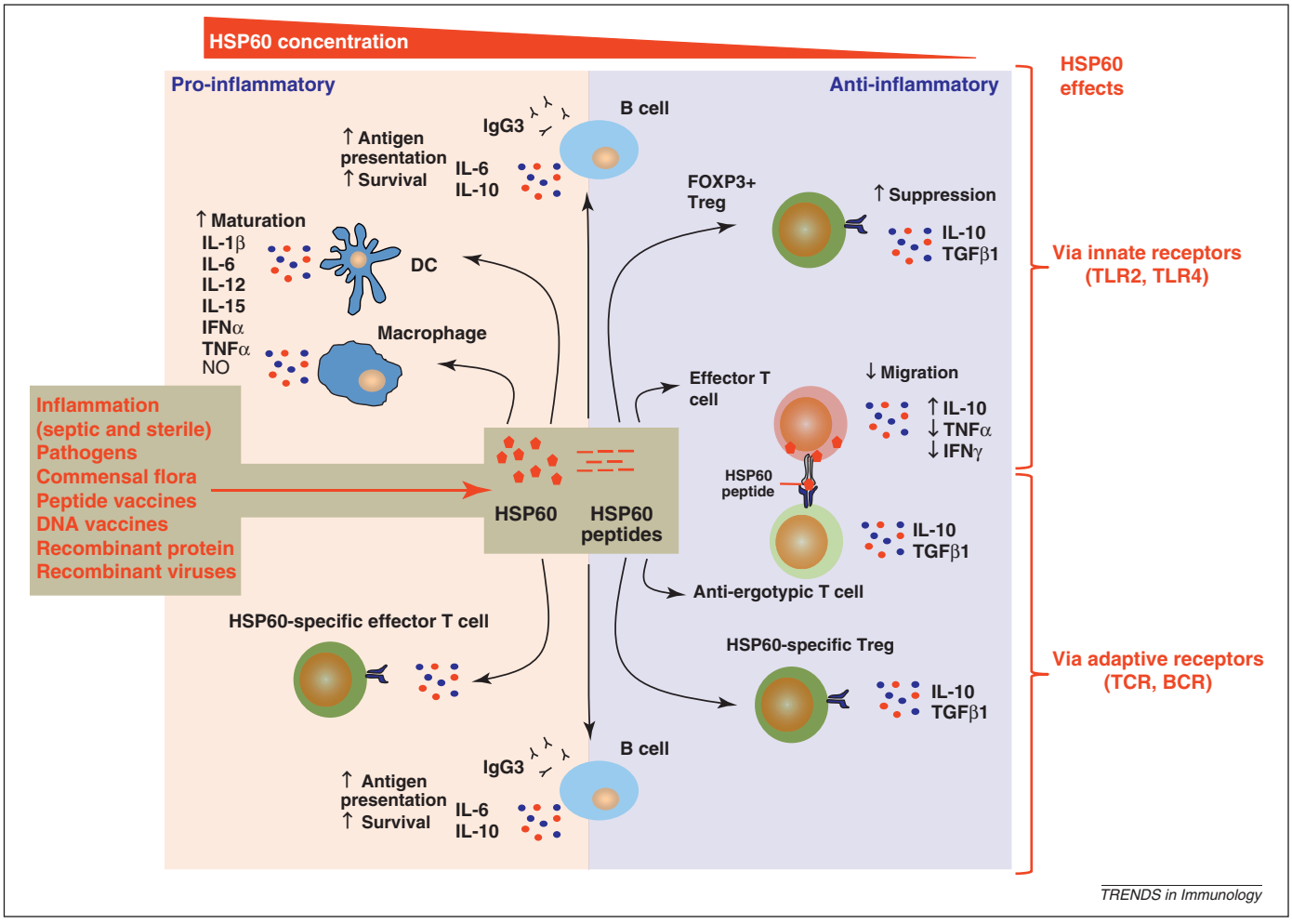

Figure 1. The HSP60 immune network. The immune effects of HSP60 are mediated both by innate TLR signaling (upper segment) and adaptive T cell and B cell antigen receptors (lower segment). The local concentration of HSP60 determines whether the pro-inflammatory (left half) or the anti-inflammatory (right half) functions of HSP60 prevail. The activation of TLR signaling (mainly via TLR4) in DCs and macrophages by high concentrations of HS60 promotes inflammation by diverse mechanisms, such as maturation of DCs, increased antigen presentation and the secretion of proinflammatory cytokines. Conversely, low concentrations favor the arrest of inflammation via TLR2 signaling in effector and CD4+CD25+ (FOXP3+) regulatory T cells. The recognition of HSP60 peptides by specific T cells via their TCRs can both promote inflammation (via effector T cells) and arrest inflammation (via anti-ergotypic T cells). HSP60-specific regulatory T cells function naturally during the resolution of inflammation; they can be induced therapeutically by administering HSP60 or HSP60 peptides, DNA vaccines or recombinant viruses. T cell arrest of inflammation is mediated by the secretion of suppressive cytokines and by anti-ergotypic regulatory mechanisms. B cells are positioned both at the innate (upper) and adaptive (lower) segments, and at the inflammatory border, because they can mediate both pro-inflammatory effects (via antibodies and antigen presentation) and anti-inflammatory effects (via IL-10 production).

inflammatory conditions [80,81]. Moreover, this recognition would automatically induce an immune reaction. It thus makes good sense for HSP60, an intracellular regulator of cell state, to function at the cell surface and in the extracellular space both as an immune biomarker and an inducer of immune regulation.

How might HSP60 be used as a biomarker and therapeutic agent?

The complexities of the HSP60 network are only just being worked out. Nevertheless, we can already benefit from the emerging information. If HSP60 is used as a biomarker by the immune system, we can use it also. An antigen microarray chip has been developed to extract biomarker profiles from autoantibody repertoires to various body components including HSP molecules [82]. HSP60 is an informative antigen: antibodies