Do reciprocal interactions between cell stress proteins and cytokines create a new intra-/extra-cellular signalling nexus?

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Abstract Cytokine biology began in the 1950s, and by 1988, a large number of cytokines, with a myriad of biological actions, had been discovered. In 1988, the basis of the protein chaperoning function of the heat shock, or cell stress, proteins was identified, and it was assumed that this was their major activity. However, since this time, evidence has accumulated to show that cell stress proteins are secreted by cells and can stimulate cellular cytokine synthesis with the generation of pro- and/or anti-inflammatory cytokine networks. Cell stress can also control cytokine synthesis, and cytokines are able to induce, or even inhibit, the synthesis of selected cell stress proteins and may also promote their release. How cell stress proteins control the formation of cytokines is not understood and how cytokines control cell stress protein synthesis depends on the cellular compartment experiencing stress, with cytoplasmic heat shock factor 1 (HSF1) having a variety of actions on cytokine gene transcription. The endoplasmic reticulum unfolded protein response also exhibits a complex set of behaviours in terms of control of cytokine synthesis. In addition, individual intracellular cell stress proteins, such as Hsp27 and Hsp90, have major roles in controlling cellular responses to cytokines and in controlling cytokine synthesis in response to exogenous factors. While still confusing, the literature supports the hypothesis that cell stress proteins and cytokines may generate complex intra- and extra-cellular networks, which function in the control of cells to external and internal stressors and suggests the cell

stress response as a key parameter in cytokine network generation and, as a consequence, in control of immunity.

Keywords Cytokines · Cell stress proteins · Cytokine networks · Unfolded protein response · Systems biology

Introduction

As humans, we are excited, intrigued, even fearful of the fact that we live in the ‘information (communication) age’ with, as a recent book has proclaimed in its title, the ‘rise of the network society (Castells 2011). Communication is built into the foundation of Biology from birdsong, to mating displays, to pheromones and other olfactory cues (Bradbury and Vehrencamp 1998) and so on. Even bacteria are now known to actively communicate between themselves in processes such as quorum sensing (Diggle et al. 2007). It is assumed that communication in the biological sense involves the passage of information. However, it is interesting that Maynard Smith and Harper (2003) define a ‘signal’ as ‘any act or structure which alters the behaviour of other organisms, which evolved because of that effect, and which is effective because the receiver’s response has also evolved’. This definition does not involve ‘information’, and it is surprising to find that there is no clear definition of the term ‘communication’ as it pertains to Biology (Scott-Phillips 2008).

If we descend from the organism to its component parts, then the problems of communication and information passage becomes clear. The average human organism consists of 10^{13} cells and a complex collection of microorganisms, largely bacteria, which outnumber the eukaryotic host cells by 10:1 (Wilson 2008). This article will only concentrate on the eukaryotic components of the human, but readers should be aware that there must be a huge amount of inter-kingdom signalling at epithelial and other sites of colonisation by the microbiota. So, how do the 10^{13} cells of the average human

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communicate to maintain organismal homeostasis? Currently, only three communication systems are recognised. The first such system to be identified, in the eighteenth century, was the system of nerves that permeate the body and give rise to long distance/rapid communication between brain and body (Ochs 2005). It was Starling, a University College London physiologist, with his brother-in-law, Bayliss, who discovered secretin in 1902 and coined the term ‘hormone’ in 1905, that led to our understanding of the role of endocrine hormones in cellular communication (Modlin et al. 2000). It was not until the 1940s/1950s that another potential form of cellular communicating agent was suspected. The first such molecule was termed endogenous pyrogen, or leucocyte pyrogen, and was proposed to be the cause of fever due to infection. This was the genesis of the discovery of the interleukin-1 proteins and of the cytokines generally (Dinarello 1989). The cytokines normally function as paracrine and autocrine signals and often induce and interact with other low molecular mass signals such as the numerous lipidic eicosanoids and related molecules (Petho and Reeh 2012), which will not be dealt with in this article. Since these early discoveries of cytokines, well over a hundred cytokines and chemokines have been identified (Manjili 2012). Their importance in homeostasis and pathology is now clearly seen by the enormously beneficial effects that can be obtained by cytokine-blocking antibodies and other anti-cytokine therapeutics (Higgs and Henderson 2000; Dinarello 2007).

Thus, there are three major classes of cellular communication identified in vertebrates, with the cytokines being recognised for being responsible for much of the paracrine and autocrine elements of cell–cell communication.

Cytokines and cellular communication

Neural and endocrine hormone signalling appears to be a straightforward linear signalling system in which a controlling cell (neurone or endocrine organ cell) releases a signal (nerve impulse or hormone), which has influence on one particular target cell. In contrast, cytokines are produced by most cells (even the anucleate platelet—Li et al. 2012) and all cells can respond to many different cytokines (Ibelgaufts 2012). It is now established that many cytokines are pleiotropic—that is, they can induce in cells many different biological effects (Ozaki and Leonard 2002). In other proteins, this phenomenon would be termed protein moonlighting (reviewed in Henderson and Pockley 2010). Cytokines also exhibit significant redundancy. A good example of this is the closely overlapping functions of the structurally distinct proteins: interleukin-1 beta (IL-1 β) and tumor necrosis factor alpha (TNF- α ; Ozaki and Leonard 2002). These properties are linked to the facts that many different cells can share cytokine receptors and that cytokine receptors

also show the evolutionary propensity to share receptor chains—although this does not explain the similarities between IL-1 and TNF signalling. As will be discussed, cell stress proteins also exhibit many of these intriguing properties of the cytokines.

These properties of cytokines, and the fact that there are a very large number of these proteins in the vertebrate [for example, there are currently 48 chemokines identified (Blanchet et al. 2012) and the latest interleukin to be named is IL-38 (van de Veerdonk et al. 2012)], results in complex behaviour being generated. Thus cytokines can act in a synergistic fashion. For example, injection of IL-1 β and TNF- α into rabbit knee joints results in marked synergy with respect to the numbers of infiltrating neutrophils, but with no synergistic interaction in terms of articular cartilage breakdown (Henderson and Pettipher 1989). In addition to synergism, there are examples of cytokines [e.g. interferon gamma (IFN- γ) and IL-4] acting antagonistically (e.g. Serpier et al. 1997). Thus, in general, the signalling mechanisms produced by cytokines seem to be much more complex than that controlled by endocrine hormones and this is exemplified by the concept of the cytokine network (Frankenstein et al. 2006). This concept of cytokine network behaviour is still in development, and any cytokine network will be dependent on the exogenous inputs controlling cytokine synthesis.

The induction of cytokine synthesis by DAMPs

Cytokine biology has largely focused on the role of cytokines and chemokines in immunity and inflammation, and this has enabled a wide range of microbial cytokine-inducing components, such as the pathogen-associated molecular pattern (PAMP) class of microbial molecule to be identified (Wilson et al. 1998; Kumar et al. 2011). Thus, the recognition of PAMPs by the growing number of families of pattern-recognition receptors leads on to the production of, it is assumed, selected cytokine networks, which activate the correct cells to deal with whatever microbe is infecting the host. Cytokine synthesis clearly goes on all the time and so endogenous cytokine inducers must also be active in the absence of infection. Cytokines are, themselves, powerful inducers of cytokine synthesis. A classic early example of this is the finding that IL-1 β stimulates the synthesis of IL-6 (Sironi et al. 1989). Many more examples of this have since been discovered including examples of cytokines antagonising each other's synthesis—a good example being IL-10 inhibiting macrophage synthesis of a range of pro-inflammatory cytokines, including TNF- α (de Waal et al. 1991). It is this stimulatory and antagonistic behaviour of cytokines that underpins the generation and stability of cytokine networks.

