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Heat Shock Proteins and Regulation of Cytokine Expression

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KEY WORDS
heat shock proteins; cytokines; heat shock factor

It has become clear in recent years that heat shock proteins (hsps) play a major role in the acute inflammatory response and inflammatory diseases.[1,2] Heat shock proteins were originally characterized as a highly conserved group of proteins involved in the response of cellular organisms to stresses such as heat shock.[3,4] These proteins appear to have acquired more specialized roles within the immune response in higher eukaryotes. Heat shock proteins released into the circulation from infectious organisms or host cells can induce cytokine release from monocytes and provoke an immune response.[1,2,5] In addition, induction of the heat shock response and the intracellular accumulation of hsps appears to play an anti-inflammatory role in monocytes and macrophages, reducing the expression of cytokines and other pro-inflammatory molecules.[6–11] Transcriptional activation of the heat shock response involves the induction of heat shock factor (HSF), a sequence-specific transcription factor that binds to heat shock elements (HSE) in the promoters of heat shock genes.[12,13] Mammalian cells contain a structurally-related family of *HSF* genes, each of which binds to HSE.[13] Heat shock factor-1 (HSF1), the best characterized family member, is a constitutive protein expressed as a monomer in the cytoplasm that migrates to the nucleus when stressed, forms a homotrimer, and binds to the promoters of heat shock genes.[14,15] In this manuscript, we aim to explore mechanisms involved in transcriptional repression of cytokine genes by molecular components of the mammalian heat shock response including hsps and HSF1.

SUBJECTS AND METHODS

Northern Analysis

Total RNA was isolated from THP-1 human monocytic cells, fractionated on 1% agarose/formaldehyde gels, and transferred to membranes as described elsewhere.[9] Specific messenger RNA (mRNA) was detected by hybridization to an interleukin (IL) 1β complementary DNA probe, an oligonucleotide probe against the human tumor necrosis factor-α (TNF-α) gene, and a 2.0-kb fragment of the human β-actin gene.

Transient Transfection, Luciferase, and β-Galactosidase Assay

The IL1β promoter-luciferase reporter construct pGL3-HT contained the TATA proximal promoter region (HT fragment) of the human IL1β gene ligated into the pGL3 basic vector (Promega, Madison, WI).[9,16] Expression plasmid pHSF1, containing the human HSF1 gene inserted into the polycloning site of the pcDNA3.1 cytomegalovirus-based eukaryotic expression vector was prepared as described previously.[17] For transfection, Chinese hamster ovary K1 (CHO K1) cells were dispensed into six well plates at 2.2×10^5 cells per well and

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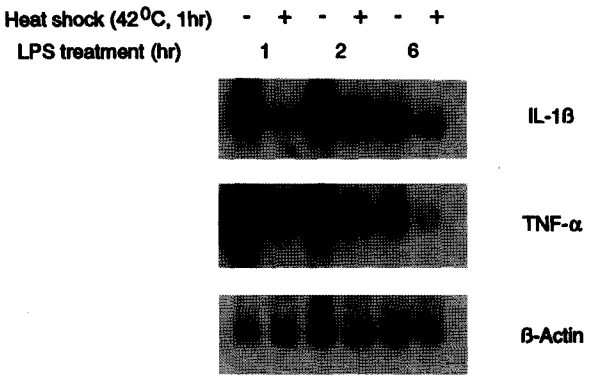

Fig. 1. Effects of elevated temperatures on expression of cytokine mRNA in human monocytes.

left for 20–24 hr prior to liposome-mediated transfection with reporter plasmids, expression plasmids, and control (pHSF-β-galactosidase expression plasmids) as described elsewhere.[17] Cell extract preparation, luciferase, and β-galactosidase assays were performed according to the manufacturer's protocol (Promega). Luciferase activity was normalized to β-galactosidase activity, which was used as an internal transfection efficiency control. Results are expressed as fold activity of control. For statistical analysis of luciferase values, ANOVA with Tukey's multiple range test was employed.

