Unfolding the relationship between secreted molecular chaperones and macrophage activation states

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Abstract Over the last 20 years, it has emerged that many molecular chaperones and protein-folding catalysts are secreted from cells and function, somewhat in the manner of cytokines, as pleiotropic signals for a variety of cells, with much attention being focused on the macrophage. During the last decade, it has become clear that macrophages respond to bacterial, protozoal, parasitic and host signals to generate phenotypically distinct states of activation. These activation states have been termed ‘classical’ and ‘alternative’ and represent not a simple bifurcation in response to external signals but a range of cellular phenotypes. From an examination of the literature, the hypothesis is propounded that mammalian molecular chaperones are able to induce a wide variety of alternative macrophage activation states, and this may be a system for relating cellular or tissue stress to appropriate macrophage responses to restore homeostatic equilibrium.

Keywords Molecular chaperones · Macrophages · Macrophage activation · Inflammation

Introduction

Metchnikoff’s studies of macrophages in transparent organisms (a century before the Zebra fish became popular)

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would not have prepared him for the enormous importance of the mononuclear phagocyte lineage in current biomedicine. These cells, in various cellular guises, populate all of the tissues of the body, playing a multitude of roles including: anti-microbial defences, regulation of immune responses, promotion of wound healing and control of bone remodelling. New functions for these cells are continuously appearing. For example, the microglia of the brain are increasingly being regarded as having key neuroprotective functions, rather than just conventional pro-inflammatory actions (Glezer et al. 2007). Less benignly, tumour-associated macrophages (TAMs) are induced in tumours and appear to enhance tumour growth and invasiveness (Sica et al. 2008). The role of macrophages in lipid metabolism and in the pathologies associated with such metabolism is now emerging (reviewed by Gordon 2007). Of course, macrophages are capable of producing a cocktail of noxious agents, including free radicals, proteases and cytokines—all of which can be damaging to the phagocyte itself. The influence of oxidative stress on macrophages has recently been called “the enemy within” (Splettstoesser et al. 2002) and highlights the hypothesis that this cell population is the most stressed in the body. This may explain the findings of the last decade, which are increasingly supportive of the hypothesis that controlling factors in macrophage activation include the molecular chaperones and protein-folding catalysts. Both are vital proteins involved in protecting cells against internal and external stress. In the context of this review, these classes of protein-folding proteins will be jointly referred to as molecular chaperones. The reader should be aware that new guidelines for the nomenclature of the human heat shock protein (HSP) families have recently been suggested (Kampinga et al. 2008). These include HSPH (HSP110), HSPC (HSP90), HSPA (HSP70), DNAJ (HSP40) and

HSPB (small HSP) as well as for the human chaperonin families HSPD/E (HSP60/HSP10) and CCT (TRiC). These have not been used in this review to avoid confusion.

The cell stress response and molecular chaperones

The finding that cells respond to stress was made in the early 1960s, but the molecular mechanisms underpinning this vital cellular response were only identified in the 1980s with the discovery of intracellular molecular chaperones and protein-folding catalysts (Gething et al. 1997). The former are proteins which non-enzymatically aid in the folding or refolding of proteins. The latter are enzymes such as thioredoxin (Trx) or peptidyl-prolyl isomerases (PPIs) whose enzymic activity is required for the correct folding of client proteins. Unfolded proteins in the different compartments of the cell give rise to coordinated responses known as the unfolded protein response (UPR). The UPR in the endoplasmic reticulum (ER) has been most closely scrutinized, and it is now recognised that one molecular chaperone in particular, BiP, acts as the key controller of the response, which is designed to inhibit the production of protein in the ER or, if this is not possible, to switch on the mechanism leading to apoptosis (Rutkowski and Kaufman 2004).

Secreted and extracellular molecular chaperones

For adherents of Thomas Kuhn, science is supposedly littered with examples of paradigm revolutions. Possibly one of the quickest revolutions was of the paradigm that molecular chaperones are intracellular proteins. Indeed, some 30 years ago, 1 year before the discovery of the first molecular chaperone, a circulating immunosuppressive activity, termed early pregnancy factor (EPF), was discovered (Morton et al. 1977). This turned out to be the molecular chaperone, chaperonin (Cpn)/heat shock protein (Hsp)10, a mitochondrial protein (Cavanagh and Morton 1994). Co-incidentally, both Hsp10 and the co-chaperone Hsp60 are now implicated as cell surface proteins essential for the capacitation of sperm, revealing a 'prior role' for these proteins in pregnancy (Walsh et al. 2008). However, it was Hightower, 1 year after the description of the mechanism of action of Hsp60, who first revealed that a molecular chaperone, in this case Hsp70, was released by cells (Hightower and Guidon 1989). Since then, a growing number of cell stress proteins have been identified in body fluids or in the medium maintaining cultured cells (Table 1). One of the major criticisms directed at these findings is the lack of mechanisms to explain the release of these variegated proteins. This criticism is generally made by

Table 1 Extracellular cell stress proteins

Hsp10 (chaperonin 10)
Thioredoxin (Trx)
Thioredoxin 80 (Trx80)
Thioredoxin reductase
Glutaredoxin
Peroxiredoxin
Cyclophilin A
Cyclophilin B
Hsp27
Protein disulfide isomerase (PDI)
Calnexin
Calreticulin
Hsp54
Hsp60 (chaperonin 60)
Hsp70
Hsp72
BiP
Hsp90
Gp96
Clusterin
Haptoglobin

Only some of these proteins have thus far been shown to be actively secreted from cells

individuals who fail to realise that both prokaryotes and eukaryotes have diverse and non-overlapping secretion pathways and that new secretion pathways (e.g. bacterial type VI secretion—Cascales 2008) are being discovered from time to time. It has recently been discovered that Hsp70, Hsp90 and calnexin are released from cells in small vesicles called exosomes (Fevrier and Raposo 2004). In addition, Hsp70 has been reported to be released from cells by a mechanism similar to that used by the key early response cytokine, interleukin-1 (Mambula et al. 2007). Intriguingly, another class of non-folding stress protein (metallothionein) also appears in the extracellular pool under cell stress (Lynes et al. 2006), suggesting that the release of such proteins may be a general cellular response to stress.

Macrophage activation in response to infection

Macrophages populate the tissues of the body and are a short-term resident population of the blood in the form of the circulating monocyte. The wide range of morphologies assumed by the tissue macrophages, from the simple rounded pleural macrophage to the microglia with its enormously long ramified processes, to the multinucleate osteoclast population of bone, reveals the phenotypic plasticity of this cell lineage. Elie Metchnikoff was the first to report that animals resistant to specific bacterial

infections had macrophages capable of killing these bacteria. However, it was the work of George Mackaness on facultatively and obligate intracellular parasites of macrophages who introduced the concept of macrophage activation and defined its role in immune responses to infections (Mackaness 1962; Adams and Hamilton 1984). The mechanism by which T cells were able to activate macrophages remained a mystery until Carl Nathan showed that a soluble factor must be responsible and then identified this factor as the cytokine, interferon (IFN)- γ (Nathan et al. 1983). Another key factor in macrophage activation is the external coat material of Gram-negative bacteria, which includes the amphiphilic molecule known as lipopolysaccharide (LPS; Henderson et al. 1998). It has been known for decades that LPS is pyrogenic and has pro-inflammatory properties. It was not until the 1950s–1970s that the role of LPS in macrophage activation and in the induction of pro-inflammatory cytokines from monocytes/macrophages was elucidated (Bomford and Henderson 1989). The activation state of macrophages induced by exposure to IFN- γ and/or LPS is now termed classical macrophage activation (Gordon 1999), although Gordon has recently suggested the term innate activation for the activation state induced by microbial products such as LPS (Gordon 2007).

Over the last two decades, it has come to be recognised that macrophages can respond to extracellular signals in distinct ways from that induced by ‘classical activation’ signals. These distinct activation phenotypes have been termed alternative activation pathways (Stein et al. 1992; Goerdts et al. 1999). Monocytes exposed to LPS and/or IFN- γ exhibit properties consistent with antibacterial activity and antigen presentation including synthesis of IL-12, nitric oxide (NO) and oxygen-derived free radicals (these are termed classically activated macrophages—caMØ or M1 cells). IL-4 and IL-13 were once thought to be natural inhibitors of macrophage activation. However, this phenomenon has proved to be more complex, as it has been shown that cells exposed to either of these cytokines will increase the production of scavenger receptors, IL-10 and arginase among other things. These cells are now described as alternatively activated macrophage—aaMØ (or M2; Gordon 2003, 2007).

The M2 phenotype has been further subdivided into M2a (after exposure to IL-4 or IL-13), M2b (exposure to immune complexes in combination with IL-1 β or LPS) and M2c (exposure to IL-10, TGF β or glucocorticoids; Martinez et al. 2008; Table 2). Gordon has recently suggested that the M2c state should be termed ‘innate and acquired inactivation’ (Gordon 2007). M1 macrophages exhibit potent microbicidal properties and promote strong IL-12-mediated Th1 responses, whilst M2 support Th2-associated effector functions. The M2 polarised macrophages play a role in resolution of inflammation through high endocytic clearance capacities and trophic factor

Table 2 Factors involved in classical and alternative macrophage activation

Macrophage activation state	Stimuli	Proposed function
Innate activation (Gordon 2007)	Microbial pro-inflammatory components such as LPS, peptidoglycan etc	Induction of innate immune mechanisms with anti-bacterial consequences
Classical activation (M1) (Martinez et al. 2008)	IFN- γ	Induction of cellular mechanisms aiding the killing of intracellular bacteria
Alternative activation (M2) (Martinez et al. 2008)	IL-4 or IL-13	Parasitic and allergic immunity
Alternative activation (M2b) (Martinez et al. 2008)	immune complexes in combination with IL-1 β or LPS	Parasite and allergic immunity
Alternative activation (M2c) Martinez et al. 2008) or Innate and acquired inactivation (Gordon 2007)	IL-10, TGF β or glucocorticoids	Anti-inflammatory activity

synthesis, accompanied by reduced pro-inflammatory cytokine secretion (Mantovani et al. 2004; Martinez et al. 2008). TAMs also display an alternative-like activation phenotype and function to promote tumour growth (Porta et al. 2007). Of interest is the recent report that regulatory T cells are capable of inducing alternative macrophage activation. These regulatory T cells are now believed to control immune responses and may do so, in part, through controlling macrophage activation (Tiemessen et al. 2007).