In recent years, another group of endogenous cytokine inducers have been proposed. This has been based on Matzinger's vision of the immune response as being a mechanism for recognising danger signals (Matzinger 2002). This gave rise to the concept that the immune system could recognise and respond (by producing cytokines) to so-called danger molecules. Among the various components of cells that were seen to be danger molecules were free radicals, nucleotides, urate crystals, matrix breakdown products, and heat shock proteins (Gallucci and Matzinger 2001). Over the past decade, Matzinger's danger molecules, and the late Charles Janeway's concept of PAMPs, have merged to generate the acronym, DAMPs, which now stands, largely, for damage-associated molecular patterns (Nace et al. 2012).

There is now a wide variety of body components coming under the umbrella of DAMPs, which commonly includes the heat shock or cell stress proteins. For the purposes of this article, the term cell stress proteins will encompass both molecular chaperones and protein-folding catalysts. The description will also include intracellular proteins such as ubiquitin and ISG15, which are not involved in protein folding but bind to proteins destined for proteolysis (Fu et al. 2012). Notably, the inclusion of cell stress proteins as DAMPs has recently been challenged by two articles published in cell stress and chaperones (van Eden et al. 2012; Henderson and Pockley 2012). One obvious part of the argument in these articles is that cell stress proteins can be found in the circulation in healthy individuals. Moreover, we propose that the relationship between cell stress proteins and cytokines may be much more complex than has been thought, and the following sections introduce the reader to the concept that cell stress proteins and cytokines may have a reciprocal relationship inasmuch as each can induce the synthesis of the other. This suggests the hypothesis that cell stress proteins may be homeostatic controllers of cytokine synthesis set within a reciprocal network-based control system of cell stress protein synthesis and secretion. Together, this reciprocity of interaction may represent a novel communication system, which links the cell stress response to an active extracellular communication network involving both cell stress proteins and cytokines. In addition, strong evidence exists for the hypothesis that intracellular cell stress proteins are major controllers of cell responses to cytokines and cytokine synthesis by cells responding to cytokine-inducing signals. As cytokines are the key regulatory molecules of immunity, it is likely that cytokine/cell stress protein interactions are an inherent part of the control of this key protective process.

Extracellular cell stress proteins as cytokine inducers and controllers

The concept that cell stress proteins in the extracellular milieu can induce the synthesis of cytokine networks can

be traced back to pioneering studies of adult T cell leukaemia (ATL) in Japan, which led to the finding of a novel cytokine secreted by these cells and termed ATL-derived factor (ADF). This cytokine functioned as an inducer of the synthesis of the 55 kDa IL-2 receptor (Teshigawara et al. 1985). It took 4 years before it was discovered that ADF was, in fact, human thioredoxin (Trx)-1 (Tagaya et al. 1989). Curiously, monocytes secrete a breakdown product of thioredoxin, termed Trx80 (denoting how many residues this peptide has), which has pro-inflammatory autocrine effects on myeloid cells (Cortes-Bratti et al. 2011). This was the beginning of studies, which some workers still regard as controversial, that cell stress proteins are potent inducers of cytokine synthesis. This literature has now been extensively reviewed by the main author (Henderson and Pockley 2005, 2010, 2012) and will not be dealt with in any detail. The salient features of this literature are found in Table 1. In 2013, the literature appears unequivocal. At least 20 distinct cell stress proteins are secreted from eukaryotic cells, can be found in the circulation, and have the properties of interacting with leucocytes or vascular endothelial cells to induce, *or inhibit*, the synthesis of selected cytokines. If all secreted cell stress proteins did was to induce cytokine synthesis, then this may just have been an evolutionary act of seriality, as defined by Paul Kammerer. However, from the limited publications on this subject, it is clear that different cell extracellular stress proteins exhibit differences in their cytokine-inducing potencies and in the patterns of cytokines that they induce. Some reports of human cell stress proteins suggest that these proteins, once released from cells, can be active at picomolar concentrations, which is as potent as the most potent cytokines (Zanin-Zhorov et al. 2006). The available evidence would support the hypothesis that cell stress proteins such as Trx80, cyclophilin A, Hsp60, Hsp70 and Gp96 are pro-inflammatory proteins, while Hsp10, Trx-1, Hsp27 and BiP (Grp78) are anti-inflammatory in nature. Thus, for example, both Hsp10 (Johnson et al. 2005) and Trx-1 (Billiet et al. 2005) inhibit lipopolysaccharides (LPS)-induced stimulation of pro-inflammatory cytokine synthesis by macrophages. These findings have resulted in cell stress proteins being either considered as therapeutic targets or as therapeutic agents. Thus, cyclophilin A (Yurchenko et al. 2010) is being considered as a general therapeutic target for inflammatory disease, and Gp96 is suggested to be a therapeutic target in rheumatoid arthritis (Huang and Pope 2012). In contrast, human Hsp10 has shown limited clinical benefit in the treatment of rheumatoid arthritis (Vanags et al. 2006), and Hsp90 α is showing promise in the treatment of diabetic skin ulcers (Cheng et al. 2011).

Thus, the simplest hypothesis to explain the above data is that some, many or probably all cell stress proteins are secreted molecules with the ability to signal to other cells, principally leucocytes and vascular endothelial cells, and to induce the synthesis of cytokines. What is the evolutionary 'purpose' of this cytokine-stimulating ability? This is the

Table 1 Secreted cell stress proteins with cytokine inducing actions

Cell stress protein	Signalling properties	References
Ubiquitin	CXCR4 agonist and leucocyte modulator	Saini et al. 2010
ISGN15	Induces anti-viral actions in mice but anti-mycobacterial in humans	Wang et al. 2012a, b; Bogunovic et al. 2012
Hsp10	Anti-inflammatory effects on macrophages	Johnson et al. 2005
Trx80	Alternative macrophage activator	Cortes-Bratti et al. 2011
Thioredoxin (Trx)	Multiple effects on leucocytes and acts a chemoattractant	Mahmood et al. 2013
Glutaredoxin	No specific information to date	Hanschmann et al. 2013
Cyclophilin A	Pro-inflammatory actions on myeloid cells and chemoattractant	Yurchenko et al. 2010
Cyclophilin B	Inhibits LPS-induced TNF- α synthesis by macrophages	Marcant et al. 2012
Hsp27	Up-regulates macrophage IL-10 synthesis and inhibits dendritic cell formation	De et al. 2000
Peroxioredoxins	Pro-inflammatory effects through TLR4 signalling	Riddell et al. 2010
		Ishii et al. 2012
Hsp40	Up-regulates anti-inflammatory cytokine synthesis	Tukaj et al. 2010
Protein disulphide isomerase (PDI)	Monocyte cytokine induction and influence of neutrophil recruitment	Henderson (unpublished); Hahm et al. 2013
Hsp47	No information to date	
Hsp60	Multiple cytokine induction from all leucocytes	Henderson et al. 2013
Hsp70 (HSPA1A)	Stimulates macrophage pro-inflammatory synthesis	Asea et al. 2000
HspB1	Stimulates VEGF-induced angiogenesis	Lee et al. 2012
BiP (HSPA5)	Stimulate anti-inflammatory cytokine synthesis	Panayi and Corrigan 2008
Hsp90	Molecule has growth factor properties	Cheng et al. 2011
Gp96	Pro-inflammatory cytokine inducer	Huang and Pope 2012
Clusterin	Induces TNF- α synthesis and chemotaxis	Shim et al. 2012

