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Role of hsp70 in cytokine production

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Abstract. Interleukin-1 and tumor necrosis factor-alpha are potent, multifunctional cytokine mediators of inflammation and immune responses that are produced primarily by activated monocytes and macrophages. Three published papers by different groups have shown that heat shock and chemical stress with heavy metal salts or sulfhydryl reagents, all of which induce the expression of heat shock protein 70 (hsp70), concomitantly inhibit the production of these cytokines in human monocytes and mouse macrophages activated by lipopolysaccharide. These papers are reviewed and discussed in some detail. Other studies suggest that various anti-inflammatory drugs, including acetylsalicyclic acid, auranofin and dexamethasone, can also facilitate HSP expression in macrophages. However, while these studies are interesting, it is clear that not a great deal of work has been done and/or published in this area. Since many pharmaceutical companies are developing cytokine synthesis inhibitors as potential anti-inflammatory drugs, one aim of this article is to emphasize that understanding the molecular mechanism(s) that lead to increased HSP expression and decreased cytokine biosynthesis may assist in achieving this goal.
Key words. hsp70; cytokines; macrophages; anti-inflammatory drugs.

Introduction

Heat shock proteins

A nearly universal cellular response to a variety of environmental stresses or unfavorable conditions is the rapid expression of families of related and highly conserved proteins called heat shock proteins (HSP). In fact, many HSP are constitutively expressed and they play an essential role as molecular chaperones in the folding of newly synthesized proteins in cells. When a cell is subjected to stress, it is thought that HSP play a similar role in protecting proteins, and that they assist in the refolding of damaged proteins or facilitate the degradation of proteins damaged beyond repair. The importance of the heat shock response has been elegantly demonstrated by the lack of survival of heat-shocked fibroblasts microinjected with antibodies against hsp70 (ref. 27). The stress response can be induced in mammalian cells cultured in vitro by a wide variety of factors including temperatures a few degrees above 37 °C (heat shock), toxic chemicals, oxidative stress due to anoxia or reactive oxygen intermediates etc. and this review will concentrate on in vitro studies using mouse and human cells of the macrophage lineage. However, it is clear that fever caused by bacterial or viral infections, oxidant injury and inflammation also induce heat shock responses in vivo. For further general reading on the subject of HSP see references 4, 15, 20, 21, 23 and this series.

Cytokines

Cytokines are proteins important for the regulation and maintenance of immunity and hematopoeisis. They usually act locally as autocrine or paracrine cellular signals having pleiotropic effects on many cell types. The central role played by cytokines in mediating a number of physiological and pathological mechanisms makes them an important area of both basic and applied biomedical research[7, 19, 32]. The current article will centre around two cytokines, interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α). These are potent, multifunctional mediators of inflammation and immune responses that are produced primarily by activated monocytes and macrophages.

Interleukin-1. Two forms of IL-1, α and β, that are encoded for by distinct genes and which have quite dissimilar sequences are produced, however they share most biological properties (reviewed in refs 8, 10). Both IL-1α and β lack a typical secretory signal peptide that allows transport into the endoplasmic reticulum. IL-1α is thought to remain cell-associated, while IL-1β has been shown to be released through a novel secretory pathway[29]. Both forms of IL-1 are synthesized as 33 kD precursor molecules that are processed and secreted as 17 kD fragments. Pro-IL-1α is active unlike pro-IL-1β, where processing by a recently described IL-1 converting enzyme (ICE) yields the bioactive 17 kD fragment[17]. The processing of pro-IL-1β by ICE appears to be coupled to its secretion from the cell. Both forms of IL-1 bind to the same high affinity receptor that is expressed on many cell types and mediates the pleiotropic effects of IL-1 on various cells and tissues.

Tumor necrosis factor-α. TNF-α was originally identified by its ability to cause necrosis of tumors in animals, but now it is recognized to play an important role in immunoregulation, inflammatory diseases, and in antimicrobial and antiviral defence[3, 24, 32]. TNF-α is

synthesized as a 26 kD precursor that is processed and secreted as a 17 kD protein produced by activated macrophages, lymphocytes and mast cells, whereas the related TNF-β is a 25 kD protein produced only by lymphocytes (hence its original name of lymphotoxin). However, as with IL-1α and -β, both TNF-α and -β bind to the 55 kD and 75 kD TNF receptors that mediate the diverse effects of TNF on different cell types. Recent findings have shown that TNF-α is the major cytokine involved in the development and maintenance of arthritis in animal models and in man[11,34].