It is now recognised that the aaMØ is a natural phenomenon in parasitic infections (Gordon 2003; Mantovani et al. 2004), raising the question of how parasites induce this state. It has recently been reported that the helminth, *Fasciola hepatica*, induces the aaMØ state by secreting the protein-folding catalyst, thioredoxin peroxidase or peroxiredoxin (Donnelly et al. 2005). This finding has brought the abilities of various cell stress proteins to modulate mononuclear phagocyte behaviour into focus and suggests a new paradigm for these proteins. Again, this may represent a common theme for stress proteins generally, such as metallothionein, mentioned earlier, which is also able to modulate the function of macrophages (Youn et al.

1995). The established factors responsible for classical or alternative macrophage activation are delineated in Table 2.

Molecular chaperones as modulators of macrophage activation

The authors propose the hypothesis that secreted molecular chaperones exert another layer of control over mononuclear phagocyte homeostasis. While an unusual proposal, it must be seen against the background of the emerging evidence for the diverse moonlighting actions of cell stress proteins. To highlight this, the prototypic molecular chaperone, Hsp60, has recently been identified as: (1) a human cell surface protein involved in sperm capacitation (Asquith et al. 2004); (2) an insect neurotoxin (Yoshida et al. 2001) and (3) a human protein acting as a tethering ligand for the alcohol acetaldehyde dehydrogenase of *Listeria monocytogenes*, thus allowing this bacterium to adhere to human cells and colonise humans (Kim et al. 2006). This is a remarkably diverse set of functions for any protein and suggests many more surprises are in store for researchers working with chaperonin 60 and presumably with all other stress response proteins. With regard to cell stress proteins and macrophage activation, we can, at present, roughly divide these proteins into those that appear to induce classical activation and those that induce some form of alternative activation. It should be borne in mind that apart from the peroxiredoxin of *F. hepatica*, there have been no real attempts made to assess cell stress proteins for their ability to induce alternative macrophage activation. Indeed, many of the activation states to be described in response to cell stress proteins seem to suggest ‘alternatives’ to the currently defined alternative activation states. Of course, this may simply reflect our lack of knowledge of what constitutes an alternatively activated macrophage (Table 3).

Hsp60 The second molecular chaperone identified, Hsp60, is recognised as being a highly conserved protein (Hill et al. 2004); yet, it is clear that, depending on the source, this protein can exhibit a bewildering diversity of biological actions. There are now many publications reporting that Hsp60 stimulates human monocytes to produce pro-inflammatory cytokines, suggesting induction of a cMØ state. The Hsp60 proteins from *Homo sapiens* and *Chlamydia trachomatis* stimulate human monocytes via a CD14/TLR2/TLR4-dependent mechanism (toll-like receptor, TLR). In contrast, the same proteins from *Escherichia coli* or *Helicobacter pylori* are not dependent on CD14/TLR2/4 for monocyte activation (reviewed by Maguire et al. 2002). Indeed, the *H. pylori* Hsp60 activates macrophages

in a TLR2/4 and myeloid differentiation factor 88 (Myd-88)-independent manner (Gobert et al. 2004). Furthermore, one of the three Hsp60 proteins of *Rhizobium leguminosarum* fails to activate human monocytes (Lewthwaite et al. 2002a).

One of the most unexpected findings from the study of the human Hsp60 protein is that it is present in the blood of >50% of the normal adult population (Shamaei-Tousi et al. 2007a). Most proteins in the circulation exist within very sharp concentration ranges. In contrast, Hsp60 can be found in human plasma at levels ranging from 1 ng/ml to >1 mg/ml. This is a million-fold range, and this range of levels encompasses both the anti- and pro-inflammatory effects of these proteins. The author's group have shown a relationship between circulating Hsp60 and cardiovascular disease, and this may relate to the influence of these proteins on macrophage function (Lewthwaite et al. 2002b; Halcox et al. 2005; Shamaei-Tousi et al. 2007b).

The in vivo breakdown of bone is driven by a specific monocyte population known as the osteoclast. Gram-negative bacterial Hsp60 proteins have been shown to stimulate both osteoclast formation and activation (Kirby et al. 1995; Reddi et al. 1998) as has human Hsp60 (Meghji et al. 2003). However, it was found that the homologous proteins (Hsp60.2) from *Mycobacterium tuberculosis* and *Mycobacterium leprae* had no such activity (Kirby et al. 1995).

The principal author and his colleagues have been involved in an increasingly detailed analysis of the Hsp60 proteins of *M. tuberculosis* (Mtb), which is revealing the unusual nature of these proteins and their interactions with macrophages. The mycobacteria generally have two genes for Hsp60 proteins, and in Mtb, these proteins are referred to as Hsp60.1 and Hsp60.2. The latter is the well-known Hsp65 protein. Indeed, one of the first reports of the signalling actions of molecular chaperones was that the *M. tuberculosis* Hsp60.2 protein activated human monocytes to synthesise pro-inflammatory cytokines (Friedland et al. 1993). Both proteins, when used in vitro at microgram per milliliter concentrations stimulate human monocytes to secrete a range of pro-inflammatory cytokines (Lewthwaite et al. 2001). These findings suggest that the *M. tuberculosis* chaperonins are inducers of the classically activated state. Attempts to inactivate the genes encoding the Hsp60 and Hsp10 proteins in Mtb have revealed that Hsp60.2 and Hsp10 are indispensable proteins. However, the gene encoding the Hsp60.1 protein can be inactivated without any significant effect on the cellular behaviour of *M. tuberculosis*. Indeed, *M. tuberculosis* lacking a function *hsp60.1* gene responds identically to the wild-type organism when exposed to a wide range of antibacterial stressors. This would not be expected if the Hsp60.1 protein

Table 3 Eukaryotic molecular chaperones that modulate macrophage activity

Hsp10 (Johnson et al. 2005)	Inhibits LPS-induced caMØ activation
Thioredoxin (Trx)80 (Pekkari et al. 2005)	Induce novel macrophage activation state
Trx (Billiet et al. 2005)	Inhibits LPS-induced caMØ activation
Cyclophilin A (Sherry et al. 1992)	Induces a caMØ state
Peroxiredoxin (Donnelly et al. 2005)	Induces an aaMØ state
Hsp27 (De et al. 2000)	Induces IL-10 synthesis but not TNF- α presumably an aaMØ state
Hsp60 (Maguire et al. 2002) ^a	Induces caMØ state
Hsp70 (Vabulas et al. 2002)	Induces caMØ state
BiP (Panayi and Corrigan 2008)	Inhibits monocyte activation and similar to Hsp27, presumably and aaMØ state
Gp96 (Vabulas et al. 2002a)	Induces caMØ state
Clusterin (Xie et al. 2005)	Induces what appears to be caMØ
Haptoglobin (Moestrup and Møller 2004)	Induces an aaMØ state

caMØ classically activated macrophage, aaMØ alternatively activated macrophage

^a *Mycobacterium tuberculosis* Hsp60 induces alternative activation state

functioned as a cell stress protein. However, when used to infect mice and guinea pigs, the $\Delta hsp60.1$ mutant, although growing to the same numbers as the wild type, failed to induce a granulomatous response. These findings suggest that the Mtb Hsp60.1 is a direct-acting mycobacterial virulence factor able to induce the formation of granulomas (Hu et al. 2008). Giant cells are a characteristic feature of granulomas, and we wondered if the *M. tuberculosis* Hsp60.1 protein had any influence on their generation. This idea came from our findings of the effects of Hsp60.1 on osteoclast formation (reported below). To test this proposal, we have used an in vitro human blood granuloma formation assay (Puissegur et al. 2004) in which we have added wild type, mutant and complemented *M. tuberculosis* with blood for various times and then enumerated the numbers of multinucleate giant cells. This has revealed that the isogenic mutant lacking the Hsp60.1 protein produces less than 10% of the multinucleate giant cells generated by human blood cultured with either the wild type or complemented *M. tuberculosis* (Henderson, Coates, Altare, unpublished data).