proverbial \$64,000 dollar question, which remains unanswered. The authors would like to suggest the hypothesis that the signalling actions of cell stress proteins is part of an unexplored nexus of signalling between intra- and extra-cellular cell stress proteins and intra- and extra-cellular cytokines, which form part of a homeostatic system for controlling the organismal response to stress. The strongest evidence in support of this hypothesis is the finding that cytokines can control the synthesis of cell stress proteins.

The influence of cytokines on cell stress protein synthesis

For didactic purposes the relationship between cytokines and cell stress proteins will be divided into: (1) the relationship with cytosolic/mitochondrial stress and (2) the relationship with ER stress. In the early 1990s, the first reports of cytokine induction of cell stress protein synthesis began to appear. Isolated pancreatic islet cells exposed to IL-1 β showed upregulation of what was termed Hsp73, Hsp90 and haeme oxygenase. Surprisingly, given the enormous overlap in biological activity, TNF- α did not induce such protein synthesis. Furthermore, IL-1 β did not induce the synthesis of these cell stress proteins in a range of other cell populations, including human monocytes (Helqvist et al. 1991). In cardiac myocytes, exposure to IL-1, IL-2, IL-3, IL-6 or TNF- α stimulated the synthesis of a

30-kDa cell stress protein. IL-1 also promoted formation of two of the Hsp70 family of proteins (Löw-Friedrich et al. 1992). In another early study, the human monocyte cell line THP-1 was induced to increase levels of Hsp60 when cultured with IFN- γ and/or TNF- α (Ferm et al. 1992). The mechanism of this increase in Hsp60 synthesis is unclear. In addition to the above, pro-inflammatory cytokines, another early study reported that transforming growth factor beta 1 (TGF- β 1), a cytokine recognised for its largely anti-inflammatory actions (Han et al. 2012), induced the formation of Hsp70 and Hsp90 in chicken embryo cells. In contrast, PDGF, FGF and EGF failed to stimulate cell stress protein synthesis (Takenaka and Hightower 1992). These initial studies reveal that different cytokines have different effects on the synthesis of selected cell stress proteins, suggesting that there is a level of complexity in the relationship between cytokine interactions with cells and the generation of patterns of cell stress proteins.

A number of studies of the cell stress response of various cell types to exogenous cytokines have now been published and the details are highlighted in Table 2. Cell stress protein synthesis can be rapid. For example, with resident mouse peritoneal macrophages exposed to macrophage colony-stimulating factor, cell stress protein synthesis can be detected within 1 h (Teshima et al. 1996). This is similar to the rapid synthesis of IL-1 β and TNF- α by monocytes exposed to bacterial factors. It is for this reason that these molecules

Table 2 Cell stress protein synthesis in cells exposed to various cytokines

Cytokine	Stress proteins induced/inhibited	Cell type	References
IL-1 β (TNF inactive)	Hsp73, Hsp90	Islet cells	Helqvist et al. 1991
<i>IL-1β</i>	<i>None</i>	Range of other cell types	Helqvist et al. 1991
IL-1 β	BiP	Pancreatic epithelial MIA PaCa-2 cells	Verma and Datta 2010
<i>IL-1/TNF/PDGF</i>	<i>Hsp27 (no increase in synthesis)</i>	Human astrocytes	Satoh and Kim 1995
<i>IL-1α/TNF-α</i>	<i>No influence on Hsp60, Hsc70, Hsp70</i>	Myoblasts	Ji et al. 1998
IL-1/2/3/6, TNF- α	30 kDa protein	Cardiac myocytes	Löw-Friedrich et al. 1992
IL-1	30 kDa plus two Hsp70 proteins	Cardiac myocytes	Löw-Friedrich et al. 1992
IL-6	Hsp90	HuH7 hepatoma cells and PBMCs	Stephanou et al. 1997
Cardiotrophin-1 ^a	Hsp70, Hsp90	Cardiac myocytes	Stephanou et al. 1998
Cardiotrophin-1	Hsp56 (also known as FKBP59)	Cardiac myocytes	Railson et al. 2001
IL-11	Hsp27 and Hsp70	Colonic epithelial cells	Ropeleski et al. 2003
IL-11	BiP (and stimulates secretion)	Human extravillous trophoblasts	Sonderegger et al. 2011
IFN- γ , TNF- α	Hsp60	THP-1 human monocyte	Ferm et al. 1992
IFN- γ , TNF- α	Hsp70	Granulosa-luteal cells	Kim et al. 1996
<i>IL-1</i>	<i>No stimulation</i>	Granulosa-luteal cells	Kim et al. 1996
IFN- γ	Thioredoxin (thioredoxin induces IFN- γ)	Monocytes	Kang et al. 2008
IFN- γ	Hsp10, Hsp60	C6 astrogloma cells	Kim and Lee 2007
IFN- γ +TNF- α	Hsp25/27 and Hsp70 in vitro and in vivo	Colon or colonic cells	Hu et al. 2007
IFN- γ /IL-2	Gp96	LG2 cells	Chen et al. 2003
IFN- $\alpha\beta$	ISG15 (ubiquitin homologue)	PBMCs	D'Cunha et al. 1996
Macrophage colony-stimulating factor	Hsp60, Hsp70, Hsp90	Mouse peritoneal macrophages	Teshima et al. 1996
TGF- β 1	Hsp70, Hsp90	Chicken embryo cells	Takenaka and Hightower 1992
TGF- β 1	Hsp47	Osteoblasts, myoblasts	Yamamura et al. 1998
TGF- β 1/IL-1	Hsp47	Human fibroblasts	Sasaki et al. 2002
TGF- β 1	Peroxiredoxin-1	A549 epithelial cells	Chang et al. 2006
TGF- β 1/IL-6	Hsp47	Fibroblasts	Martelli-Junior et al. 2003
IFN- γ	Hsp47	Fibroblasts	Martelli-Junior et al. 2003
TGF- β 1	clusterin	Mammary epithelial cells	Itahana et al. 2007
TGF- β 2	Hsp27	Optic head astrocytes	Yu et al. 2008

Cytokines in italics have no effect on cell stress protein synthesis

^a An IL-6 family member

are called early response cytokines (Dinarello 2010). The induction of cell stress proteins by cytokines actually appears to be a two-way process as IFN- γ can induce expression of Trx-1, and this cell stress protein can, in turn, stimulate synthesis of IFN- γ (Kang et al. 2008) (Fig. 1). This would strongly suggest that these two classes of proteins are indulging in some form of feedback network behaviour. Hightower's group have made the interesting observation that Hsp70B (HSPA6) promoter activation is, beyond a certain population size, highly dependent on cell number, and this is enhanced in cell populations with limited cell matrix attachment. This is not related to HSF1 activity and is in line with the concept of an autocrine feedback control

loop (possibly through some cytokine) controlling Hsp70B expression in cells (Noonan et al. 2007).