Hsp70 and cytokine production

Only three full reports on the effect of heat shock or chemical stressors on cytokine production have been published and these are reviewed below.

1) The first published report, by Dinarello et al. in 1986[9], examined the effect of heat shock on the production of various cytokines (assessed in bioassays) by stimulated human peripheral blood mononuclear cells. Consistent with the later molecular studies described below, this group showed that the production of IL-1 by LPS-stimulated monocytes was decreased after incubation at 39 °C for 24 h. In this study, IL-1 and IL-2 concentrations in supernatants were assessed in a thymocyte proliferation bioassay. In order to examine the effect of heat shock on IL-2 (T cell growth factor) and granulocyte-macrophage colony stimulating factor (GM-CSF) production, enriched peripheral blood T cells were stimulated with phytohaemagglutinin (a T cell mitogen) at 39 °C for 3 days. IL-2 levels in supernatants assayed by the thymocyte proliferation assay, were decreased in heat shocked cultures by 50%, and GM-CSF levels assessed in a bone marrow colony-forming assay were decreased by 60–80% at 39 °C compared to cells cultured at 37 °C. This study also rather surprisingly showed that the production of IL-1 and IL-2 was greater at 34 °C than at 37 °C, although the production of GM-CSF was similar at both temperatures. Interestingly, heat shock at 39 °C was shown to increase cytotoxic T cell (Tc) induction and killing activity. Therefore the febrile reactions that are often seen in cancer patients given cytokines such as IL-2 in order to stimulate anti-tumoral Tc activity may enhance the effectiveness of such therapy.

2) The report published by Schmidt and Abdulla in 1988[30], may well become a seminal paper in this field. The majority of their work used the human myelomonocytic cell line THP-1 which synthesizes pro-IL-1β (subsequently referred to as p35 as in ref. 30) in response to stimulation with LPS. As shown in figure 1a, incubation of L-[^{35}S]methionine-labelled THP-1 cells with LPS at 39 °C or 41 °C for 4 h stimulates an accumulation of hsp70 and hsp90 in whole cells. On the left side of figure 1a, it can be seen that cells incubated with (+) LPS at 37 °C express p35 as shown by immunoprecipitation with an anti-IL-1β antiserum, but not with preimmune serum (P). As expected, p35 was not expressed in the absence (−) of LPS stimulation. At 39 °C the amount of p35 is reduced compared to 37 °C and at 41 °C, p35 is undetectable. These decreases in p35 levels clearly correlate with the increase in HSP expression. The decrease in cellular p35 was not due to increased secretion of pro-IL-1β or processed IL-1β from the cells, since supernatant levels of any form of IL-1β were not detectable under any of the experimental conditions. The immunoprecipitated p35 bands were quantitated by scanning densitometry and plotted relative to the value of cells at 37 °C + LPS (= 100%) as shown in figure 1b, which also shows whole cell protein levels determined by trichloroacetic acid precipitation of [^{35}S]methionine-labelled proteins. The decrease of p35 levels at 39 °C and 41 °C appears to be a selective effect since whole cell protein levels do not decrease at these temperatures. At 43 °C very little cellular protein is detectable, presumably due to cell death and lysis at this temperature. Importantly, it was shown that increasing the temperature did not prevent THP-1 cell responsiveness to LPS, since heat also negatively regulated p35 levels in LPS preinduced cells. In addition, enhanced degradation of p35 at higher temperatures was ruled out by pulse-chase experiments that indicated a constant half-life of 2.5 h for p35. The effect of heat on IL-1 biosynthesis was shown to be at the transcriptional level since IL-1 mRNA levels were found to be markedly reduced.

Certain chemicals, including heavy metals e.g. Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Hg^{2+}, and sulfhydryl reagents such as sodium arsenite and iodoacetamide, have been shown to induce the heat shock response[18,21]. Schmidt and Abdulla also examined the effect of such chemical inducers of the heat shock response on p35 biosynthesis in LPS-stimulated THP-1 cells and showed that, as with heat, there was a strong correlation between increased hsp70 and hsp90 expression and a decrease in p35 biosynthesis that was not due to cytotoxicity as shown by normal levels of total cellular protein. In their studies with chemicals, it was of particular interest that a gold salt, auranofin, which is currently used for the therapy of chronic inflammatory diseases such as rheumatoid arthritis[1], also induces hsp70 and inhibits p35 synthesis, perhaps indicating the mechanism of action of this compound. Another gold salt, aurothiomalate, did not have this effect on hsp70 and p35 levels. However, auranofin is an orally active compound where the gold moiety is more freely available to cells which may explain its greater effects on HSP induction. Finally, it is noteworthy that induction of hsp70 and decreased synthesis of p35 was also demonstrated with normal human peripheral blood monocytes, although the effect was not as pronounced as in THP-1 cells.