to epitopes of HSP60 can help discriminate between different clinical types of multiple sclerosis [34], characterize the state of a tumor in an experimental mouse system [55], and mark chronic rejection of lung allografts [83]. Thus, although the functions of HSP60 autoantibodies in health or during inflammatory disease are largely unknown, they might provide a biomarker to monitor the immune status of the individual [78,84].

HSP60 is a natural immune regulator; so, we might use it therapeutically also, particularly to arrest pathogenic inflammation perpetrated by inadequately regulated effector T cells. The administration of HSP60 is now being explored in various ways. Clinical trials of the administration of HSP60 peptides to downregulate uncontrolled inflammation have been undertaken in rheumatoid arthritis and in type 1 diabetes. The HSP60 peptide termed DiaPep277 was successful in preserving beta cell function in Phase II trials [85–88], and is now being tested in advanced Phase III trials in new-onset type 1 diabetes.

The administration of HSP60 has shown promise in various animal models of experimental autoimmune diseases: we have administered whole HSP60, specific HSP60 peptides, and HSP60 naked DNA to inhibit arthritis [63,66] and type 1 diabetes [30,56,65,89–93] in animal

models, and others have applied HSP60 peptides to inhibit experimental arthritis [60–64].

The role of HSP60 peptide p458 as a potential activator of TLR4 signaling in macrophages and DCs [27] has been exploited in the development of anti-microbial vaccines. Defined multi-component vaccines conjugated to peptide p458 were found to induce IgG antibodies to otherwise poorly immunogenic capsular polysaccharide antigens of salmonella [94], pneumococci [27,95], and meningococci [96]; these vaccines were found to be effective even without added adjuvants. The HSP60 peptide was also able to enhance the immunogenicity of peptides from viruses such as cytomegalovirus [97] and West Nile virus [98]. Thus, HSP60 functions as the body's natural adjuvant [26], probably as a result of its ability to activate the innate and the adaptive immune response. A natural adjuvant such as HSP60 has survived millions of years of immune system evolution, and so is likely to be effective, and yet safer than an artificial adjuvant devised by humans.