Some cells respond to certain cytokines without the induction of cell stress protein synthesis (e.g. Satoh and Kim 1995). There are also two reports of cytokines inhibiting the synthesis of cell stress proteins. IFN- γ is reported to inhibit the synthesis of the collagen-folding chaperone, Hsp47, in fibroblasts (Martelli-Junior et al. 2003) and the combination of IFN- γ plus TNF- α inhibits the production of Hsp27 and Hsp70 (Hu et al. 2007). It is possible that cytokine-induced inhibition of cell stress protein synthesis is also a prominent cellular response. These findings would also conform to a network view of the interactions of these two classes of proteins.

Fig. 1 Potential reciprocal relationship between target cells, exogenous cytokines and cell stress proteins. Currently it is known that cell stress proteins (CSPs) can induce the synthesis and secretion of a range of pro- and anti-inflammatory cytokines. In turn, cytokines can induce aspects of cytosolic and ER UPRs and can induce both cytoplasmic and mitochondrial CSPs. It is not known if cytokines induce CSP secretion.

However, if they do, one can envisage that both cytokines and CSPs can induce autocrine (red arrows) and paracrine (blue arrows) signalling resulting in the generation of network interactions to control local cell activity. Added to this is the known actions of intracellular CSPs such as Hsp27, Hsp70 and Hsp90, which can control cellular responses to cytokines and cytokine inducers (including CSPs?).

A key question, which needs to be addressed, is whether cytokines, as well as inducing cell stress protein synthesis, also promote the secretion of cell stress proteins and thus generate autocrine/paracrine feedback loops (Fig. 1). One interesting protein is the interferon-inducible ubiquitin homologue called ISG15 (Loeb and Haas 1992). Like ubiquitin, this protein binds to cellular proteins and, like ubiquitin, it is also secreted (D'Cunha et al. 1996). ISG15 is induced by IFN α/β , is secreted from leucocytes and functions as a cytokine in that it can induce the formation of the third interferon family member, IFN- γ (D'Cunha et al. 1996). This is the first example of a protein-folding protein being induced to be released from cells by cytokines. Whether this is specific to ISG15 or is it the general rule needs to be determined. Unexpectedly, it turns out that ISG15 has a moonlighting biological activity that was only discovered when individuals lacking the gene for this protein were identified. It has emerged that secreted ISG15 plays an essential role as an IFN- γ -inducing molecule for optimal anti-mycobacterial immunity and may be one explanation for the condition, Mendelian susceptibility to mycobacterial disease in which individuals are highly susceptible to infection with mycobacterial species (Bogunovic et al. 2012).

Influence of cell stress on cytokine synthesis

There is incontrovertible evidence that extracellular cell stress proteins stimulate cytokine synthesis. This raises the

obvious question of the role that the cell stress process itself has on cytokine synthesis. This has not been an area of particularly vigorous study, but there are a collection of reports that do not, as yet, make a consistent story, but do show some influence of stress on cellular cytokine synthesis. An early study by one of the pioneers of interleukin-1, Charles Dinarello, examined on the influence of hyperosmolarity on pro-inflammatory cytokine synthesis by human monocytes (Shapiro and Dinarello 1995). Exposure of cells to solutions to which 12–50 mM monovalent salts (e.g. KI, NaCl) had been added, promoted the synthesis of IL-8, but not IL-1 α , IL-1 β or TNF- α . This effect could be blocked by selective inhibitors of p38 MAP kinase. In contrast, lowering the pH of the media supporting human monocytes to pH 6.5 stimulated the synthesis of IL-1 β , without stimulating the synthesis of IL-6 or TNF- α . In addition, this lower pH did not enhance cell surface expression of the major monocyte activation markers: CD40, CD80/86 and HLA-DR—so cytokine production was not directly due to monocyte activation. Curiously, maturing monocytes to generate macrophages blocked this IL-1 β activation effect of lower pH (Jancic et al. 2012). Tissue acidification is generally due to the production of lactic acid. It has been reported that lactic acid blocks TNF- α synthesis by LPS-activated human monocytes. However, this effect seems to be related to the ability of lactate to inhibit glycolysis rather than a direct effect of pH (Dietl et al. 2010). Of interest is the report that addition of *Mycobacterium tuberculosis* Hsp60.2 to monocytes induced

pro-inflammatory cytokine synthesis without promoting cell surface expression of HLA-DR (Peetermans et al. 1994), suggesting that this chaperone was acting in a similar manner to that of low pH.

Oxygen and other free radicals are well-known cell stressors, and there is evidence that they can induce cytokine synthesis. Of course, it is also well known that certain cytokines can induce cells, such as macrophages, to generate bacteriostatic/cidal-free radicals (Karhumäki and Helin 1987). One of the first studies reported that NK-enriched cell fractions exposed to hydrogen peroxide produced IFN- γ (Munakata et al. 1985). Ozone also stimulates leucocytes to synthesise IFN- γ (Bocci and Paulesu 1990). Dendritic cells exposed to hydrogen peroxide produced both TNF- α and IL-8 (Verhasselt et al. 1998). Exposure of vascular endothelial cells to hypoxia/reperfusion stimulated IL-1 α/β and IL-6 synthesis, and this could be blocked by free radical scavenging enzymes (Ala et al. 1992). The role of oxidative stress in the induction/inhibition of cytokine synthesis is complex and may be involved in various disease states including diabetes (see review by Elmarakby and Sullivan 2012). This topic is still confusing, with early evidence being presented that oxidative species activate nuclear factor kappa B (NF- κ B), and this, in turn, would give rise to cytokine synthesis (e.g. Schreck et al. 1991). However, this hypothesis was disputed by Hayakawa and coworkers, who produced evidence that endogenous reactive oxygen species (ROS) actually lowered NF- κ B activation (Hayakawa et al. 2003). The evidence supporting the hypothesis that hydrogen peroxide is not a direct activator of NF- κ B has recently been reviewed and reveals the technical problems, which still bedevil this area of cellular research (Oliveira-Marques et al. 2009).

How do cells respond to the classic cell stress—heat stress—in terms of cytokine synthesis? Bacterially stimulated monocytes heated to 40.5–43° C were more cytotoxic to target cells and produced more TNF- α than non-heated cells (Tomasovic et al. 1989). However, a later report on the mouse monocyte cell line RAW 264.7 revealed that heating LPS-stimulated cells to 40 °C reduced TNF- α secretion by 90 % and intracellular messenger RNA (mRNA) levels for this cytokine by >40 % by affecting posttranscriptional processes including decreasing stability of the TNF- α mRNA (Ensor et al. 1995). There are various other reports in the literature on the effect of heat on cells, but the results are indirect and, at the present time, it is still unclear what role elevated temperature plays in cellular cytokine synthesis.