Macrophages can undergo fusion to produce either giant cells or osteoclasts (Helming and Gordon 2008), and this may be thought of as an alternative form of macrophage activation. Macrophage fusion is a complex process (Helming and Gordon 2008) and one of the main cell surface proteins involved in the process, dendritic cell-specific transmembrane protein (DC-STAMP), has recently been shown to be differentially regulated in osteoclasts and in macrophage giant cells (Yagi et al. 2007). The finding that the Mtb Hsp60.2 protein, unlike other Hsp60 proteins, did not promote osteoclast formation and bone remodelling (Kirby et al. 1995) stimulated an examination of the bone destroying actions of the Mtb Hsp60.1 protein. Surprisingly, this recombinant protein did not promote osteoclast formation (Meghji et al. 1997). This raised the question as

to whether the Mtb Hsp60 proteins could inhibit bone resorption. Although the two Mtb Hsp60 proteins share over 60% sequence identity, the Hsp60.2 protein has no influence on the process of osteoclast formation, while the Hsp60.1 protein turns out to be a potent inhibitor of murine osteoclast formation both in vitro and in vivo (Winrow et al. 2008). The process of osteoclast formation is multi-staged and takes up to 7 days. It has been found that Mtb Hsp60.1 can block osteoclast formation in vitro even after the process has been going for 4 days (Winrow et al. 2008). A partial explanation for the inhibitory effect of Mtb Hsp60.1 is that it inhibits the transcription of a key osteoclast transcription factor, nuclear factor of activated T cells (NFATc1). This, it is believed, accounts for inhibition over the first few days of the process of osteoclastogenesis. However, it is thought that the later stages must involve inhibition of other signalling processes such as those related to DC-STAMP (Winrow et al. 2008). These findings clearly reveal that in spite of very high sequence conservation, individual Hsp60 proteins can have very distinct extracellular signalling actions. Surprisingly, administration of *M. tuberculosis* Hsp60.1 to rats with adjuvant arthritis completely blocked the osteoclastic bone destruction that is the characteristic of this disease without inhibiting the joint inflammation. Thus, *M. tuberculosis* Hsp60.1 may have therapeutic potential.

Macrophages are important bacterial killing machines, and this is the function of the classical activation pathway in response to IFN- γ or LPS. Tuberculosis is a disease in which it is well known that the macrophages are inhibited, and rather than an antibacterial Th1 response, tubercular patients appear to exhibit more of a Th2 phenotype (Rook et al. 2005). Testing for tuberculosis sensitivity often uses an extract of whole Mtb called purified protein derivative (PPD). This response is known to be suppressed in individuals infected with Mtb (Wilsher et al. 1999). It has

recently been reported that Mtb Hsp60.1 inhibits the PPD-induced formation of the key cytokine IL-12 p40 subunit. IL-12 is responsible for activating Th1 responses. The mechanism of action of Mtb Hsp60.1 is by inducing synthesis of TLR2 and then binding to this receptor to suppress intracellular signalling, specifically by suppression of nuclear c-rel, which leads to inhibition of IL-12p40 (Khan et al. 2008). This is a novel mechanism for inhibiting the classical activation of macrophages.

One spin-off from these studies is the potential therapeutic efficacy of the mycobacterial Hsp60 proteins. Gelfand and co-workers have reported that the *M. leprae* Hsp60.2 protein is a potent inhibitor of experimental allergic asthma in the mouse (Rha et al. 2002) but that a range of other Hsp60 proteins, including the *M. tuberculosis* Hsp60.2, were inactive. The author's group has also shown that *M. tuberculosis* Hsp60.1 can inhibit the same experimental asthma model in the mouse but, like Gelfand, found that the Mtb Hsp60.2 protein was inactive (Riffo-Vasquez et al. 2004). Since the two mycobacterial Hsp60.2 proteins share 96% sequence identity, this suggests that very small sequence differences can have profound effects on the biological actions of these proteins.

The surprising finding from these studies is the marked range of distinct and non-overlapping biological actions exerted by different Hsp60 proteins. This contradicts the current view that sequence homology relates to functional homology. It is not known if these major differences in extracellular signalling actions of the Hsp60 proteins are specific to this family of proteins or if this is a general feature of cell stress proteins.

Hsp70 family members There are at least 11 members of the Hsp70 gene family if one includes the Hsp110 and Grp170 family members (Daugaard et al. 2007). The reader should be aware that the literature is often somewhat confusing as to the nature of the Hsp70 protein(s) being used in published studies. Initial studies of human Hsp70 (presumably Hsp70-1a) reported that it stimulated human monocytes to produce pro-inflammatory cytokines by a mechanism involving rapid intracellular calcium flux. Of importance, this calcium flux is not seen in cells stimulated with LPS (Asea et al. 2000). The early studies suggested that Hsp70-1a stimulated monocytes by binding to TLR2 and TLR4 and to CD14 (Asea et al. 2000, 2002; Vabulas et al. 2002a). The cellular receptors for LPS include TLR4 and CD14. Questions about the role of LPS contamination in cell stress protein preparations have dogged the literature. Surprisingly, similar questions are rarely addressed in experiments with the many hundreds, if not thousands, of bacterial and mammalian recombinant proteins expressed in *E. coli*. However, most active research groups in this cell stress protein field have taken great care

to exclude the role of LPS contamination, and this is particularly true of those studying the Hsp70 proteins. The formulation of LPS controls in this type of study has been reviewed by Lehner (2005). Thus, the weight of evidence would suggest that human Hsp70 proteins stimulate a classically activated macrophage phenotype.

Lehner has studied the effect of Hsp70 from *M. tuberculosis* on human monocyte cell lines and human peripheral blood monocytes. It produces a classical activation state with the production of TNF- α , IL-12, nitric oxide (NO) and the chemokines CCL3, CCL4 and CCL5. Initial studies revealed that the activation of these cells was not through binding to CD14 or a TLR but was the result of interaction with CD40, a member of the TNF receptor family (Wang et al. 2001, 2002, 2005). In a later work, Lehner's group have found that Mtb Hsp70 also binds to the chemokine receptor CCR5, which is the co-receptor for HIV-1 (Whittall et al. 2006). The synergistic relationship between AIDS and tuberculosis is now seen as a major global health problem (Young et al. 2008). It is therefore important to note that *M. tuberculosis* Hsp70 can dose-dependently inhibit the infection of CD4 T cells by HIV-1, the active site in this protein being the C-terminal epitope 407–426. The Hsp70 apparently works by blocking the CCR5 co-receptor and by inhibition of CC-chemokines (Babaahmady et al. 2007). This suggests a therapeutic potential for this protein and a re-examination of the *M. tuberculosis*/HIV-1 relationship.

Surprisingly, the human Hsp70 protein has been claimed to bind to a number of membrane-bound targets including: CD14, TLR2, TLR4, CD40, LOX-1 and CD91. Calderwood has recently claimed that Hsp70 binds most strongly to scavenger receptor (SR) family and C-type lectins of the NK family (Theriault et al. 2006). Obviously, further studies are needed to clearly identify the range of receptors and the intracellular signalling contributions made by them when bound to Hsp70 proteins.

Thus, the Hsp70-1a and the *M. tuberculosis* Hsp70 proteins bind to human monocytes and induce a classical activation pattern of gene transcription. The endoplasmic reticulum stress protein, BiP is another member of the Hsp70 family, which demonstrates 64% sequence identity to Hsp70-1a. In a search for the autoantigen in rheumatoid arthritis, Panayi and co-workers used proteomics, with human rheumatoid serum, to identify immunoreactive proteins in human articular chondrocytes. One of the strongest interactions turned out to involve BiP. On the assumption that this protein would be a potent inducer of immunity, BiP was tested in animals. Surprisingly, it turned out that the administration of BiP to rodents with experimental arthritis actually inhibited the symptoms of disease (Corrigall et al. 2001). An explanation for this

finding was that the addition of BiP to human monocytes induced the formation of IL-10 rather than the pro-inflammatory cytokine TNF- α (Corrigall et al. 2005). Microarray analysis of human monocytes exposed to BiP for 24 h has revealed a decrease in the transcription of pro-inflammatory cytokines and chemokines and reduced transcription of co-stimulatory proteins such as HLA-DR and CD86 and increased transcription of anti-inflammatory proteins such as TNF receptor II and IL-1 receptor antagonist (Corrigall et al. 2005). These findings strongly support the hypothesis that BiP is an alternative macrophage activator.

Hsp10 (chaperonin 10/EPF) As described already, Hsp10 was the first molecular chaperone identified in human blood. It is found in the blood during the first trimester of pregnancy (Morton et al. 1977) and was rapidly recognised as an immunosuppressive factor (Noonan et al. 1979). Recombinant human Hsp10 inhibits experimental autoimmune encephalomyelitis in Lewis rats (Harness et al. 2003) and graft versus host disease (Johnson et al. 2005). In the latter study, Hsp10 also inhibited the LPS-induced activation of monocyte pro-inflammatory cytokine synthesis, thus showing the capacity to inhibit the induction of the classically activated macrophage state. The potential importance of such macrophage-inhibiting activity is seen in the report that human recombinant Hsp10, given to patients with rheumatoid arthritis, has therapeutic activity (Vanags et al. 2006).

Recent information suggests that human and rodent Hsp10 has a range of biological actions both inside cells and when released from stressed cells (reviewed by Czarnecka et al. 2006). The concentration of Hsp10 has been measured in the plasma of controls and in patients with chronic periodontal disease undergoing treatment. The surprising finding from this study is that levels of Hsp10 were low in the blood of periodontal patients compared to comparable controls. However, after successful therapy, the levels returned to those found in healthy controls. This suggests that Hsp10 is a circulating anti-inflammatory factor, which is consumed during inflammation (perhaps to inhibit macrophage activation) and only returns to the blood once the inflammation has been controlled (Shamaei-Tousi et al. 2007b).

Hsp27 There are a growing number of examples in which this small heat shock protein plays important roles in intracellular signalling (Arrigo 2007). Extracellular Hsp27 has also been shown to influence monocyte/macrophage/dendritic cells maturation and signalling. The first evidence for this was the finding that the addition of exogenous Hsp27 to human monocytes resulted in a high IL-10 to low

TNF- α ratio similar to that described for BiP (De et al. 2000). It has been further shown that Hsp27 inhibits the differentiation of monocytes into macrophages and of monocytes into immature dendritic cells by both IL-10-dependent and IL-10-independent pathways (Laudanski et al. 2007). One emerging concept in immunology is that the immune system is more concerned with signals that cause damage than it is with self/non-self (Matzinger 2002). These danger signals have been proposed to include certain molecular chaperones (Williams and Ireland 2008). This has led to the suggestion that Hsp27 functions as an 'anti-danger' signal (Miller-Graziano et al. 2008).