The natural roles of HSP60 in immune regulation recommend that we continue to explore its potential both in the context of cancer and autoimmune disorders. Its dual role as a biomarker and an immune modulator offer a physiologic avenue to monitor the inflammatory response in health and disease, and to amend the architecture of immune networks for therapeutic purposes.

References

1 Barabasi, A.L. (2003) *Linked. How Everything is Connected to Everything Else and What it Means for Business, Science and Everyday Life*, Perseus Publishing
2 Borges, J.C. and Ramos, C.H. (2005) Protein folding assisted by chaperones. *Protein Pept. Lett.* 12, 257–261
3 Bendz, H. *et al.* (2007) Human heat shock protein 70 enhances tumor antigen presentation through complex formation and intracellular antigen delivery without innate immune signaling. *J. Biol. Chem.* 31688–31702
4 Kunisawa, J. and Shastri, N. (2006) Hsp90alpha chaperones large C-terminally extended proteolytic intermediates in the MHC class I antigen processing pathway. *Immunity* 523–534
5 Bohen, S.P. *et al.* (1995) Hold 'em and fold 'em: chaperones and signal transduction. *Science* 268, 1303–1304
6 Pratt, W.B. (1997) The role of the hsp90-based chaperone system in signal transduction by nuclear receptors and receptors signaling via MAP kinase. *Annu. Rev. Pharmacol. Toxicol.* 37, 297–326
7 Henry, E.C. and Gasiewicz, T.A. (1993) Transformation of the aryl hydrocarbon receptor to a DNA-binding form is accompanied by release of the 90 kDa heat-shock protein and increased affinity for 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Biochem. J.* 294 (Pt 1), 95–101
8 Kimura, A. *et al.* (2008) Aryl hydrocarbon receptor regulates Stat1 activation and participates in the development of Th17 cells. *Proc. Natl. Acad. Sci. U. S. A.* 105, 9721–9726
9 Quintana, F.J. *et al.* (2008) Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. *Nature* 23, 23
10 Veldhoen, M. *et al.* (2008) The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature* 453, 106–109
11 Apetoh, L. *et al.* (2010) The aryl hydrocarbon receptor (AhR) interacts with c-Maf to promote the differentiation of IL-27-induced regulatory type 1 (TR1) cells. *Nat. Immunol.* In press
12 Gandhi, R. *et al.* Aryl hydrocarbon receptor activation induces human Tr1-like and Foxp3+ Treg cells. *Nat. Immunol.* In press
13 Henderson, B. and Pockley, A. (2010) Molecular chaperones and protein-folding catalysts as intercellular signaling regulators in immunity and inflammation. In *J. Leukoc. Biol.* jlb.1209779v1209771
14 Pockley, A.G. *et al.* (2008) The dual immunoregulatory roles of stress proteins. *Trends Biochem. Sci.* 71–79
15 Srivastava, P. (2002) Roles of heat-shock proteins in innate and adaptive immunity. *Nat. Rev. Immunol.* 185
16 Moudgil, K.D. and Durai, M. (2008) Regulation of autoimmune arthritis by self-heat-shock proteins. *Trends Immunol.* 29, 412–418
17 Schwartz, M. and Ziv, Y. (2008) Immunity to self and self-maintenance: a unified theory of brain pathologies. *Trends Immunol.* 29, 211–219
18 Gastpar, R. *et al.* (2005) Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells. *Cancer Res.* 65, 5238–5247
19 Bausero, M.A. *et al.* (2005) Alternative mechanism by which IFN-gamma enhances tumor recognition: active release of heat shock protein 72. *J. Immunol.* 175, 2900–2912