RESULTS AND DISCUSSION

Cytokine Expression Is Reduced During Heat Shock

Previous studies have indicated that cytokine expression is inhibited at elevated temperatures in human and murine cells.[6–11] Figure 1 shows the effects of elevated temperatures on the expression of IL1β and TNF-α in cells treated with bacterial lipopolysaccharide (LPS). Hyperthermia for 1 hr at 42°C led to marked inhibition of LPS induction of both genes, while the constitutive expression of the control mRNA (β-actin) was maintained (Fig. 1). The effects of heat shock were particularly marked immediately after 1 hr of heat shock. This led us to develop a hypothesis to explain temperature-dependent repression of cytokine gene expression involving the induction of HSF1 by heat shock and the repression of cytokine promoters by HSF1.[9] Activation of HSF1 by stress is a rapid, transient phenomenon that reaches a maximum at 1 hr in many cells.[10,18] However, heat shock is a pleiotropic agent and can affect gene expression at many levels.[19] We therefore examined whether HSF1 expression in the absence of stress could repress the IL1β promoter.[9] We found that both exposure to elevated temperatures and heat-independent HSF1 expression repressed the transcription of IL1β, and repression was strictly dependent on an intact consensus HSE in the IL1β promoter to which HSF1 bound.[9] This was the first demonstration of HSF1 as a transcriptional repressor and suggests a role for the factor in the counter-regulation of cytokine gene transcription. Figure 2 shows more recent data confirming these findings, indicating profound repression of IL1β by HSF1, while a non-DNA binding form of HSF1 fails to repress IL1β. We have also shown that HSF1 binds specifically to the IL1β promoter and that repression depends upon the presence of an intact HSE at position −67 within the promoter.[9] A model for repression based on competition for binding sites on the IL1β promoter between HSF1 and the essential transcription factors NF-IL6 and Pu-1/*Spi-1* seems unlikely, as repeated studies show that neither factor is displaced from the IL1β promoter by HSF1 binding. However repression did require a functional NF-IL6 binding canonical CCAAT/enhancer binding protein (C/EBP) site at −91, suggesting that repression is mediated through cooperative interactions between HSF1 and C/EBP factors on the IL1β promoter.

Studies carried out in parallel on other systems indicate that HSF1 is a versatile gene repressor (Table 1). Although the mechanism of gene repression by HSF1 is still uncertain, a number of common features have been discerned. Repression of most promoters examined so far does not require the possession of a functional HSE binding domain in HSF1 (Table 1). At least in the case of the c-*fos* gene, no evidence was obtained for binding of HSF1 to the −395 to +12 region of the promoter repressed by HSF1.[17] IL1β appears to be an exception in this respect, and IL1β repression does not occur if the HSE-like sequence in IL1β is mutated[9] or in mutants of HSF1 that do not bind HSE (Fig. 2). Our working hypothesis, therefore, is that HSF1 represses target promoters by interaction with factors involved in regulating their transcription in a mechanism that does not require HSF1 to bind DNA or displace target factors. Mutagenesis studies aimed at understanding functional domains in HSF1 involved in repression indicate that the