When Hsp27 over-expressing mice were crossed with apoE negative mice (which spontaneously develop atherosclerosis), female offspring demonstrated a tenfold increase in circulating Hsp27 and a significant inhibition of atherogenesis. Of relevance to this review, the Hsp27 inhibited the binding of acetylated low-density lipoprotein (acLDL) to macrophages by competing with the binding of this protein-lipid complex to the scavenger receptor A. In addition, extracellular HSP27 decreased acLDL-induced release of the pro-inflammatory cytokine IL-1 β and increased the release of the anti-inflammatory cytokine IL-10. It is therefore hypothesised that HSP27 is atheroprotective because of its actions on the macrophage population either or both competing for the uptake of atherogenic lipids or attenuating inflammation. (Rayner et al. 2008).

Thioredoxin superfamily members The thioredoxin superfamily (containing the conserved CXXC motif) consists of oxidoreductases with the ability to catalyse oxidative protein folding via protein-protein interactions and covalent catalysis and therefore to act as chaperones and isomerases of disulfides to generate a native fold (Yamawaki and Berk 2005; Berndt et al. 2008). Thioredoxin (Trx) is the 12-kDa prototypic protein of this superfamily, which was originally discovered in *E. coli* as an electron donor to ribonucleotide reductase, an enzyme essential for DNA synthesis (Holmgren 1985). The human *Trx* gene was originally cloned as the result of the identification of a cytokine released by retrovirally transformed human T lymphocytes and was originally called adult T cell leukaemia-derived factor. This protein is an inducer of one subunit (IL-2R α) of the T cell IL-2 receptor (Tagayi et al. 1989). Human Trx is chemotactic for monocytes, polymorphonuclear leukocytes and T lymphocytes, with a comparable potency to known chemokines but with a different mechanism of action (Bertini et al. 1999; Nakamura 2001a). A high plasma Trx level in HIV-infected individuals with low CD4 counts is associated with increased mortality, which is thought to be due to the high chemotactic activity in the plasma inhibiting trafficking of

leukocytes into tissues (Nakamura et al. 2001b). Thioredoxin is released by mononuclear phagocytes (Bertini et al. 1999), but only a few studies have been directed at the effects of Trx on macrophages and inflammation. Addition of Trx to human macrophages, which have been stimulated by exposure to LPS, reduced both IL-1 β messenger RNA (mRNA) levels and IL-1 protein synthesis in a dose-dependent manner. This effect was largely mediated through a reduction of NF- κ B activation (Billiet et al. 2005). Administration of Trx to mice with myosin-induced autoimmune myocarditis (Liu et al. 2004) or with experimental ulcerative colitis (Tamaki et al. 2006) inhibits inflammation, strongly supporting the hypothesis that Trx is an alternative macrophage activator (Nakamura 2008).

More is known about a naturally produced truncated form of Trx, which contains 80 or 84 of the N-terminal residues of this protein (termed Trx80). This was initially discovered as an eosinophil cytotoxicity-enhancing factor in the blood of patients with schistosomiasis (Silberstein et al. 1989). Thioredoxin 80 is produced and released by monocytes, presumably as a Trx cleavage product, and is reported to induce a novel state of macrophage activation with increased mannose receptor expression but lower amounts of MHC class II receptors. Activated cells have higher pinocytotic activity, release significant amounts of both pro- and anti-inflammatory cytokines and have a lower allogeneic proliferative response (Pekkari et al. 2005). It is currently unclear how this state relates to the alternative macrophage activation states described by various workers (Gordon 2003; Mantovani et al. 2004; Martinez et al. 2008). Thioredoxin reductase, a catalyst required for the reduction of Trx, is also secreted by macrophages and is found in the blood (Soderberg et al. 2000) and may also be shown to modulate mononuclear phagocyte function in future studies.

Macrophage migration inhibitory factor (MIF) is a well-known cytokine, originally discovered as a lymphokine involved in delayed hypersensitivity and various other macrophage functions, including production of proinflammatory cytokines, glucocorticoid-induced immunomodulation, and natural killer cell inhibitory factor production, regulation of toll-like receptor expression and macrophage adherence and phagocytosis. This protein has now been shown to be a member of the thioredoxin family (Kleemann et al. 1998). It has now been shown that MIF binds to the chemokine receptors CXCR2 and CXCR4 to form a functional complex leading to G(α i)- and integrin-dependent arrest and chemotaxis of monocytes and T cells, rapid integrin activation and calcium influx (Bernhagen et al. 2007).

Peroxiredoxin, initially known as macrophage 23-kDa stress protein, is now recognised to be part of a family of

enzymes (currently, there are six members of this family in humans) that contain a conserved Cys residue that undergoes a cycle of peroxide-dependent oxidation and thiol-dependent reduction during catalysis (Rhee et al. 2005). Using peroxiredoxin II-deficient macrophages, it has recently been established that *intracellular* peroxiredoxin II is an essential negative regulator of macrophage activation induced by LPS. The mechanism of action is the modulation of oxygen-derived free radical formation via nicotinamide adenine dinucleotide phosphate (reduced form) oxidase activity (Yang et al. 2007a). There is also evidence that intracellular peroxiredoxin I is involved in the generation of foam cells. These are modified forms of macrophages involved in atherogenesis and can be considered to be alternative states of macrophage activation (Conway and Kinter 2006). Alternatively, activated macrophages are found in the chronic stages of parasitic infections and are associated with the development of a polarised Th2 response. The liver fluke, *F. hepatica*, induces a polarised Th2 response in BALB/c mice during both the latent and chronic stage of disease. This alternative activation state was induced by both live flukes and also by parasite excretory–secretory (ES) products. The active component of the ES products is a peroxiredoxin, and the addition of recombinant *F. hepatica* peroxiredoxin to the murine monocyte cell line, RAW 264.7, converts these cells to an alternatively activated phenotype characterised by the production of high levels of interleukin-10 (IL-10), prostaglandin (PG)E₂, and correspondingly low levels of IL-12 (Donnelly et al. 2005).

Peptidylprolyl *cis-trans* isomerases These proteins catalyse the *cis-trans* isomerisation of proline residues in proteins and are also termed cyclophilins and immunophilins, as the potent immunosuppressants cyclosporine and FK506 act by inhibiting these proteins. There are four classes of peptidyl prolyl isomerases (PPIs) termed cyclophilins, FK506 binding proteins (FKBPs), parvulins and trigger factors. Humans have 16 cyclophilins, 15 FKBPs and two parvulins (Fanghanel and Fischer 2004). Cyclophilin A was found to be secreted from LPS-stimulated RAW264.7 cells, a murine macrophage cell line. The purified protein was, in turn, found to have pro-inflammatory effects on macrophages including acting as a chemoattractant (Sherry et al. 1992). The signalling receptor for the human cyclophilin A and B is CD147, otherwise known as extracellular matrix metalloproteinase inducer (EMMPRIN) (Yurchenko et al. 2002). Like *M. tuberculosis* Hsp70, cyclophilin A has been reported to inhibit the uptake of HIV-1 by macrophages and T lymphocytes (Sherry 1998). The protozoan parasite, *Toxoplasma gondii*, has been reported to release cyclophilin-18 (C-18), and the recombinant form of this protein is a high affinity signalling ligand for CCR5, inducing the

synthesis of IL-12 by dendritic cells (Aliberti et al. 2003). Thus, this PPI appears to induce a classical activation response, which the authors claim may benefit parasite transmission by stopping the protozoan killing its intermediate hosts. These workers have also reported that *T. gondii* cyclophilin-18 (C-18) inhibits HIV-1 cell fusion and infection with cell-free virus. This same protein also blocked syncytium formation between human T cells and effector cells expressing R5 but not X4 HIV strains (Golding et al. 2003). These two HIV virus strains show markedly different properties in their interaction with human T cells (Cicala et al. 2005).

Gp96 This endoplasmic reticulum molecular chaperone was thought to be essential for cell viability. However, in a screen for B cell mutants lacking responsiveness to LPS, a cell lacking the Gp96 gene was identified. Further analysis of this mutant revealed that the major defect in this cell was in its cell surface expression of TLRs, revealing a hitherto unknown role for Gp96 in inserting the TLR proteins into the plasma membrane (Randow and Seed 2001). In a later study, a macrophage-specific Gp96-deficient mouse was created (Yang et al. 2007b). Macrophages from these mice developed normally and were responsive to a range of cytokines including: IFN- γ , TNF- α and IL-1 β . However, Gp96-deficient macrophages cannot respond to ligands of both cell-surface and intracellular TLRs including TLR2, TLR4, TLR5, TLR7 and TLR9. GP96-deficient mice are resistant to endotoxic shock but were susceptible to infection with the intracellular bacterium, *L. monocytogenes*. These findings suggest that Gp96 plays a major role in the folding and disposition of the TLRs. This study also reveals that macrophages, and not other myeloid cells, are the main source of pro-inflammatory cytokines in response to systemic endotoxin and *L. monocytogenes* infection (Yang et al. 2007b).