20 Lancaster, G.I. and Febbraio, M.A. (2005) Exosome-dependent trafficking of HSP70: a novel secretory pathway for cellular stress proteins. *J. Biol. Chem.* 280, 23349–23355
21 Basu, S. *et al.* (2000) Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. *Int. Immunol.* 12, 1539–1546
22 Tsan, M.F. and Gao, B. (2009) Heat shock proteins and immune system. *J. Leukoc. Biol.* 85, 905–910
23 Henderson, B. *et al.* (2010) Caught with their PAMPs down? The extracellular signalling actions of molecular chaperones are not due to microbial contaminants. *Cell Stress Chaperones* 123–141
24 Bulut, Y. *et al.* (2002) Chlamydial heat shock protein 60 activates macrophages and endothelial cells through Toll-like receptor 4 and MD2 in a MyD88-dependent pathway. *J. Immunol.* 168, 1435–1440
25 Osterloh, A. *et al.* (2007) Synergistic and differential modulation of immune responses by Hsp60 and lipopolysaccharide. *J. Biol. Chem.* 4669–4680
26 Quintana, F.J. and Cohen, I.R. (2005) Heat shock proteins as endogenous adjuvants in sterile and septic inflammation. *J. Immunol.* 175, 2777–2782
27 Cohen, N. *et al.* (2008) Pneumococcal capsular polysaccharide is immunogenic when present on the surface of macrophages and dendritic cells: TLR4 signaling induced by a conjugate vaccine or by lipopolysaccharide is conducive. *J. Immunol.* 180, 2409–2418
28 Kaufmann, S.H. (1989) Immunity to bacteria. *Curr. Opin. Immunol.* 2, 353–359
29 Abulafia-Lapid, R. *et al.* (2003) T cells and autoantibodies to human HSP70 in type 1 diabetes in children. *J. Autoimmun.* 20, 313–321
30 Elias, D. *et al.* (1990) Induction and therapy of autoimmune diabetes in the non-obese diabetic (NOD/Lt) mouse by a 65-kDa heat shock protein. *Proc. Natl. Acad. Sci. U. S. A.* 87, 1576–1580
31 Quintana, F.J. *et al.* (2003) Cluster analysis of human autoantibody reactivities in health and in type 1 diabetes mellitus: a bio-informatic approach to immune complexity. *J. Autoimmun.* 21, 65–75
32 Quintana, F.J. *et al.* (2004) Functional immunomics: microarray analysis of IgG autoantibody repertoires predicts the future response of mice to induced diabetes. *Proc. Natl. Acad. Sci. U. S. A.* 101 (Suppl. 2), 14615–14621
33 Danieli, M.G. *et al.* (1992) Juvenile rheumatoid arthritis patients manifest immune reactivity to the mycobacterial 65-kDa heat shock protein, to its 180-188 peptide, and to a partially homologous peptide of the proteoglycan link protein. *Clin. Immunol. Immunopathol.* 64, 121–128
34 Quintana, F.J. *et al.* (2008) Antigen microarrays identify unique serum autoantibody signatures in clinical and pathologic subtypes of multiple sclerosis. *Proc. Natl. Acad. Sci. U. S. A.* 105, 18889–18894
35 Dieude, M. *et al.* (2004) Induction of endothelial cell apoptosis by heat-shock protein 60-reactive antibodies from anti-endothelial cell autoantibody-positive systemic lupus erythematosus patients. *Arthritis Rheum.* 50, 3221–3231
36 Xu, Q. *et al.* (1993) Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis. *Lancet* 341, 255–259
37 Tanaka, T. *et al.* (1999) Behcet's disease and antibody titers to various heat-shock protein 60 s. *Ocul. Immunol. Inflamm.* 7, 69–74
38 Stevens, T.R. *et al.* (1992) Circulating antibodies to heat-shock protein 60 in Crohn's disease and ulcerative colitis. *Clin. Exp. Immunol.* 90, 271–274
39 Quintana, F.J. *et al.* (2008) Induction of IgG3 to LPS via Toll-like receptor 4 co-stimulation. *PLoS One* 3, e3509