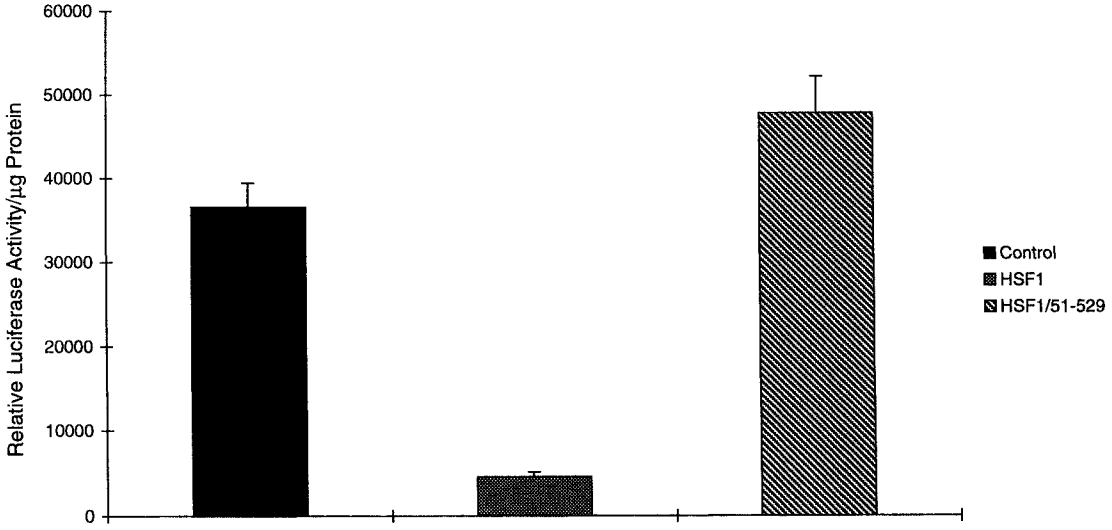

Fig. 2. Effects of overexpression of wild-type HSF1 and a mutant form ($HSF1^{51-529}$) that does not bind DNA on the activity of the IL1β promoter.

TABLE 1. Genes repressed by HSF1[a]

Gene	Species	Characteristics
IL1β	Human	DNA binding, NF-IL6
c-fos	Mouse	DNA binding independent
uPa	Mouse	DNA binding independent
c-fms	Mouse	DNA binding independent
HIV-1 LTR	Viral	DNA binding independent
SV-40	Viral	—

[a]uPa, urokinase-type plasminogen activator; HIV-1 LTR, human immunodeficiency virus type 1-long terminal repeat; SV-40, simian immunodeficiency virus type 40.

transcriptional activation domains are largely dispensable for repression and that HSF1 contains a centrally located domain involved in the repression of a number of genes (Xie Y, Zhong R, Chen C, and Calderwood SK, *in preparation*).

Although HSF1 can repress a number of quite dissimilar promoters, there is so far no indication that HSF1 can target the general transcription apparatus (Xie Y, Zhong R, Chen C, and Calderwood SK, *in preparation*). In the case of IL1β and c-*fms*, two genes involved in monocyte function, the C/EBP family member NF-IL6 is strongly implicated as a major target for HSF1(Xie Y, Zhong R, Chen C, and Calderwood SK, *in preparation*).[9] Other mechanisms have also been described in examination of interactions of the heat shock system with monocytic genes: hsp70 has been shown to repress NFkB-dependent activation of the inducible nitric oxide synthase (iNOS) gene by a mechanism involving the inhibition of nuclear localization of the factor.[11,20] It is not clear whether similar mechanisms might be involved in cytokine repression during heat shock. In addition, other factors involved in cytokine expression, such as STAT3, CREB, and AP-1, which might also be targeted for repression, have not yet been investigated. Another area in the regulation of cytokine gene expression that might be potentially targeted during heat shock is signal transduction through the MAP kinase pathways. Heat shock protein 70 has been shown to inhibit the activity of the stress inducible stress activated protein kinase (SAPK) and p38 pathways that are upstream from AP-1 and NF-IL6, respectively, and might thus impact on gene expression during the inflammatory response.[21]

Gene Repression by Heat Shock Factor 1: Potential Roles in Inflammatory Processes and as Targets for Anti-Inflammatory Therapy

Although the significance of gene repression by HSF1 in the physiological function of IL1β has not been fully addressed, our findings and results from other groups have implications in terms of IL1β regulation during whole body stress and infection and other pro-inflammatory molecules.[6–11] It should be pointed out that many of these experiments involve exposing cells to temperatures such as 42°C and above that are above the fever range. However, fever involves the exposure of cells to temperatures in the 39–40°C temperature range for extended periods (up to 80 hr), and these condi-

tions are also known to inhibit cytokine expression. The biological effects of exposure to heat shock are a function of both time and temperature; thus, temperatures of 42°C and above, although nonphysiological, may reflect the effects of a longer exposure at temperatures in the fever range.[9] Repression of IL1β by HSF-1 under fever conditions could thus play a role in limiting potentially damaging responses, such as toxic shock, fever, and inflammation, which are mediated in part by the rapid and abundant expression of IL1β in blood monocytes and tissue macrophages. Our findings, therefore, suggest a novel role for HSF1 in gene repression during the heat shock response and in the feedback regulation of cytokines in the acute phase and febrile responses. In addition, we and others have shown that pro-inflammatory agonists such as LPS and other inducers can directly activate HSF1 and induce hsp70 by unknown upstream mechanisms (Cahill C and Calderwood SK, unpublished).[22,23] By these mechanisms, IL1β and other genes may thus be subjected to feedback regulation both directly due to inflammatory mediators and indirectly by secondary induction of fever.