In addition to its intracellular role in chaperoning TLRs, it has been reported that extracellular Gp96 can induce classical macrophage activation in murine RAW264.7 macrophages through binding to TLR2 and TLR4 (Vabulas et al. 2002b)

Clusterin and haptoglobin The accepted paradigm is that all molecular chaperones are intracellular proteins, which may be secreted by certain cells under certain circumstances. Clusterin is the first member of this group of proteins, which is a constitutively secreted extracellular chaperone capable of binding to exposed hydrophobic regions on unfolded proteins. Clusterin has been proposed to control levels of unfolded extracellular proteins by promoting receptor-mediated endocytosis and intracellular lysosomal degradation (Wilson and Easterbrook-Smith

2000). Clusterin has been reported to induce rodent microglia to produce both NO and TNF- α , suggesting it acts as a classical macrophage activator (Xie et al. 2005).

Haptoglobin is an acute phase protein whose function is to scavenge hemoglobin after hemolysis. Haptoglobin complexes with soluble hemoglobin and the complex are taken up by the exclusive monocyte/macrophage receptor CD163. This receptor is overexpressed in alternatively activated macrophages with CD163 being reported to directly induce intracellular signaling leading to secretion of anti-inflammatory cytokines. In addition, CD163-mediated uptake of hemoglobin may create an anti-inflammatory response because heme metabolites have potent anti-inflammatory effects (Moestrup and Moller 2004). Evidence exists for the association between haptoglobin levels and inflammatory diseases, and it is proposed that this protein has an anti-inflammatory/immunomodulatory function (Quaye 2008). In recent years, it has been proposed, on the basis of structural and functional analysis, that haptoglobin is, like clusterin, a secreted molecular chaperone (Ettrich et al. 2002; Yerbury et al. 2005). If this evidence is correct, then we can add another molecular chaperone to the list of alternative macrophage activators.

Conclusions

The response of macrophages to bacteria was termed 'macrophage activation' by George Mackaness in 1962, although Metchnikoff had reported similar findings at the turn of the twentieth century (Metchnikoff 1905). Mackaness's work led to the discovery of IFN- γ as a macrophage-activating cytokine (Nathan et al. 1983) and to the concept of classically activated macrophages whose major function is to combat bacterial infection including instances of intracellular bacterial infection (Han and Kauffmann 1982). The concept of alternatives to this mechanism of macrophage activation have only appeared in relatively recent years, even though it has been appreciated that macrophages in different sites in the body have widely different functions. For example, alveolar macrophages have long been recognised for their anti-inflammatory/immunomodulatory actions (Holt 1986). However, there is now much excitement about the mechanisms responsible for inducing multiple forms of activation states in mammalian macrophages and the role these activation states can have in homeostasis, infection and idiopathic disease. Currently, only a small number of factors have been reported to induce classical or alternative macrophage activation (Table 2). A word of caution has to be introduced about the absolute differences between classical and alternative activation, as the few microarray

studies that have been conducted with macrophages responding to protozoan parasites (Rodriguez et al. 2004) or bacteria (Goldmann et al. 2007) tend to show a mixture of phenotypes at the mRNA level.

Since the early 1990s, evidence has begun to accumulate to support the hypothesis that secreted molecular chaperones can interact with monocytes/macrophages and cause various states of activation. Much more analysis of the actions of these proteins on macrophage function is needed before a definitive conclusion can be reached. However, at the time of writing, it would appear that monocytes/macrophages respond to various extracellular recombinant molecular chaperones in ways that either resemble classically and alternatively activated macrophages or that resemble neither of these states. Macrophages are among the key sentinel cells in the body, and it is not difficult to imagine that evolution has moulded their behaviour by developing a response to molecular elements of the cell stress response. This raises several questions: (1) what receptors and signalling mechanisms are involved in macrophage recognition of molecular chaperones; (2) do molecular chaperones exhibit network activity with emergent properties (that is, functions which arise from the interaction of simpler entities, none of which show the fully formed and complex function); and (3) are bacterial and host molecular chaperones complementary or antagonistic (or both) in function?

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References

- Adams DO, Hamilton TA (1984) The cell biology of macrophage activation. *Annu Rev Immunol* 2:283–318 doi:10.1146/annurev. iy.02.040184.001435
- Aliberti J, Valenzuela JG, Carruthers VB et al (2003) Molecular mimicry of a CCR5 binding-domain in the microbial activation of dendritic cells. *Nat Immunol* 4:485–490 doi:10.1038/ni915
- Arrigo AP (2007) The cellular “networking” of mammalian Hsp27 and its functions in the control of protein folding, redox state and apoptosis. *Adv Exp Med Biol* 594:14–26 doi:10.1007/978-0-387-39975-1_2
- Asea A, Kraeft S-K, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW, Koo GC, Calderwood SK (2000) Hsp70 stimulates cytokine production through a CD14-dependent pathway, demonstrating its dual role as a chaperone and cytokine. *Nature Med* 6:435–442 doi:10.1038/74697
- Asea A, Rehli M, Kabingu E, Boch JA, Bare O, Auron PE, Stevenson MA, Calderwood SK (2002) Novel signal transduction pathway utilized by extracellular HSP70: role of toll-like receptor (TLR) 2 and TLR4. *J Biol Chem* 277:15028–15034 doi:10.1074/jbc. M200497200
- Asquith KL, Baleato RM, McLaughlin EA, Nixon B, Aitken RJ (2004) Tyrosine phosphorylation activates surface chaperones facilitating sperm-zona recognition. *J Cell Sci* 117:3645–3657 doi:10.1242/jcs.01214
- Babaahmady K, Oehlmann W, Singh M, Lehner T (2007) Inhibition of human immunodeficiency virus type 1 infection of human CD4+ T cells by microbial HSP70 and the peptide epitope 407–426. *J Virol* 81:3354–3360 doi:10.1128/JVI.02320-06
- Berndt C, Lillig CH, Holmgren A (2008) Thioredoxins and glutaredoxins as facilitators of protein folding. *Biochim Biophys Acta* 1783:641–650 doi:10.1016/j.bbamcr.2008.02.003
- Bernhagen J, Krohn R, Lue H et al (2007) MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Nat Med* 13:587–596 doi:10.1038/nm1567
- Bertini R, Howard OMZ, Dong H-F et al (1999) Thioredoxin, a redox enzyme released in infection and inflammation, is a unique chemoattractant for neutrophils, monocytes and T cells. *J Exp Med* 189:1783–1789 doi:10.1084/jem.189.11.1783
- Billiet L, Furman C, Larigauderie G, Copin C, Brand K, Fruchart JC, Rouis M (2005) Extracellular human thioredoxin-1 inhibits lipopolysaccharide-induced interleukin-1 β expression in human monocyte-derived macrophages. *J Biol Chem* 280:40310–40318 doi:10.1074/jbc.M503644200
- Bomford R, Henderson B (1989) Interleukin-1, inflammation and disease. North Holland, Elsevier
- Cascales E (2008) The type VI secretion toolkit. *EMBO Rep* 9:735–741 doi:10.1038/embor.2008.131
- Cavanagh AC, Morton H (1994) The purification of early-pregnancy factor to homogeneity from human platelets and identification as chaperonin 10. *Eur J Biochem* 222:551–560 doi:10.1111/j.1432-1033.1994.tb18897.x
- Cicala C, Arthos J, Martinelli E, Censoplano N, Cruz CC, Chung E et al (2005) R5 and X4 HIV envelopes induce distinct gene expression profiles in primary peripheral blood mononuclear cells. *Proc Natl Acad Sci USA* 103:3746–3751 doi:10.1073/pnas.0511237103
- Conway JP, Kinter M (2006) Dual role of peroxiredoxin I in macrophage-derived foam cells. *J Biol Chem* 281:27991–28001 doi:10.1074/jbc.M605026200
- Corrigall VM, Bodman-Smith MD, Fife MS et al (2001) The human endoplasmic reticulum molecular chaperone BiP is an autoantigen for rheumatoid arthritis and prevents the induction of experimental arthritis. *J Immunol* 166:1492–1498
- Corrigall VM, Bodman-Smith M, Panayi GS (2005) BiP stimulation induces and anti-inflammatory gene activation profile in monocytes. *Rheumatology* 44(Suppl):48
- Czarnecka AM, Campanella C, Zummo G, Capello F (2006) Heat shock protein 10 and signal transduction: a ‘capsula eburnea’ of carcinogenesis? *Cell Stress Chaperones* 11:287–294 doi:10.1379/CSC-200.1
- Daugaard M, Rohde M, Jaattela M (2007) The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. *FEBS Letts* 581:3702–3710 doi:10.1016/j.febslet.2007.05.039
- De AK, Kodys KM, Yeh BS, Miller-Graziano C (2000) Exaggerated human monocyte IL-10 concomitant to minimal TNF α induction by heat-shock protein 27 (Hsp27) suggests Hsp27 is primarily an antiinflammatory stimulus. *J Immunol* 165:3951–3958
- Donnelly S, O’Neill SM, Sekiya M, Mulcahy G, Dalton JP (2005) Thioredoxin peroxidase secreted by *Fasciola hepatica* induces