40 Herlands, R.A. *et al.* (2008) T cell-independent and toll-like receptor-dependent antigen-driven activation of autoreactive B cells. *Immunity* 29, 249–260
41 Cohen-Sfady, M. *et al.* (2005) Heat shock protein 60 activates B cells via the TLR4-MyD88 pathway. *J. Immunol.* 175, 3594–3602
42 Cohen-Sfady, M. *et al.* (2009) Heat shock protein 60, via MyD88 innate signaling, protects B cells from apoptosis, spontaneous and induced. *J. Immunol.* 183, 890–896
43 Madi, A. *et al.* (2009) Organization of the autoantibody repertoire in healthy newborns and adults revealed by system level informatics of antigen microarray data. *Proc. Natl. Acad. Sci. U. S. A.* 106, 14484–14489
44 Merbl, Y. *et al.* (2007) Newborn humans manifest autoantibodies to defined self molecules detected by antigen microarray informatics. *J. Clin. Invest.* 117, 712–718
45 Frankenstein, Z. *et al.* (2006) The immune-body cytokine network defines a social architecture of cell interactions. *Biol. Direct* 1, 32
46 Chen, W. *et al.* (2003) Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J. Exp. Med.* 198, 1875–1886
47 Bettelli, E. *et al.* (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 441, 235–238
48 Veldhoen, M. *et al.* (2006) TGFbeta in the context of an inflammatory cytokine milieu supports *de novo* differentiation of IL-17-producing T cells. *Immunity* 24, 179–189
49 Korn, T. *et al.* (2007) IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature* 448, 484–487
50 Kaufmann, S.H. (1990) Heat shock proteins and the immune response. *Immunol. Today* 11, 129–136
51 Bonneville, M. *et al.* Gammadelta T cell effector functions: a blend of innate programming and acquired plasticity. *Nat. Rev. Immunol.* 10, 467–478
52 O'Brien, R.L. *et al.* (1992) Heat shock protein Hsp60-reactive gamma delta cells: a large, diversified T-lymphocyte subset with highly focused specificity. *Proc. Natl. Acad. Sci. U. S. A.* 89, 4348–4352
53 Munk, M.E. *et al.* (1989) T lymphocytes from healthy individuals with specificity to self-epitopes shared by the mycobacterial and human 65-kilodalton heat shock protein. *J. Immunol.* 143, 2844–2849
54 Ramage, J.M. *et al.* (1999) T cell responses to heat-shock protein 60: differential responses by CD4+ T cell subsets according to their expression of CD45 isotypes. *J. Immunol.* 162, 704–710
55 Merbl, Y. *et al.* (2009) A systems immunology approach to the host-tumor interaction: large-scale patterns of natural autoantibodies distinguish healthy and tumor-bearing mice. *PLoS One* 4, e6053
56 Elias, D. *et al.* (1991) Vaccination against autoimmune mouse diabetes with a T-cell epitope of the human 65-kDa heat shock protein. *Proc. Natl. Acad. Sci. U. S. A.* 88, 3088–3091
57 Abulafia-Lapid, R. *et al.* (1999) T cell proliferative responses of type 1 diabetes patients and healthy individuals to human hsp60 and its peptides. *J. Autoimmun.* 12, 121–129
58 de Graeff-Meeder, E.R. *et al.* (1995) Juvenile chronic arthritis: T cell reactivity to human HSP60 in patients with a favorable course of arthritis. *J. Clin. Invest.* 95, 934–940
59 de Kleer, I.M. *et al.* (2003) The spontaneous remission of juvenile idiopathic arthritis is characterized by CD30+ T cells directed to human heat-shock protein 60 capable of producing the regulatory cytokine interleukin-10. *Arthritis Rheum.* 48, 2001–2010
60 Anderton, S.M. *et al.* (1995) Activation of T cells recognizing self 60-kD heat shock protein can protect against experimental arthritis. *J. Exp. Med.* 181, 943–952
61 Moudgil, K.D. *et al.* (1997) Diversification of T cell responses to carboxy-terminal determinants within the 65-kD heat-shock protein is involved in regulation of autoimmune arthritis. *J. Exp. Med.* 185, 1307–1316
62 Prakken, B.J. *et al.* (2002) Inhibition of adjuvant-induced arthritis by interleukin-10-driven regulatory cells induced via nasal administration of a peptide analog of an arthritis-related heat-shock protein 60 T cell epitope. *Arthritis Rheum.* 46, 1937–1946
63 Quintana, F.J. *et al.* (2003) DNA fragments of the human 60-kDa heat shock protein (HSP60) vaccinate against adjuvant arthritis: identification of a regulatory HSP60 peptide. *J. Immunol.* 171, 3533–3541