This section on cell stress and cytokine production has focused on the cytosolic cell stress response or unfolded protein response (UPR). The next section will deal with the role of heat shock factors in controlling cytokine synthesis and the section following that will consider the role played by the endoplasmic reticulum unfolded protein response (ER/UPR) in the reciprocal signalling with cytokines.

Mechanism of control of cytoplasmic cell stress protein synthesis and trafficking by cytokines

Details of the transcriptional or translational control of cytokine synthesis in cells exposed to cell stress proteins have not been provided because this has not been an active area of research and because there are multiple potential intracellular control systems. With cytokine-stimulated cell stress protein synthesis, the most obvious mechanism for stimulation/inhibition of synthesis is through regulation of the four vertebrate cytoplasmic heat shock factors (HSFs1–4, Björk and Sistonen 2010). The other obvious control systems that cytokines could interact with are the transcriptional and translational control of the various UPRs, which exist in the ER, mitochondria, nucleus and lysosomes. Currently, we know most about the control of the ER/UPR (Walter and Ron 2011)—see next section.

IL-1 β is the most potent cytokine and the first pyrogenic cytokine to be identified (Dinarello 1989). Thus, the possibility that increased body temperature induced a heat shock response that could control the synthesis of pyrogenic cytokines was an obvious area of study. This was first examined by Schmidt and Abdulla (1988) who reported that a range of inducers of the cell stress response inhibited the synthesis of IL-1 β . Heat shock was also found to block synthesis of TNF- α (Snyder et al. 1992). HSF1 was reported to block IL-1 β synthesis by binding a heat shock element in the IL-1 β gene promoter, suggesting a mechanism for stress down-regulating pro-inflammatory cytokine synthesis (Cahill et al. 1996). Further work from this group identified that HSF1-repressed LPS-induced IL-1 β transcription through interaction with nuclear factor of interleukin-6 (NF-IL6) also called CCAAT enhancer binding protein (C/EBP β), which is required for IL-1 β transcription. As C/EBP β is involved in transcriptional regulation of a wide range of genes, this interaction could influence other pro-inflammatory cytokine genes (Xie et al. 2002; Inouye et al. 2007).

Another key pyrogenic cytokine, and major mediator of the acute phase response, is IL-6. Heat shock blocks synthesis of this cytokine, and here the mechanism appears to be the HSF1-induced expression of activating transcription factor (ATF) 3 (Takii et al. 2010). One possible explanation is that HSF1 changes the structure of the IL-6 promoter allowing other proteins to bind to it, such as ATF3 (Inouye et al. 2007). It is not clear if this factor is also involved in other HSF1-inhibitable cytokines. Thus, granulocyte-colony stimulating factor (G-CSF) gene expression is up-regulated in HSF-1 knock-out mice and transcription of this gene and secretion of the protein are inhibited by HSF1 (Takii et al. 2010). The activity of HSF1 is related to the presence of NF-IL-6/CCAAT binding sites in the G-CSF promoter, and thus, HSF1 would appear to be a direct negative regulator of G-CSF synthesis (Zhang et al. 2011). G-CSF has cardioprotective activity in mice, and this is related to the interaction of HSF1 with the

cytoplasmic cytokine transcription factor STAT3 (Ma et al. 2012) revealing the potential complexities in the reciprocal relationships between cytokines and cell stress proteins.

One group of cytokines that have come to prominence in the last two decades is the chemokines. Originally discovered as leucocyte chemoattractants, it is now established that these proteins have a wide range of cellular actions and roles in human disease (Sharma 2010). It has been proposed that certain of these proteins, particularly the CXC chemokines, may be upregulated by the cell stress response. Indeed, the promoters of these proteins contain multiple copies of the HSF1 binding sequence, resulting in the suggestion that the CXC chemokines are, themselves, heat shock proteins (Nagarsekar et al. 2005). A later study revealed that the heat shock response elements (HSEs) in the IL-8 promoter were responsible for heat shock enhancement of TNF- α -induced IL-8 synthesis in respiratory epithelial cells (Singh et al. 2008). This has raised the possibility that the CXC chemokine genes may have incorporated control elements of the cell stress response to enhance neutrophil chemoattraction during febrile conditions. Indeed, 28 of 29 human and mouse CXC chemokine genes were found to have multiple HSEs in their promoters. Recruitment of HSF1 to the promoters of five human CXC genes (CXCL-1,2,3,5,8) was analysed by chromatin immunoprecipitation (ChIP) assay and all but one gene (CXCL-3) showed positive responses. Curiously, HSF1 binding causes a variable effect on chemokine gene transcription, with CXCL8 being significantly increased, but CXCL5 expression being significantly repressed in cells exposed to heat shock and TNF- α . Moreover, expression of CXCL1/2 was not influenced by heat shock exposure (Maity et al. 2011). This suggest, as some of the previous reports also do, that HSF1 interactions with selected genes may encompass a range of other interacting transcription elements such as ATF3 (Inouye et al. 2007) or even other HSFs (He et al. 2003). TNF- α and Fas ligand (FasL) induce a variety of chemokines (CCL2/3 and CXCL2/10) in cultured astrocytes. Of interest, inhibition of protein synthesis enhanced chemokine induction, suggesting a control mechanism employing newly synthesised protein inhibitors. The enhancement of chemokine synthesis was related to HSF1 binding. With the exception of CXCL10, the extracellular signal regulated protein kinase (ERK) inhibitor, PD98059, blocked chemokine expression by a NF- κ B-independent pathway, revealing another example of the variability of cytokine expression in response to the cell stress response (Choi et al. 2011).

In addition to variability of cytokine expression in response to the cell stress/heat shock response reported, there also appears to be differences in response in different cell populations. For example, it has been reported that HSF1 is activated at lower temperatures in mouse T cells than in other cells (Murapa et al. 2011).

The relationship of the endoplasmic reticulum UPR to cytokine synthesis

There is growing evidence for a role for the ER/UPR (simply termed UPR hereafter) in the control of inflammation and inflammatory diseases (Adolph et al. 2012). The UPR is initiated by the presence of unfolded proteins in the ER, leading to a stereotypical set of control systems involving the key ER chaperone, Grp78/BiP/HSPA5 and two sets of proximal sensor proteins: (1) PERK, ATF6 and IRE1 and (2) the transcriptional effectors ATF4, ATF6 and the XBP1s. The interaction of these various proteins is shown schematically in Fig. 2.

Increased concentrations of unfolded protein in the ER competes for the binding of BiP to PERK, IRE1 and ATF6 resulting in these proteins becoming free and, therefore, active and inducing major changes in protein synthesis (it is decreased) and in the transcription of a large number of responsive genes including increased amounts of molecular chaperones and protein-folding catalysts. Many signals can induce the UPR including: hypoxia, nutrient (principally glucose) deprivation, alterations in redox state, changes in ER Ca^{2+} concentrations, failure of posttranslational protein modification and increases in protein synthesis. A range of chemicals have been implicated in inducing the UPR including thapsigargin, an inhibitor of sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA); A23187, a calcium ionophore; tunicamycin, which prevent protein glycosylation; dithiothreitol, which inhibits disulphide bridge formation and; 2-deoxyglucose—a non-metabolisable glucose analogue, which inhibits protein glycosylation (Walter and Ron 2011). As can be seen, the ER is very susceptible to developing its UPR, and it is not clear how the initiation of the UPR influences cell control mechanisms including cytokines.