- the alternative activation of macrophages. *Infect Immun* 73:166–173 doi:[10.1128/IAI.73.1.166-173.2005](https://doi.org/10.1128/IAI.73.1.166-173.2005)
- Ettrich R, Brandt W Jr, Kopecký V, Baumruk V, Hofbauerová K, Pavlíček Z (2002) Study of chaperone-like activity of human haptoglobin: conformational changes under heat shock conditions and localization of interaction sites. *Biol Chem* 383:1667–1676 doi:[10.1515/BC.2002.187](https://doi.org/10.1515/BC.2002.187)
- Fanghänel J, Fischer G (2004) Insights into the catalytic mechanism of peptidyl prolyl cis/trans isomerases. *Front Biosci* 9:3453–3478 doi:[10.2741/1494](https://doi.org/10.2741/1494)
- Fevrier B, Raposo G (2004) Exosomes: endosomal-derived vesicles shipping extracellular messages. *Curr Opin Cell Biol* 16:415–421 doi:[10.1016/j.ceb.2004.06.003](https://doi.org/10.1016/j.ceb.2004.06.003)
- Friedland JS, Shattock R, Remick DG, Griffin GE (1993) Mycobacterial 65-kDa heat shock protein induces release of proinflammatory cytokines from human monocytic cells. *Clin Exp Immunol* 91:58–62
- Gething M-J (1997) Guidebook to molecular chaperones and protein-folding catalysts. University Press, Oxford
- Glezer I, Simard AR, Rivest S (2007) Neuroprotective role of the innate immune system by microglia. *Neuroscience* 147:867–883 doi:[10.1016/j.neuroscience.2007.02.055](https://doi.org/10.1016/j.neuroscience.2007.02.055)
- Gobert AP, Bambou JC, Werts C, Balloy V, Chignard M, Moran AP, Ferrero RL (2004) *Helicobacter pylori* heat shock protein 60 mediates interleukin-6 production by macrophages via a toll-like receptor (TLR)-2-, TLR-4-, and myeloid differentiation factor 88-independent mechanism. *J Biol Chem* 279:245–250 doi:[10.1074/jbc.M307858200](https://doi.org/10.1074/jbc.M307858200)
- Goerdts S, Politz O, Schledzewski K et al (1999) Alternative versus classical activation of macrophages. *Pathobiology* 67:222–226 doi:[10.1159/000028096](https://doi.org/10.1159/000028096)
- Golding H, Aliberti J, King LR, Manischewitz J, Andersen J, Valenzuela J, Landau NR, Sher A (2003) Inhibition of HIV-1 infection by a CCR5-binding cyclophilin from *Toxoplasma gondii*. *Blood* 102:3280–3286 doi:[10.1182/blood-2003-04-1096](https://doi.org/10.1182/blood-2003-04-1096)
- Goldmann O, von Köckritz-Blickwede M, Hölte C, Chhatwal GS, Geffers R, Medina E (2007) Transcriptome analysis of murine macrophages in response to infection with *Streptococcus pyogenes* reveals an unusual activation program. *Infect Immun* 75:4148–4157 doi:[10.1128/IAI.00181-07](https://doi.org/10.1128/IAI.00181-07)
- Gordon S (1999) Macrophages and the immune system. In: Paul WE (ed) *Fundamental immunology*. 4th edn. Lippincott-Raven, Philadelphia, pp 533–545
- Gordon S (2003) Alternative activation of macrophages. *Nat Rev Immunol* 3:23–35 doi:[10.1038/nri978](https://doi.org/10.1038/nri978)
- Gordon S (2007) Macrophage heterogeneity and tissue lipids. *J Clin Invest* 117:89–93 doi:[10.1172/JCI30992](https://doi.org/10.1172/JCI30992)
- Hahn H, Kaufmann SH (1982) T lymphocyte-macrophage interactions in cellular antibacterial immunity. *Immunobiology* 161:361–368
- Halcox JP, Shamaei-Tousi A, Steptoe A, Coates AR, Henderson B, Deanfield J (2005) Circulating human heat shock protein 60 in the blood of healthy teenagers: a novel determinant of endothelial dysfunction and early vascular injury? *Arterioscler Thromb Vasc Biol* 25:141–142 doi:[10.1161/01.ATV.0000185832.34992.ff](https://doi.org/10.1161/01.ATV.0000185832.34992.ff)
- Harness J, Cavanagh A, Morton H, McCombe P (2003) A protective effect of early pregnancy factor on experimental autoimmune encephalomyelitis induced in Lewis rats by inoculation with myelin basic protein. *J Neurol Sci* 216:33–41 doi:[10.1016/S0022-510X\(03\)00212-0](https://doi.org/10.1016/S0022-510X(03)00212-0)
- Helming L, Gordon S (2008) The molecular basis of macrophage fusion. *Immunobiol* 212:785–793 doi:[10.1016/j.imbio.2007.09.012](https://doi.org/10.1016/j.imbio.2007.09.012)
- Henderson B, Poole S, Wilson M (1998) Bacteria-cytokine interactions in health and disease. Portland, London
- Hightower LE, Guidon PT (1989) Selective release from cultured mammalian cells of heat-shock (stress) proteins that resemble glia-axon transfer proteins. *J Cell Physiol* 138:257–266 doi:[10.1002/jcp.1041380206](https://doi.org/10.1002/jcp.1041380206)
- Hill JE, Penny SL, Crowell KG, Goh SH, Hemmingsen SM (2004) cpnDB: a chaperonin sequence database. *Genome Res* 14:1669–1675 doi:[10.1101/gr.2649204](https://doi.org/10.1101/gr.2649204)
- Holmgren A (1985) Thioredoxin. *Annu Rev Biochem* 54:237–271 doi:[10.1146/annurev.bi.54.070185.001321](https://doi.org/10.1146/annurev.bi.54.070185.001321)
- Holt PG (1986) Down-regulation of immune responses in the lower respiratory tract: the role of alveolar macrophages. *Clin Exp Immunol* 63:261–270
- Hu Y, Henderson B, Lund PA, Tormay P, Liu HL, Gurcha SS, Besra GS, Coates ARM (2008) A *Mycobacterium tuberculosis* mutant lacking the *groEL* homologue *cpn60.1* is viable, but fails to induce an inflammatory response in animal models of infection. *Infect Immun* 76:1535–1546 doi:[10.1128/IAI.01078-07](https://doi.org/10.1128/IAI.01078-07)
- Johnson BJ, Le TT, Dobbin CA et al (2005) Heat shock protein 10 inhibits lipopolysaccharide-induced inflammatory mediator production. *J Biol Chem* 280:4037–4047 doi:[10.1074/jbc.M411569200](https://doi.org/10.1074/jbc.M411569200)
- Kampinga HH, Hageman J, Vos MJ, Kubota H, Tanguay RM, Bruford EA, Cheetham ME, Chen B, Hightower LE (2008) Guideline for the nomenclature of the human heat shock proteins. *Cell Stress Chaperones* (in press)
- Khan N, Alam K, Mande SC, Valluri VL, Hasnain SE, Mukhopadhyay S (2008) *Mycobacterium tuberculosis* heat shock protein 60 modulates immune response to PPD by manipulating the surface expression of TLR2 on macrophages. *Cell Microbiol* 10:1711–1722
- Kim K-P, Jagadeesan B, Burkholder KM, Jaradat ZW, Wampler JL, Lathrop AA, Morgan MT, Bhunia AK (2006) Adhesion characteristics of *Listeria* adhesion protein (LAP)-expressing *Escherichia coli* to Caco-2 cells and of recombinant LAP to eukaryotic receptor Hsp60 as examined in a surface plasmon resonance sensor. *FEMS Microbiol Lett* 256:324–332 doi:[10.1111/j.1574-6968.2006.00140.x](https://doi.org/10.1111/j.1574-6968.2006.00140.x)
- Kirby AC, Meghji S, Nair SP et al (1995) The potent bone resorbing mediator of *Actinobacillus actinomycetemcomitans* is homologous to the molecular chaperone GroEL. *J Clin Invest* 96:1185–1194 doi:[10.1172/JCI118150](https://doi.org/10.1172/JCI118150)
- Kleemann R, Kapurniotu A, Frank RW et al (1998) Disulfide analysis reveals a role for macrophage migration inhibitory factor (MIF) as thiol-protein oxidoreductase. *J Mol Biol* 280:85–102 doi:[10.1006/jmbi.1998.1864](https://doi.org/10.1006/jmbi.1998.1864)
- Laudanski K, De A, Miller-Graziano C (2007) Exogenous heat shock protein 27 uniquely blocks differentiation of monocytes to dendritic cells. *Eur J Immunol* 37:2812–2824 doi:[10.1002/eji.200636993](https://doi.org/10.1002/eji.200636993)
- Lehner T, Wang Y, Whittall T, Bergmeier LA (2005) Heat shock proteins, their cell surface receptors and effects on the immune system. In: Henderson B, Pockley AG (eds) *Molecular chaperones and cell signalling*. Cambridge University Press, Cambridge, pp 160–178
- Lewthwaite JC, Coates ARM, Tormay P, Singh M, Mascagni P, Poole S, Roberts M, Sharp L, Henderson B (2001) *Mycobacterium tuberculosis* chaperonin 60.1 is a more potent cytokine stimulator than chaperonin 60.2 (hsp 65) and contains a CD14-binding domain. *Infect Immun* 69:7349–7355 doi:[10.1128/IAI.69.12.7349-7355.2001](https://doi.org/10.1128/IAI.69.12.7349-7355.2001)
- Lewthwaite JC, George R, Lund PA, Poole S, Tormay P, Sharp L, Coates ARM, Henderson B (2002a) *Rhizobium leguminosarum* chaperonin 60.3, but not chaperonin 60.1, induces cytokine production by human monocytes: activity is dependent on interaction with cell surface CD14. *Cell Stress Chaperones* 7:130–136 doi:[10.1379/1466-1268\(2002\)007<0130:RLCBNC>2.0.CO;2](https://doi.org/10.1379/1466-1268(2002)007<0130:RLCBNC>2.0.CO;2)
- Lewthwaite J, Owen N, Coates ARM, Henderson B, Steptoe AD (2002b) Circulating heat shock protein (Hsp)60 in the plasma of