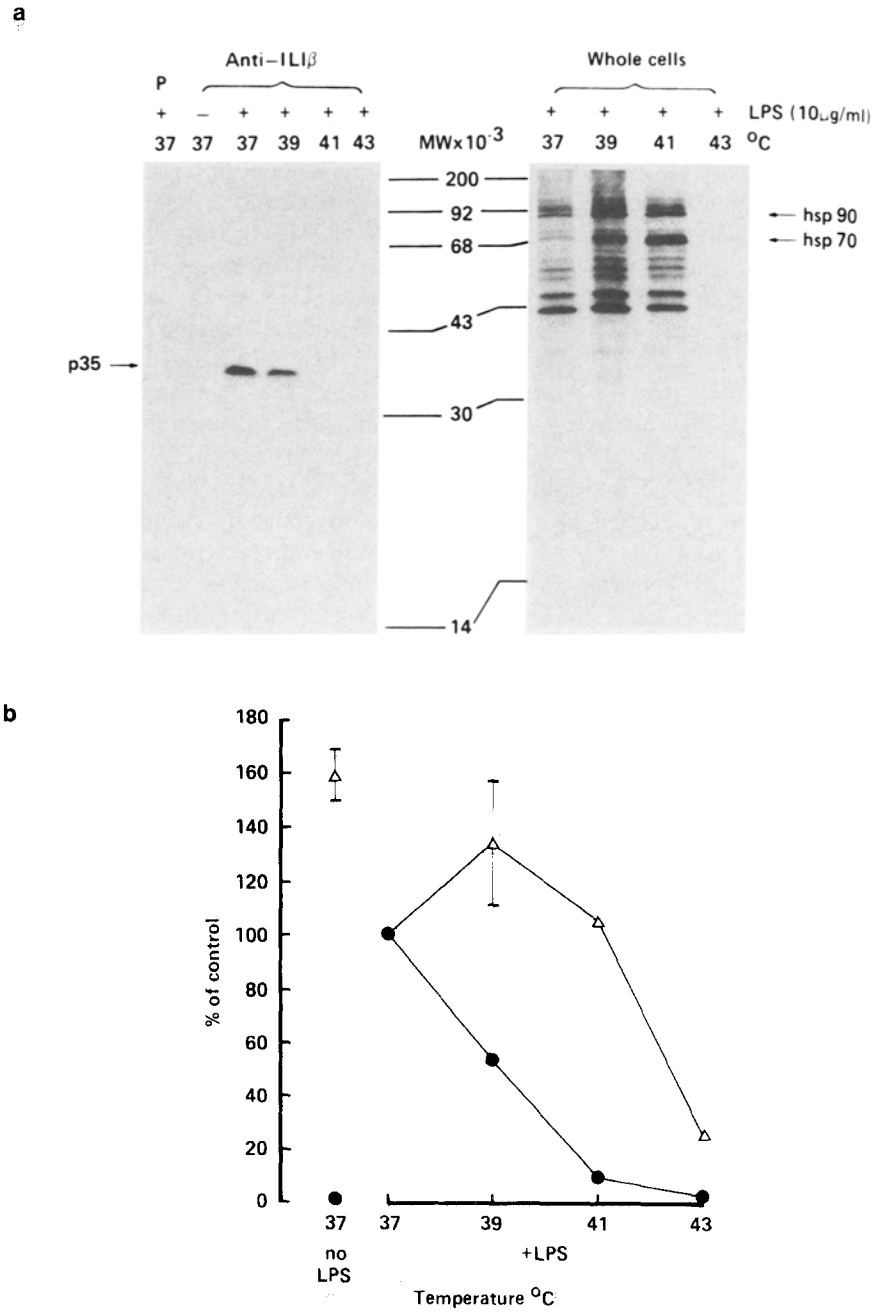

Figure 1. The effect of heat on LPS-induced IL-1β biosynthesis in THP-1 cells.
a The left panel shows fluorographs of SDS-PAGE analysis of immunoprecipitates of the p35 IL-1β precursor from cells stimulated with LPS at various temperatures. P is a preimmune serum control of the 37 °C + LPS sample. The right panel shows SDS-PAGE analysis of the corresponding whole cell proteins.
b The immunoprecipitated p35 bands in the left hand panel of *a* were quantitated by scanning densitometry and the integrals are plotted relative to the value from cells at 37 °C + LPS (●). [^{35}S]Methionine incorporation into whole cell protein was estimated by trichloroacetic acid precipitation and similarly compared at the various temperatures (△). The error bars show the variation between duplicate samples when greater than the size of the symbols. (Reproduced from reference 30 by kind permission of Dr. J. A. Schmidt and the Journal of Immunology. Copyright 1988, The Journal of Immunology.)

The results of this study convicingly demonstrate that induction of the heat shock response inhibits IL-1 biosynthesis in human monocytes. This effect was seen at physiologically relevant temperatures, suggesting that fever caused by cytokine release in response to viral or bacterial infections may be self-limiting. In this respect it is intriguing to remember that visits to hot spas were recommended for arthritis in earlier times and that heat pads, massage and heat ointments are palliative in such inflammatory diseases.

3) Finally, Snyder et al. in 1992[31] reported that heat shock at 45 °C for 12 min produced a reciprocal in-

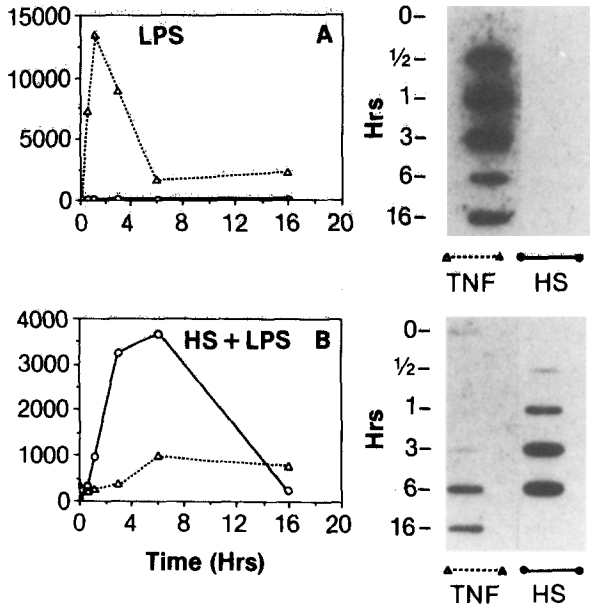

Figure 2. Kinetic analysis of TNF versus hsp70 mRNA levels after heat shock. Control *A* or heat shocked (HS) macrophages *B* were stimulated with LPS and lysed at 0, 0.5, 1, 3, 6 or 16 h later. 5 µg RNA for each time point was slot blotted and hybridized with oligonucleotide probles for hsp70 and TNF. Blots were scanned and counts quantitated by a Betagen blot analyzer. (Reproduced by kind permission of The Journal of Leukocyte Biology.)