The key questions that this article is asking are (1) do cytokines influence the UPR and thus the cell stress proteins produced by cells and/or (2) does the UPR have any effect on the networks of cytokines produced by ER stressed cells.

The study of the endoplasmic reticulum UPR has focused on the beta cells of the pancreas, where it is thought to be a contributory factor in the development of diabetes (Back and Kaufman 2012). It should be noted that other cell types can behave differently to the pancreatic beta cells in response to factors such as cytokines. An early study of the effect of cytokines on pancreatic beta cells found that the induction of NO synthesis by a combination of IL-1 β and IFN- γ decreased mRNA levels for the sarcoendoplasmic reticulum pump (Ca^{2+}) ATPase 2b (SERCA 2b) while inducing the expression of the ER stress-related and pro-apoptotic gene CHOP (CCAAT-enhancer-binding protein homologous protein) (CHOP) (Cardozo et al. 2005). The subsequent depletion of ER Ca^{2+} and the induction of the ER stress pathway both ultimately lead to beta cell apoptosis and necrosis.

Fig. 2 The interrelationship between the endoplasmic reticulum molecular chaperone BiP (HSPA5) and the unfolded protein response. The description of the events is provided in the text. From Boyce and Yuan (2006) Cell death and differentiation 13:363–373 (with permission)

Fibroblasts did not show the same sensitivity to inhibition of SERCA 2b. Interestingly, IL-1 β could induce ER stress, but not apoptosis, and IFN- γ was unable to induce either ER stress or apoptosis. Clearly, the combination of both cytokines is generating emergent properties. Indeed, it was later shown that IFN- γ could enhance ER stress and apoptosis induced by the SERCA blocker, cyclopiazonic acid (Pirot et al. 2006). TNF- α also contributes to pancreatic beta cell death. Comparison of apoptosis of pancreatic beta cells versus fibroblasts in response to IL-1 β or TNF- α revealed that the pro-apoptotic effect in the former cells was due to a sustained activation of NF- κ B compared with an oscillatory form of activation in the fibroblasts. The activation of NF- κ B in pancreatic beta cells by exposure to IL-1 β was faster and more pronounced than with exposure to TNF- α . These findings show that pancreatic beta cells respond differently to fibroblasts when exposed to cytokines and also respond differently to the two very similar major early response cytokines: IL-1 β and TNF- α (Ortis et al. 2006). In the context of this study, it is interesting that studies of the relationship between the UPR and NF- κ B suggests that in the early UPR there is activation of the NF- κ B system, while in later stages of the UPR, NF- κ B is inhibited (Kitamura 2011).

Free fatty acids are inducers of endoplasmic stress and apoptosis in pancreatic beta cells (Cnop et al. 2010), and it is thought that pro-inflammatory cytokines act in the same manner. IL-6 is an interesting cytokine with both pro- and anti-inflammatory functions, and it has been reported that this cytokine can block the pro-apoptotic effects of pro-inflammatory cytokines on pancreatic beta cells (Choi et al. 2004). However, IL-6 was unable to protect beta cells against

palmitate-induced apoptosis because it was unable to inhibit this particular form of UPR activation (Nicoletti-Carvalho et al. 2010). This shows the subtleties of the ER/UPR.

ER stress can be induced by a variety of factors including oxidants. In the fibrosarcoma cell line L929, TNF induces the production of ROS, and this is related to induction of the ER/UPR with activation of PERK and IRE1. This contrasts with hydrogen peroxide, which does not activate these key ER/UPR signalling proteins (Xue et al. 2005). In contrast, while injected LPS can induce the complete panoply of endoplasmic stress in the hypothalamus, it was found that TNF- α only promoted a partial ER stress response (Denis et al. 2010). This may be linked to a study of the anticancer compound Bortezomib (Velcade), which is a selective and potent inhibitor of the proteasome (Adams and Kauffman 2004). In spite of this, the therapeutic efficacy of this compound is limited, and it was thought that a combination of bortezomib and TNF- α may produce greater clinical effect. Using a colon cancer model in mice, the combination of TNF- α and bortezomib caused up-regulation of the ER stress proteins BiP, PDI and calnexin. TNF- α also decreased the up-regulation of Hsp27 caused by bortezomib. These effects were associated with decreased tumour growth and increased survival of animals (Nowis et al. 2007).

K-7174 is a homopiperazine compound with a number of interesting inhibitory properties through its ability to inhibit the transcription factor GATA-2 (Majik et al. 2012). This GATA-2 protein is a haematopoietic transcription factor with essential functions in development of bone marrow cell populations. K-7174 is being developed as an anti-inflammatory agent that decreases cellular responses to cytokines in target

cells. The ability of TNF- α to stimulate the chemokine CCL2 and NO was abrogated by K-7174, and this inhibition was associated with the induction of the UPR in the glomerular podocytes being studied. This led to the finding that a variety of compounds (tunicamycin, thapsigargin, A23187, etc.) able to induce the UPR response, could also block the induction of CCL2 and NO. This suggests a role for the UPR in controlling cytokine-induced inflammation (Takano et al. 2007). Similar effects were seen in glomerular mesangial cells with the anti-ulcer compound geranylgeranylacetone, which is also a known heat shock protein inducer (Ooie et al. 2001). This compound could also block the synthesis of CCL2, and activation of NF- κ B, induced by pro-inflammatory cytokines (Hayakawa et al. 2008).

Inhibition of prostanoid synthesis, through inhibition of cyclooxygenase by the use of non-steroidal drugs, dates back to the late nineteenth century, and it would be thought that we would know all about these agents. However, the CCL2 synthesis induced by TNF- α in murine podocytes is blocked by indomethacin, but not by ibuprofen. This anti-inflammatory action is related to the induction of key UPR markers including upregulation of Grp78/BiP and CHOP and repression of ER stress responsive alkaline phosphatase. That the action of indomethacin was not related to inhibition of cyclooxygenase was confirmed by the fact that two other very different non-steroidal agents, aspirin and sulindac also failed to induce the UPR. Again, the suppressive effect was mimicked by other UPR inducers (thapsigargin, A23187, etc.). The effect of indomethacin appears to be related to the induction of Grp78/BiP and the degradation of TNF- α receptor-associated factor (TRAF)2 (Okamura et al. 2008). TNF- α , in addition to inducing an ER/UPR, and thus generate apoptotic signals, also can interact with the mitochondrion, and thus, both ER and mitochondrial apoptotic pathways can be generated by this cytokine leading to cell death (Li et al. 2008).

Acute phase protein synthesis is a key indication of the presence of inflammation in an animal. CREBH is a liver-specific transcription factor that is cleaved with ER stress and activates expression of the acute phase response genes. Pro-inflammatory cytokines such as IL-1, TNF- α , and IL-6 are major inducers of the acute phase response, and this is now seen to be through activation of the UPR and the cleavage of CREBH (Zhang et al. 2006).