64 Quintana, F.J. *et al.* (2004) Inhibition of adjuvant-induced arthritis by DNA vaccination with the 70-kd or the 90-kd human heat-shock protein: immune cross-regulation with the 60-kd heat-shock protein. *Arthritis Rheum.* 50, 3712–3720
65 Quintana, F.J. *et al.* (2002) DNA vaccination with heat shock protein 60 inhibits cyclophosphamide-accelerated diabetes. *J. Immunol.* 169, 6030–6035
66 Quintana, F.J. *et al.* (2002) Inhibition of adjuvant arthritis by a DNA vaccine encoding human heat shock protein 60. *J. Immunol.* 169, 3422–3428
67 Lohse, A.W. *et al.* (1989) Control of experimental autoimmune encephalomyelitis by T cells responding to activated T cells. *Science* 244, 820–822
68 Mimran, A. and Cohen, I.R. (2005) Regulatory T cells in autoimmune diseases: anti-ergotypic T cells. *Int. Rev. Immunol.* 24, 159–179
69 Quintana, F.J. and Cohen, I.R. (2006) Anti-ergotypic immunoregulation. *Scand. J. Immunol.* 64, 205–210
70 Mimran, A. *et al.* (2004) DNA vaccination with CD25 protects rats from adjuvant arthritis and induces an antiergotypic response. *J. Clin. Invest.* 113, 924–932
71 Quintana, F.J. *et al.* (2008) HSP60 as a target of anti-ergotypic regulatory T cells. *PLoS One* 3, e4026
72 Hong, J. *et al.* (2006) CD4+ regulatory T cell responses induced by T cell vaccination in patients with multiple sclerosis. *Proc. Natl. Acad. Sci. U. S. A.* 103, 5024–5029
73 Zanin-Zhorov, A. *et al.* (2003) T cells respond to heat shock protein 60 via TLR2: activation of adhesion and inhibition of chemokine receptors. *FASEB J.* 17, 1567–1569
74 Nussbaum, G. *et al.* (2006) Peptide p277 of HSP60 signals T cells: inhibition of inflammatory chemotaxis. *Int. Immunol.* 18, 1413–1419
75 Zanin-Zhorov, A. *et al.* (2005) Heat shock protein 60 activates cytokine-associated negative regulator suppressor of cytokine signaling 3 in T cells: effects on signaling, chemotaxis, and inflammation. *J. Immunol.* 175, 276–285
76 Zanin-Zhorov, A. *et al.* (2005) Heat shock protein 60 inhibits Th1-mediated hepatitis model via innate regulation of Th1/Th2 transcription factors and cytokines. *J. Immunol.* 174, 3227–3236
77 Zanin-Zhorov, A. *et al.* (2006) Heat shock protein 60 enhances CD4+ CD25+ regulatory T cell function via innate TLR2 signaling. *J. Clin. Invest.* 116, 2022–2032
78 Cohen, I.R. (2007) Real and artificial immune systems: computing the state of the body. *Nat. Rev. Immunol.* 7, 569–574
79 Cohen, I.R. (2009) Biomarker vaccines. *Hum. Vaccin* 5, 637–639
80 Lang, A. *et al.* (2005) Heat shock protein 60 is released in immune-mediated glomerulonephritis and aggravates disease: *in vivo* evidence for an immunologic danger signal. *J. Am. Soc. Nephrol.* 383
81 Pockley, A.G. *et al.* (2000) Circulating heat shock protein 60 is associated with early cardiovascular disease. *Hypertension* 36, 303–307
82 Quintana, F.J. *et al.* (2006) Antigen-chip technology for accessing global information about the state of the body. *Lupus* 15, 428–430
83 Hagedorn, P.H. *et al.* Chronic rejection of a lung transplant is characterized by a profile of specific autoantibodies. *Immunology* 130, 427–435
84 Quintana, F.J. and Cohen, I.R. (2004) The natural autoantibody repertoire and autoimmune disease. *Biomed. Pharmacother.* 58, 276–281
85 Huurman, V.A. *et al.* (2008) Immunological efficacy of heat shock protein 60 peptide DiaPep277 therapy in clinical type I diabetes. *Clin. Exp. Immunol.* 152, 488–497
86 Raz, I. *et al.* (2007) Treatment of new-onset type 1 diabetes with peptide DiaPep277 is safe and associated with preserved beta-cell function: extension of a randomized, double-blind, phase II trial. *Diabetes Metab. Res. Rev.* 23, 292–298
87 Huurman, V.A. *et al.* (2007) Therapy with the hsp60 peptide DiaPep277 in C-peptide positive type 1 diabetes patients. *Diabetes Metab. Res. Rev.* 23, 269–275
88 Raz, I. *et al.* (2001) Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. *Lancet* 358, 1749–1753
89 Bockova, J. *et al.* (1997) Treatment of NOD diabetes with a novel peptide of the hsp60 molecule induces Th2-type antibodies. *J. Autoimmun.* 10, 323–329