- British civil servants: relationship to physiological and psychosocial stress. *Circulation* 106:196–201 doi:[10.1161/01.CIR.0000021121.26290.2C](https://doi.org/10.1161/01.CIR.0000021121.26290.2C)
- Liu W, Nakamura H, Shioji K et al (2004) Thioredoxin-1 ameliorates myosin-induced autoimmune myocarditis by suppressing chemokine expressions and leukocyte chemotaxis in mice. *Circulation* 110:1276–1283 doi:[10.1161/01.CIR.0000141803.41217.B6](https://doi.org/10.1161/01.CIR.0000141803.41217.B6)
- Lynes MA, Zaffuto K, Unfricht DW, Marusov G, Samson JS, Yin X (2006) The physiological roles of extracellular metallothionein. *Exp Biol Med* (Maywood) 231:1548–1554
- Mackness GB (1962) Cellular resistance to infection. *J Exp Med* 116:381–406 doi:[10.1084/jem.116.3.381](https://doi.org/10.1084/jem.116.3.381)
- Maguire M, Coates ARM, Henderson B (2002) Chaperonin 60 unfolds its secrets of cellular communication. *Cell Stress Chaperones* 7:317–329 doi:[10.1379/1466-1268\(2002\)007<0317:CUISOC>2.0.CO;2](https://doi.org/10.1379/1466-1268(2002)007<0317:CUISOC>2.0.CO;2)
- Mambula SS, Stevenson MA, Ogawa K, Calderwood SK (2007) Mechanisms for Hsp70 secretion: crossing membranes without a leader. *Methods* 43:168–175 doi:[10.1016/j.ymeth.2007.06.009](https://doi.org/10.1016/j.ymeth.2007.06.009)
- Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M (2004) The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 25:677–686 doi:[10.1016/j.it.2004.09.015](https://doi.org/10.1016/j.it.2004.09.015)
- Martinez FO, Sica A, Mantovani A, Locati M (2008) Macrophage activation and polarization. *Front Biosci* 13:453–461 doi:[10.2741/2692](https://doi.org/10.2741/2692)
- Matzinger P (2002) The danger model: a renewed sense of self. *Science* 296:301–305 doi:[10.1126/science.1071059](https://doi.org/10.1126/science.1071059)
- Meghji S, White PA, Nair SP et al (1997) *Mycobacterium tuberculosis* chaperonin 10 stimulates bone resorption: a potential contributory factor in Pott's disease. *J Exp Med* 186:1241–1246 doi:[10.1084/jem.186.8.1241](https://doi.org/10.1084/jem.186.8.1241)
- Meghji S, Lillcrap M, Maguire M, Tabona P, Gaston JSH, Poole S, Henderson B (2003) Human chaperonin 60 (Hsp60) stimulates bone resorption: structure/function relationships. *Bone* 33:419–425 doi:[10.1016/S8756-3282\(03\)00117-0](https://doi.org/10.1016/S8756-3282(03)00117-0)
- Metchnikoff E (1905) *Immunity to infective diseases*. Cambridge University Press, London
- Miller-Graziano CL, De A, Laudanski K, Herrmann T, Bandyopadhyay S (2008) HSP27: an anti-inflammatory and immunomodulatory stress protein acting to dampen immune function. *Novartis Found Symp* 291:196–208 doi:[10.1002/9780470754030.ch15](https://doi.org/10.1002/9780470754030.ch15)
- Moestrup SK, Møller HJ (2004) CD163: a regulated hemoglobin scavenger receptor with a role in the anti-inflammatory response. *Ann Med* 36:347–354 doi:[10.1080/07853890410033171](https://doi.org/10.1080/07853890410033171)
- Morton H, Rolfe B, Clunie GJ (1977) An early pregnancy factor detected in human serum by the rosette inhibition test. *Lancet* 1:394–397 doi:[10.1016/S0140-6736\(77\)92605-8](https://doi.org/10.1016/S0140-6736(77)92605-8)
- Nakamura H (2008) Extracellular functions of thioredoxin. *Novartis Found Symp* 291:184–192 (discussion 192–5, 221–224)
- Nakamura H, Herzenberg LA, Bai J, Araya S, Kondo N, Nishinaka Y, Herzenberg LA, Yodoi J (2001a) Circulating thioredoxin suppresses lipopolysaccharide-induced neutrophil chemotaxis. *Proc Natl Acad Sci U S A* 98:15143–15148 doi:[10.1073/pnas.191498798](https://doi.org/10.1073/pnas.191498798)
- Nakamura H, De Rosa SC, Yodoi J, Holmgren A, Ghezzi P, Herzenberg LA, Herzenberg LA (2001b) Chronic elevation of plasma thioredoxin: inhibition of chemotaxis and curtailment of life expectancy in AIDS. *Proc Natl Acad Sci U S A* 98:2688–2693 doi:[10.1073/pnas.041624998](https://doi.org/10.1073/pnas.041624998)
- Nathan CF, Murray HW, Weibe ME, Rubin BY (1983) Identification of interferon-gamma as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J Exp Med* 158:670–689 doi:[10.1084/jem.158.3.670](https://doi.org/10.1084/jem.158.3.670)
- Noonan FP, Halliday WJ, Morton H, Clunie GJ (1979) Early pregnancy factor is immunosuppressive. *Nature* 278:649–651 doi:[10.1038/278649a0](https://doi.org/10.1038/278649a0)
- Panayi GS, Corrigan VM (2008) BiP, an anti-inflammatory ER protein, is a potential new therapy for the treatment of rheumatoid arthritis. *Novartis Found Symp* 291:212–220 doi:[10.1002/9780470754030.ch16](https://doi.org/10.1002/9780470754030.ch16)
- Pekkari K, Goodarzi MT, Scheynius A, Holmgren A, Avila-Carino J (2005) Truncated thioredoxin (Trx80) induces differentiation of human CD14⁺ monocytes into a novel cell type (TAMs) via activation of the MAP kinases p38, ERK and JNK. *Blood* 105:1598–1605 doi:[10.1182/blood-2004-04-1577](https://doi.org/10.1182/blood-2004-04-1577)
- Porta C, Subhra Kumar B, Larghi P, Rubino L, Mancino A, Sica A (2007) Tumor promotion by tumor-associated macrophages. *Adv Exp Med Biol* 604:67–86 doi:[10.1007/978-0-387-69116-9_5](https://doi.org/10.1007/978-0-387-69116-9_5)
- Puissegur MP, Botanch C, Duteyrat JL, Delsol G, Caratero C, Altare F (2004) An *in vitro* dual model of mycobacterial granulomas to investigate the molecular interactions between mycobacteria and human host cells. *Cell Microbiol* 6:423–433 doi:[10.1111/j.1462-5822.2004.00371.x](https://doi.org/10.1111/j.1462-5822.2004.00371.x)
- Quaye IK (2008) Haptoglobin, inflammation and disease. *Trans R Soc Trop Med Hyg* 102:735–742
- Randow F, Seed B (2001) Endoplasmic reticulum chaperone gp96 is required for innate immunity but not cell viability. *Nat Cell Biol* 3:891–896 doi:[10.1038/ncb1001-891](https://doi.org/10.1038/ncb1001-891)
- Rayner K, Chen YX, McNulty M, Simard T, Zhao X, Wells DJ, de Bellerche J, O'Brien ER (2008) Extracellular release of the atheroprotective heat shock protein 27 is mediated by estrogen and competitively inhibits acLDL binding to scavenger receptor-A. *Circ Res* 103:133–141
- Reddi K, Meghji S, Nair SP, Arnett TR, Miller AD, Preuss M, Wilson M, Henderson B, Hill P (1998) The *Escherichia coli* chaperonin 60 (groEL) is a potent stimulator of osteoclast formation. *J Bone Miner Res* 13:1260–1266 doi:[10.1359/jbmr.1998.13.8.1260](https://doi.org/10.1359/jbmr.1998.13.8.1260)
- Rha Y, Taube C, Haczku A et al (2002) Effect of microbial heat shock proteins on airway inflammation and hyperresponsiveness. *J Immunol* 169:5300–5307
- Rhee SG, Chae HZ, Kim K (2005) Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radic Biol Med* 38:1543–1552 doi:[10.1016/j.freeradbiomed.2005.02.026](https://doi.org/10.1016/j.freeradbiomed.2005.02.026)
- Riffo-Vasquez Y, Spina D, Page C, Desel C, Whelan M, Tormay P, Singh M, Henderson B, Coates ARM (2004) Differential effects of *Mycobacterium tuberculosis* chaperonins on bronchial eosinophilia and hyperresponsiveness in a murine model of allergic inflammation. *Clin Exp Allergy* 34:712–719 doi:[10.1111/j.1365-2222.2004.1931.x](https://doi.org/10.1111/j.1365-2222.2004.1931.x)
- Rodriguez NE, Chang HK, Wilson ME (2004) Novel program of macrophage gene expression induced by phagocytosis of *Leishmania chagasi*. *Infect Immun* 72:2111–2122 doi:[10.1128/IAI.72.4.2111-2122.2004](https://doi.org/10.1128/IAI.72.4.2111-2122.2004)
- Rook GA, Dheda K, Zumla A (2005) Immune responses to tuberculosis in developing countries: implications for new vaccines. *Nat Rev Immunol* 5:661–667 doi:[10.1038/nri1666](https://doi.org/10.1038/nri1666)
- Rutkowski DT, Kaufman RJ (2004) A trip to the ER: coping with stress. *Trends Cell Biol* 14:20–27 doi:[10.1016/j.tcb.2003.11.001](https://doi.org/10.1016/j.tcb.2003.11.001)
- Shamaei-Tousi A, D'Aiuto F, Nibali L, Steptoe A, Coates AR, Parkar M, Donos N, Henderson B (2007a) Differential regulation of circulating levels of molecular chaperones in patients undergoing treatment for periodontal disease. *PLoS One* 2:e1198 doi:[10.1371/journal.pone.0001198](https://doi.org/10.1371/journal.pone.0001198)
- Shamaei-Tousi A, Steptoe A, O'Donnell K et al (2007b) Plasma heat shock protein 60 and cardiovascular disease risks: the role of psychosocial, genetic and biological factors. *Cell Stress Chaperones* 12:284–392 doi:[10.1379/CSC-300.1](https://doi.org/10.1379/CSC-300.1)
- Sherry B, Yarlett N, Strupp A, Cerami A (1992) Identification of cyclophilin as a proinflammatory secretory product of lipopolysaccharide-activated macrophages. *Proc Natl Acad Sci U S A* 89:3511–3515 doi:[10.1073/pnas.89.8.3511](https://doi.org/10.1073/pnas.89.8.3511)