A role for HSF1 in antiinflammatory therapy is suggested by the finding that the nonsteroidal antiinflammatory drugs (NSAIDs) uniformly activate HSF1, suggesting a potential role for HSF1 as a target for the NSAIDs. Our previous experiments indicated that NSAIDs activate HSF1 at concentrations similar to those found in the plasma of patients treated for rheumatoid arthritis with NSAIDs by a mechanism independent of effects on the activity of prostaglandin H synthase (PGHS) 1 or 2.[24] We therefore investigated the effects of the NSAID family member salicylic acid (SAL) on the inducible expression of IL-1β mRNA in human monocytes (Fig. 3). Salicylate led to the inhibition of IL-1β expression induced by 2-hr exposure to LPS (Fig. 3). That HSF1 may be a common target in the repression of cytokines and other monocytic genes by NSAID treatment is indicated by the finding that sodium salicylate and seven other NSAID family members coordinately inhibit the LPS-induced expression of TNF-α, IL-6, IL-8, IL-10, and intercellular adhesion molecule-1 (ICAM-1), as well as IL-1β in human monocytes.[25] A role for the participation of HSF1 in this process is indicated by the finding that the NSAIDs sodium salicylate and sulindac both repress the IL1β promoter to a similar degree to HSF1 overexpression.[25] However, the NSAID family produces pleiotropic changes in cells at the concentrations used to activate HSF1; in addition, other effects of the drugs occur, such as inhibition of NFkB, making the exact role of HSF1 in this process currently uncertain.[25] Further experiments are underway to examine the role of HSF1 in feedback responses to pro-inflammatory stimuli and as a target for antiinflammatory drugs.

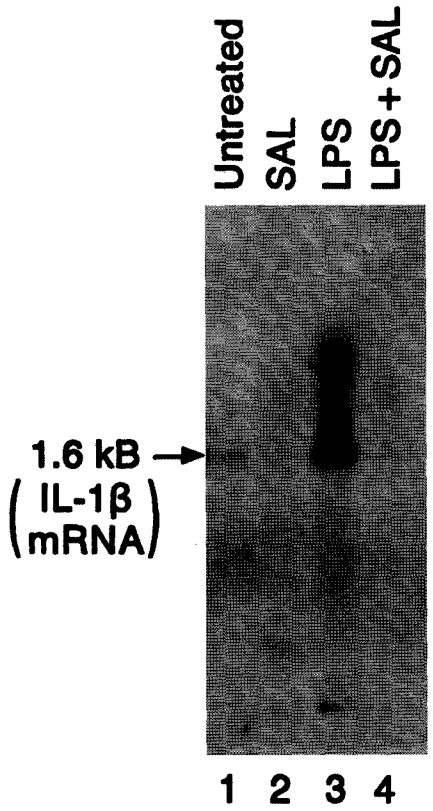

Fig. 3. Inhibition of LPS-induced IL1β mRNA expression in THP-1 human monocytes. THP-1 cells were treated for 1 hr with LPS (100 ng/mL), either with or without a 10-min pretreatment with sodium salicylate (10 mmol/L).

In conclusion, therefore, activation of the heat shock response inhibits the expression of a number of genes induced in monocytes by pro-inflammatory stimuli. A number of mechanisms may be involved in this process, including direct transcriptional repression of cytokine genes by HSF1. We have shown that HSF1 is a potent gene repressor that requires stimulation either by stress or overexpression and can then inactivate the transcription of target genes, including IL1β. Activation of HSF1 under febrile conditions or by factors re-

leased in the acute phase response (APR) may thus play a feedback role and limit the amplitude of the inflammatory response. Heat shock factor 1 may also be a potential target for pharmacological approaches and is activated by many NSAIDs under conditions in which cytokine expression and release is inhibited.

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