The above reveals that the key early response, pro-inflammatory cytokines (IL-1 β and TNF- α) can be associated with the induction of the ER/UPR. Clearly, much more work is needed to determine exactly how exogenous, and possibly also endogenous (e.g. IL-1 α —Maier et al. 1994) cytokines, control cellular UPRs. Is there evidence that the ER/UPR can exert control over the cytokine networks produced by cells? An early report suggested that ER stress activated NF- κ B by a pathway distinct from the conventional pathway and involving

Grp78/BiP (Pahl and Baeuerle 1995). Later studies reported that induction of ER stress in various cell lines could stimulate the synthesis of pro-angiogenic vascular endothelial cell growth factor (VEGF) (Abcouwer et al. 2002) as well as expression of pro-angiogenic cytokines such as IL-8 (Marjon et al. 2004). Thapsigargin was reported to increase transcription of the genes encoding VEGFA, FGF2, angiogenin, and IL-8 in various human medulloblastoma cell lines. Both ATF4 and XBP-1 were shown to be responsible for VEGF synthesis (Pereira et al. 2010). Microarray analysis of glucose-deprived tumour cells found that there was up-regulation of pro-angiogenic cytokines (VEGF, FGF-2, and IL-6) and down-regulation of cytokines that are associated with inhibition of angiogenesis (CXCL10 and CXCL14). The VEGF transcription was due to binding of ATF4. Knockdown of PERK led to decreased tumour growth and in tumour angiogenesis (Wang et al. 2012a, b). It is not clear if the UPR and angiogenic cytokines are an obligate specific pairing in physiological angiogenesis.

Monocytes/macrophages are key immune cells involved in both innate and acquired immunity. The lifestyle of the macrophage would suggest that it is one of the most stressed cells in the body (Spletstoeser and Schuff-Werner 2002). What do we know about the relationship between ER stress and cytokine synthesis/secretion with this cell population? Macrophages are key cells in responding to bacterial signals such as LPS. Induction of the macrophage UPR is associated with a log order increase in the synthesis of IFN- β by LPS-stimulated macrophages (Zeng et al. 2010). This enhanced expression of IFN- β required participation of XBP-1, but this did not bind to the *ifnb1* promoter but to another site 6 kb downstream, which acts as an enhancer of the *ifnb1* promoter (Zeng et al. 2010).

HIV protease inhibitors induce ER stress and the UPR in macrophages and increase synthesis of IL-6 and TNF- α by modulating the intracellular RNA binding protein HuR (ELAV1). The cytosolic translocation of HuR and binding to the 3' untranslated region (UTR) of the mRNAs of IL-6 and TNF- α is dependent on activation of ERK. In addition, cytokine synthesis does not take place in CHOP null cells (Chen et al. 2009).

IL-12 is a major macrophage cytokine involved in the differentiation of naive CD4 T lymphocytes into antigen-specific Th1 lymphocytes. It has been reported that the Cox-2 inhibitor Celecoxib (CE), induces tumour cell apoptosis in a Cox-2 independent manner involving inhibition of SERCA and induction of ER stress through inhibition of SERCA (Schönthal 2007). This effect has an interesting consequence in the inhibition of the folding of the two subunits of IL-12 and the inhibition of the secretion of this cytokine, without influencing the transcription of the encoding gene (Alloza et al. 2006). Secretion of the structurally related cytokine, IL-23, which shares the p40 subunit, is also blocked. Analysis

of the ER chaperones in drug-treated cells identified a seven-fold increase in transcripts for homocysteine-inducible ER protein (HERP), which forms part of the protein complex required for ER-associated protein degradation. The CE analogue, 4-trifluoromethyl CE, causes the IL-12 dimer and IL-23 to associate with HERP and leads to their degradation. This suggests an interesting target for low molecular mass anti-cytokine therapeutics (McLaughlin et al. 2010). In this context, one of the more relevant pathological inducers of the UPR is the misfolding of HLA-B27, a MHC class I allele that is a major risk factor for developing spondylarthritis (Turner et al. 2005). It is therefore curious to find that enhanced IL-23 secretion occurs in LPS-stimulated macrophages in which a UPR had been induced by HLA-B27 unfolding or by use of pharmacological agents. Such HLA-B27 unfolding could contribute to the inflammatory aspects of spondylarthritis (DeLay et al. 2009). However, a later study reported that circulating monocytes from patients with spondylarthritis produced significantly more IL-23 than did monocytes from healthy controls. Moreover, assay of ER stress in the patient's monocytes failed to find any significant increase in signs of an UPR (Zeng et al. 2011). This does not fit with the earlier studies, suggesting that the system is more complex than was previously thought. Indeed, another example of disease associated with ER protein misfolding are the serpinopathies (Roussel et al. 2011) in which mutation in the protease inhibitor superfamily, the serpins, results in misfolded proteins and protein aggregation in the ER. Curiously, the generation of such misfolded proteins in the ER stimulates NF- κ B by a mechanism that is independent of the ER/UPR, but which is regulated by intracellular calcium levels (Davies et al. 2009).

Clearly, there is a relationship between the ER/UPR and cytokines/cytokine synthesis. However, the data to date does not lead to a clear mechanism for the interaction of the genes involved in UPR and those involved in cytokine synthesis and the consequences of cytokine synthesis.

Intracellular cell stress proteins controlling responses to cytokines

Another level of integration between cell stress proteins and cytokines involves the role of these proteins in cellular responsiveness to cytokines and cytokine-inducing agents. The small heat shock protein, Hsp27 (Hsp25 in mice), was identified as a protein phosphorylated by a cytoplasmic protein kinase in IL-1 β -treated fibroblasts (Guesdon and Saklatvala 1991). TNF- α was also shown to induce phosphorylation of this cell stress protein (Guy et al. 1993) as did IL-6 (Belka et al. 1995). IL-1 β was then shown to be inducing a protein kinase cascade to phosphorylate Hsp27 that involved the, then, novel MAP kinase, p38 (Freshney et al. 1994). The role of Hsp27 in IL-1 and TNF- α signalling has been examined

using RNA interference to deplete cellular Hsp27. TNF-induced Hsp27 phosphorylation was then shown to enhance the association of Hsp27 with IKK β , resulting in decrease in the activity of this protein and thus inhibiting NF- κ B (Park et al. 2003). Overexpression of Hsp27 enhances the proteosomal degradation of ubiquitinated proteins in cells exposed to TNF- α and IL-1 β including proteins involved in inhibiting NF- κ B (Parcellier et al. 2003). Knockdown of Hsp27 blocked the migration of macrophages induced by the chemokine CXCL12 (Rousseau et al. 2006).

Another cell stress protein which appears to have a role in controlling cytokines is Hsp90. Note that this can be a two-way process with, for example, IL-6 inducing Hsp90 α synthesis (Stephanou et al. 1998). Thus, the low molecular mass Hsp90 inhibitor, geldanamycin was reported to inhibit the synthesis of IL-1, IL-6 and TNF- α in LPS-stimulated RAW 264.7 murine macrophage cells, not by inhibiting gene transcription but by causing decreased stability of transcripts (Wax et al. 2003). Inhibition of Hsp90 appears to influence a range of transcriptional systems involved in cytokine induction. Thus, it has been reported that through use of inhibitors, Hsp90 has effects on the NF- κ B activator IKK and thus on NF- κ B signalling generally (Broemer et al. 2004; Salminen et al. 2008). Hsp90 inhibition also inhibits expression of the oncogene akt as well as IKK in murine monocytes (Shimp et al. 2012).