crease in expression of hsp70 and a decrease in TNF-α and IL-1 production by mouse peritoneal macrophages stimulated with LPS. In slot blot analysis using oligonucleotide probes specific for hsp70, cytokines and β-actin, it was shown that the mRNA induction of both TNF-α and IL-1 was reduced by up to 60-fold in heat shocked macrophages stimulated with LPS, while mRNA for hsp70 increased 30-fold. In contrast to these dramatic changes, mRNA levels for β-actin were relatively constant, indicating a selective effect on LPS-induced cytokine mRNA transcription. As shown in the top panel of figure 2, LPS-stimulated macrophages at 37 °C express very low levels of hsp70 mRNA but show a rapid increase in TNF-α mRNA levels which peak at 1 h and decline by 6 h. In contrast, heat shocked LPS-stimulated macrophages express high levels of hsp70 mRNA that peak 6 h and have declined by 16 h, while TNF-α mRNA induction is strongly inhibited at all time points (lower panel of fig. 2). However, it can be seen from figure 2 that the suppression of TNF-α mRNA preceded the accumulation of hsp70 mRNA by 30 min, indicating that cellular events preceding hsp70 induction are involved in the suppression of TNF-α mRNA expression. These results are consistent with regulation of TNF-α at the transcriptional levels, as was demonstrated for IL-1β in human monocytes[30]. It was also shown that heat shock did not significantly affect the ability of macrophages to phagocytose antibody-coated erythrocytes or latex particles. Therefore, the effect of heat shock on LPS-stimulated macrophage effector functions is relatively specific for inhibition of cytokine transcription and stimulation of hsp transcription, while phagocytic function is unaffected. Although the heat shock used in these experiments was severe (12 min at 45 °C), the authors noted that exposure of cells to α- or β-interferon has been shown to reduce the temperature required to induce heat shock[22]. The expression of interferon may well occur during febrile reactions in inflammatory microenvironments with elevated cytokine levels and may therefore facilitate inhibition of cytokine production by macrophages at physiologically elevated temperatures. Interestingly, it was noted in the discussion of this paper that the role of anti-inflammatory agents in modulating hsp70 levels represents an area for further investigation.