90 Elias, D. and Cohen, I.R. (1994) Peptide therapy for diabetes in NOD mice. *Lancet* 343, 704–706
91 Elias, D. and Cohen, I.R. (1995) Treatment of autoimmune diabetes and insulitis in NOD mice with heat shock protein 60 peptide p277. *Diabetes* 44, 1132–1138
92 Elias, D. and Cohen, I.R. (1996) The hsp60 peptide p277 arrests the autoimmune diabetes induced by the toxin streptozotocin. *Diabetes* 45, 1168–1172
93 Elias, D. *et al.* (1997) Hsp60 peptide therapy of NOD mouse diabetes induces a Th2 cytokine burst and downregulates autoimmunity to various beta-cell antigens. *Diabetes* 46, 758–764
94 Konen-Waisman, S. *et al.* (1995) Self and foreign 60-kilodalton heat shock protein T cell epitope peptides serve as immunogenic carriers for a T cell-independent sugar antigen. *J. Immunol.* 154, 5977–5985
95 Amir-Kroll, H. *et al.* (2003) Proteins and their derived peptides as carriers in a conjugate vaccine for Streptococcus pneumoniae: self-heat shock protein 60 and tetanus toxoid. *J. Immunol.* 170, 6165–6171
96 Amir-Kroll, H. *et al.* (2006) A conjugate vaccine composed of a heat shock protein 60 T-cell epitope peptide (p458) and *Neisseria meningitidis* type B capsular polysaccharide. *Vaccine* 24 (42–43), 6555–6563
97 Rouvio, O. *et al.* (2005) Self HSP60 peptide serves as an immunogenic carrier for a CTL epitope against persistence of murine cytomegalovirus in the salivary gland. *Vaccine* 23, 3508–3518
98 Gershoni-Yahalom, O. *et al.* (2010) Chimeric vaccine composed of viral peptide and mammalian heat-shock protein 60 peptide protects against West Nile virus challenge. *Immunology* 130, 527–535
99 Vabulas, R.M. *et al.* (2001) Endocytosed HSP60 s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. *J. Biol. Chem.* 31332–31339
100 Osterloh, A. *et al.* (2008) Hsp60-mediated T cell stimulation is independent of TLR4 and IL-12. *Int. Immunol.* 433
101 Lehnardt, S. *et al.* (2008) A vicious cycle involving release of heat shock protein 60 from injured cells and activation of toll-like receptor 4 mediates neurodegeneration in the CNS. *J. Neurosci.* 2320
102 Kol, A. *et al.* (1999) Chlamydial and human heat shock protein 60 s activate human vascular endothelium, smooth muscle cells, and macrophages. *J. Clin. Invest.* 571–577
103 Chen, W. *et al.* (1999) Human 60-kDa heat-shock protein: a danger signal to the innate immune system. *J. Immunol.* 3212
104 Flohe, S. *et al.* (2003) Human heat shock protein 60 induces maturation of dendritic cells versus a Th1-promoting phenotype. *J. Immunol.* 2340