These are not purely in vitro effects as administration of another Hsp90 inhibitor (SNX-7081) to mice with collagen-induced arthritis, a model for rheumatoid arthritis, significantly inhibited all aspects of disease (Rice et al. 2008)—a result associated with inhibition of inflammatory cytokine synthesis. Similarly, LPS-induced liver damage has been reported to be inhibited by administration of the geldanamycin analogue, 17-dimethylamino-ethylamino-17-demethoxygeldanamycin (17-DMAG), and to result in lower levels of circulating pro-inflammatory cytokines (Ambade et al. 2012). CHIP assays found that 17-DMAG increased HSF1 binding to the TNF- α , but not IL-6 promoter and that IL-6 inhibition is indirectly blocked by induction of ATF3. Knock-down of HSF1 prevented this inhibition of cytokine synthesis and associated down-regulation of NF- κ B, thus relating Hsp90, HSF1 and pro-inflammatory cytokine synthesis (Ambade et al. 2012). A caecal-ligation and puncture model in the mouse, which mimics human polymicrobial sepsis, was found to be inhibited by the Hsp90 inhibitor radicicol. Interestingly, such treatment had no influence on colonic TNF- α levels but was associated with significant decreases in levels of CXC chemokines (Zhao et al. 2013).

Geldanamycin also inhibits the IL-2-induced proliferation of murine CTLL-2 cells (a murine cytotoxic T cell line) by blocking the phosphorylation of the transcription factor STAT5 and revealing a role for Hsp90 in this process (Xu

et al. 2004). Another cytokine transcription factor shown to be controlled by Hsp90 is STAT1, which is recruited to receptors for the various interferons and accounts for alterations in IFN- γ signalling in alveolar macrophages (Howard et al. 2010). To show the complexity of the system, a recent report has suggested that Hsp90 also influences the activity of T regulatory (T regs) lymphocytes (de Zoeten et al. 2011).

These few papers clearly show that these two intracellular cell stress proteins are having major effects on cytokine signalling and, by inference, on cytokine network control. This suggests that other cellular cell stress proteins may also influence cellular responses to cytokines and cytokine inducers.

Conclusions

The term ‘cytokine’ describes a large group of proteins which integrate the cells of the body and, in particular, control the complex cellular behaviours that is immunity and inflammation. Interference with the networks of homeostatic interactions that cytokines generate is a major cause of human idiopathic disease. The true importance of cytokines is only now being realised with the introduction of cytokines and cytokine inhibitors into clinical practice (Wilson and Barker 2013). As an example, the intractable chronic inflammatory disease, rheumatoid arthritis, can now be managed by use of biological agents, which neutralise the early response cytokine, TNF- α (Navarro-Millán and Curtis 2013). Further advances in our understanding of how to control cytokine networks, requires that the endogenous controls over cytokine synthesis be identified. Evidence emerging in the late 1980s revealed the unexpected finding that cell stress proteins could be secreted from cells and could function as cell-signalling agonists with a particular propensity to induce the formation of cytokines. At roughly the same time evidence for a relationship between cell stress, the induction of cell stress proteins and the stimulation of cytokine synthesis emerged. Over the past 20 years evidence has accumulated to suggest that: (1) exposure of cells to certain forms of stress can induce cytokine synthesis as well as the synthesis of cell stress proteins; (2) selected intracellular cell stress proteins (e.g. Hsp27, Hsp70, Hsp90) function to control cellular cytokine synthesis in response to various extracellular agonists including cytokines; (3) cell stress proteins can promote the formation of cytokines by various cell populations and these proteins can induce either pro- and/or anti-inflammatory cytokine networks; (4) cytokines can induce the expression of various cell stress proteins, although the exact mechanisms of this control are only partially understood; and (5) interactions between cytokines and cell stress proteins with cells is likely to result in the secretion of both induced cytokines and cell stress proteins. The importance of this latter process has recently been

exemplified by the finding that secretion of the ubiquitin analogue, ISG15, in humans, is essential for protection against environmental mycobacteria. This is due to the ability of secreted ISG15 to induce formation of the anti-mycobacterial cytokine, IFN- γ (Bogunovic et al. 2012).

The literature is currently unable to generate a coherent picture of the interactions that are occurring between the >100 cell stress proteins (assuming all such proteins can be secreted) and the >100 cytokines that are known to be synthesised by eukaryotic cells. If these proteins, and their associated transcriptional and translational control pathways, are truly part of a network of interactions then there must exist an enormously complex system connecting the stress response of cells with their expression of cytokine networks. It is interesting to compare cell stress proteins with cytokines in terms of their cellular behaviours. The current, albeit limited, literature on cell stress proteins would support the hypothesis that, like cytokines, cell stress proteins also exhibit the properties of redundancy and pleiotropy, plus having effects on many cell types. This supports the earlier proposal of the author that cell stress proteins should be classified as *stress cytokines* (Panayi et al. 2004).

Based on the evidence presented in this review, the authors predict that the control of cell behaviour in the multicellular organism is controlled, at the local (paracrine/autocrine) level not simply by networks of interacting cytokines, but by an interacting network of both cytokines and cellular/secreted cell stress proteins. This network will clearly be dependent on local levels of stress adding on to the existing cell stress protein/cytokine reciprocal networking behaviours. In addition, increased levels of secreted cell stress proteins in times of stress will also be reflected in increased levels of intracellular cell stress proteins, which will also influence the intracellular control systems on cytokine synthesis and on cytokine reception by cells. As we know very little about the control of cytokine networks by cells this hypothesis suggests that cellular control has to be very much more complex than we ever expected it to be. In spite of this, if we look at controlling cell aberrant behaviour due to disease, it is clear that removal of one cytokine (e.g. TNF- α ; Piguet et al. 1992) or one cell stress protein (e.g. Hsp90; Rice et al. 2008) can block the enormously complex process that we call rheumatoid arthritis (or in these cases, the mouse equivalent—collagen-induced arthritis). This suggests that human disease states will have a new range of additional therapeutic targets in terms of the blockade of cell stress proteins—both intracellularly and extracellularly. This distinction between intra- and extra-cellular cell stress proteins may introduce more specificity into future treatment for human disease states.

The key problem to understanding cytokine/cell stress protein interactions is generating the experimental and theoretical tools to comprehend such enormous cellular and molecular complexity. It is possible that this complexity will

be exacerbated by the fact that each cell stress protein may exhibit a range of distinct, and unexpected, biological actions. This turns out to be the situation with the Hsp60 (HSPD1) family of proteins, which exhibit a simply incredible range of biological actions ranging from enzymes, to transcription factors to insect toxins to apoptotic controllers. Indeed, the human protein alone has well over a dozen different biological actions (Henderson et al. 2013). These additional functions have been termed moonlighting functions and Hsp60 is one of a handful of proteins, which exhibit such multiple functions. If all cell stress proteins exhibit this multiplicity of function then the complexity of cell stress protein/cytokine interactions becomes simply mind-boggling.

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