- Sherry B, Zybarth G, Alfano M, Dubrovsky L, Mitchell R, Rich D, Ulrich P, Bucala R, Cerami A, Bukrinsky M (1998) Role of cyclophilin A in the uptake of HIV-1 by macrophages and T lymphocytes. *Proc Natl Acad Sci U S A* 95:1758–1763 doi:10.1073/pnas.95.4.1758
- Sica A, Allavena P, Mantovani A (2008) Cancer related inflammation: the macrophage connection. *Cancer Lett* 267:204–215
- Silberstein DS, Ali MH, Baker SL, David JR (1989) Human eosinophil cytotoxicity-enhancing factor. Purification, physical characteristics, and partial amino acid sequence of an active polypeptide. *J Immunol* 143:979–983
- Soderberg A, Sahaf B, Rosen A (2000) Thioredoxin reductase, a redox-active selenoprotein, is secreted by normal and neoplastic cells: presence in the human plasma. *Cancer Res* 60:2281–2289
- Spletstoeser WD, Schuff-Werner P (2002) Oxidative stress in macrophages—“the enemy within”. *Micros Res Tech* 57:441–445 doi:10.1002/jemt.10098
- Stein M, Keshav S, Harris N, Gordon S (1992) Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J Exp Med* 176:287–292 doi:10.1084/jem.176.1.287
- Tagayi Y, Maeda Y, Mitsui A et al (1989) ATL-derived factor (ADF), and IL-2 receptor/Tac inducer homologous to thioredoxin; possible involvement in dithiol reduction in the IL-2 receptor induction. *EMBO J* 8:757–764
- Tamaki H, Nakamura H, Nishio A et al (2006) Human thioredoxin-1 ameliorates experimental murine colitis in association with suppressed macrophage inhibitory factor production. *Gastroenterology* 131:1110–1121 doi:10.1053/j.gastro.2006.08.023
- Thériault JR, Adachi H, Calderwood SK (2006) Role of scavenger receptors in the binding and internalization of heat shock protein 70. *J Immunol* 177:8604–8611
- Tiemessen MM, Jagger AL, Evans HG, van Herwijnen MJ, John S, Taams LS (2007) CD4+CD25+Foxp3+regulatory T cells induce alternative activation of human monocytes/macrophages. *Proc Natl Acad Sci U S A* 104:19446–19451 doi:10.1073/pnas.0706832104
- Vabulas RM et al (2002) HSP70 as endogenous stimulus of the Toll/interleukin-1 receptor signal pathway. *J Biol Chem* 277:15107–15112
- Vabulas RM, Ahmad-Nejad P, Ghose S, Kirschning CJ, Issels RD, Wagner H (2002a) Hsp70 as endogenous stimulus of the Toll/interleukin-1 receptor signal pathway. *J Biol Chem* 277:15107–15112 doi:10.1074/jbc.M11204200
- Vabulas RM, Braedel S, Hilf N et al (2002b) The endoplasmic reticulum-resident heat shock protein Gp96 activates dendritic cells via the Toll-like receptor 2/4 pathway. *J Biol Chem* 277:20847–20853 doi:10.1074/jbc.M200425200
- Vanags D, Williams B, Johnson B, Hall S, Nash P, Taylor A, Weiss J, Feeney D (2006) Therapeutic efficacy and safety of chaperonin 10 in patients with rheumatoid arthritis: a double-blind randomised trial. *Lancet* 368:855–863 doi:10.1016/S0140-6736(06)69210-6
- Walsh A, Whelan D, Bielancowicz A, Skinner B, Aitken RJ, O'Bryan MK, Nixon B (2008) Identification of the molecular chaperone, heat shock protein 1 (chaperonin 10), in the reproductive tract and in capacitating spermatozoa in the male mouse. *Biol Reprod* 78:983–993 doi:10.1095/biolreprod.107.066860
- Wang Y, Kelly CG, Karttunen JT et al (2001) CD40 is a cellular receptor mediating mycobacterial heat shock protein 70 stimulation of CC-chemokines. *Immunity* 15:971–983 doi:10.1016/S1074-7613(01)00242-4
- Wang Y, Kelly CG, Singh M, McGowan EG, Carrara A-S, Bergmeier LA, Lehner T (2002) Stimulation of Th1-polarizing cytokines, C-chemokines, maturation of dendritic cells, and adjuvant function by the peptide binding fragment of heat shock protein 70. *J Immunol* 169:2422–2429
- Wang Y, Whittall T, McGowan E, Younson J, Kelly C, Bergmeier LA, Singh M, Lehner T (2005) Identification of stimulating and inhibitory epitopes within the heat shock protein 70 molecule that modulate cytokine production and maturation of dendritic cells. *J Immunol* 174:3306–3316
- Whittall T, Wang Y, Younson J, Kelly C, Bergmeier L, Peters B, Singh M, Lehner T (2006) Interaction between the CCR5 chemokine receptors and microbial HSP70. *Eur J Immunol* 36:2304–2314 doi:10.1002/eji.200635953
- Williams JH, Ireland HE (2008) Sensing danger—Hsp72 and HMGB1 as candidate signals. *J Leukoc Biol* 83:489–492 doi:10.1189/jlb.0607356
- Wilsher ML, Hagan C, Prestidge R, Wells AU, Murison G (1999) Human *in vitro* immune responses to *Mycobacterium tuberculosis*. *Tuber Lung Dis* 79:371–377 doi:10.1054/tuld.1999.0223
- Wilson MR, Easterbrook-Smith SB (2000) Clusterin is a secreted mammalian chaperone. *TIBS* 25:95–98 doi:10.1016/S0968-0004(99)01534-0
- Winrow VR, Mesher J, Meghji S, Morris CJ, Fox S, Coates ARM, Tormay P, Blake D, Henderson B (2008) The two homologous chaperonin 60 proteins of *Mycobacterium tuberculosis* have distinct effects on monocyte differentiation into osteoclasts. *Cell Microbiol* 10:2091–2104 doi:10.1111/j.1462-5822.2008.01193.x
- Xie Z, Harris-White ME, Wals PA, Frautschy SA, Finch CE, Morgan TE (2005) Apolipoprotein J (clusterin) activates rodent microglia *in vitro* and *in vivo*. *J Neurochem* 93:1038–1046 doi:10.1111/j.1471-4159.2005.03065.x
- Yagi M, Ninomiya K, Fujita N et al (2007) Induction of DC-STAMP by alternative activation and downstream signalling mechanisms. *J Bone Miner Res* 22:992–1001 doi:10.1359/jbmr.070401
- Yamawaki H, Berk BC (2005) Thioredoxin: a multifunctional antioxidant enzyme in kidney, heart and vessels. *Curr Opin Nephrol Hypertens* 14:149–153 doi:10.1097/00041552-200503000-00010
- Yang CS, Lee DS, Song CH et al (2007a) Roles of peroxiredoxin II in the regulation of proinflammatory responses to LPS and protection against endotoxin-induced lethal shock. *J Exp Med* 204:583–594 doi:10.1084/jem.20061849
- Yang Y, Liu B, Dai J, Srivastava PK, Zammit DJ, Lefrançois L, Li Z (2007b) Heat shock protein gp96 is a master chaperone for toll-like receptors and is important in the innate function of macrophages. *Immunity* 26:215–226 doi:10.1016/j.immuni.2006.12.005
- Yerbury JJ, Rybchyn MS, Easterbrook-Smith SB, Henriques C, Wilson MR (2005) The acute phase protein haptoglobin is a mammalian extracellular chaperone with an action similar to clusterin. *Biochemistry* 44:10914–10925 doi:10.1021/bi050764x
- Yoshida N, Oeda K, Watanabe E, Mikami T, Fukita Y, Nishimura K, Komai K, Matsuda K (2001) Chaperonin turned insect toxin. *Nature* 411:44 doi:10.1038/35075148
- Youn J, Borghesi LA, Olson EA, Lynes MA (1995) Immunomodulatory activities of extracellular metallothionein. II. Effects on macrophage functions. *Toxicol Env Health* 45:397–413
- Young DB, Perkins MD, Duncan K, Barry CR (2008) Confronting the scientific obstacles to global control of tuberculosis. *J Clin Invest* 118:1255–1265 doi:10.1172/JCI34614
- Yurchenko V, Zybarth G, O'Connor M et al (2002) Active site residues of cyclophilin A are crucial for its signalling activity via CD147. *J Biol Chem* 277:2259–22965 doi:10.1074/jbc.M201593200