Discussion

These 3 reports show that heat shock or chemical stress that induce an HSP response concomitantly inhibit IL-1β and TNF-α production at the transcriptional level. However, it would appear that HSP themselves may not be directly involved in the inhibition of cytokine production, since the time course studies of Snyder et al. show that inhibition of TNF-α mRNA expression precedes the expression of hsp70 mRNA by at least 30 min (see fig. 2 and ref.[31]). Schmidt and Abdulla[30] noted that there are sequences upstream of the IL-1β gene that are similar to heat shock response elements. In addition, serum regulatory elements that control HSP gene expression are also found upstream of the IL-1β gene and, interestingly, in the IL-2 gene which is also regulated by elevated temperatures as described above[9]. There has been a considerable increase in the number of transcription factors identified and understanding of their interactions with gene regulatory elements in the last few years. For example, an AP-1 binding site has been identified at position −2892 upstream of the IL-1β gene[2]. In addition, interferon response elements have been identified upstream of both the IL-1β and TNF-α genes[33]. It seems likely that a common mechanism(s) will be identified that is responsible for enhancing HSP and inhibiting cytokine gene expression.

It is noteworthy that a number of reports have shown that mitogens, bacterial products such as LPS and cytokines themselves can stimulate heat shock expression in lymphocytes and monocytes (reviewed in ref. 26). Thus, IL-2 was shown to induce hsp70 mRNA and protein synthesis, indicating a role for hsp70 in T lymphocyte cycle progression[12]. In human monocytes, TNF, LPS and also adherence to plastic were shown to induce hsp70 (interferon-γ and GM-CSF had no effect), indicating that HSP induction may be associated with monocytic differentiation[13]. In addition, cytotoxicity induced by TNF-α and the toxicity of IL-1 to pancreatic

β-cells can be prevented by heat shock or by introducing hsp70 into the target cells (see the article by Jacquier-Sarlin et al. in this series).

Finally, many pharmaceutical companies are interested in cytokine modulation as an approach to identifying novel anti-inflammatory drugs (reviewed in refs 6, 7, 19). Numerous cytokine inhibitors have been reported e.g. tenidap (Pfizer; ref. 25), SKF-105,685 (SmithKline Beecham), IX 207–887 (Sandoz)[6], CGP 47969A (Ciba-Geigy; ref. 16) and RP 54745 (Rhone-Poulenc Rorer ref. 14), but their mechanism(s) of action are not well defined. It is interesting that some of the classic anti-inflammatory compounds including aspirin, salicylate, indomethacin and dexamethasone, have recently been reported to induce HSP synthesis directly or to facilitate the heat shock response at 39 °C in human peripheral blood mononuclear cells[5]. In fact Ritossa, who first described the heat shock response in 1962, noted that sodium salicylate mimiced the heat shock response in drosophila[28]. As noted above, HSP may not directly modulate cytokine production, but identification of the molecular mechanisms involved in HSP induction may reveal novel molecular targets for the development of drugs that inhibit cytokine production.

Note added in proof

A recent paper by Ensor et al.[(1)] reported the effect of heat shock at 40 °C on the production of TNF-α and IL-6 (another multifunctional cytokine involved in inflammatory responses and haematopoiesis) by LPS-stimulated human peripheral blood monocytes. The results confirmed previous reports showing that TNF-α production was inhibited by an effect at the transcriptional level, and there was a concomitant 75-fold increase in inducible hsp72 expression. However, IL-6 production in the same cells was unaffected by heat shock and it was suggested that IL-6 serves an antiinflammatory role since it suppresses macrophage expression of TNF-α and IL-1[(2)].

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