Heat Shock Factor 1 Represses Transcription of the IL-1β Gene through Physical Interaction with the Nuclear Factor of Interleukin 6*

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Heat shock factor (HSF) 1 is the major heat shock transcription factor that regulates stress-inducible synthesis of heat shock proteins and is also essential in protection against endotoxic shock. Following our previous study, which demonstrated the transcriptional repression of the IL-1β gene by HSF1 (Cahill, C. M., Waterman, W. R., Xie, Y., Auron, P. E., and Calderwood, S. K. (1996) J. Biol. Chem. 271, 24874–24879), we have examined the mechanisms of transcriptional repression. Our studies show that HSF1 represses the lipopolysaccharide-induced transcription of the IL-1β promoter through direct interaction with the nuclear factor of interleukin 6 (NF-IL6, also known as CCAAT enhancer binding protein (C/EBPβ), an essential regulator in IL-1β transcription. We show for the first time that HSF1 binds directly to NF-IL6 in vivo and antagonizes its activity. The HSF1/NF-IL6 interaction involves a sequence of HSF1 containing the trimerization and regulatory domains and the bZip region of NF-IL6. HSF1 has little effect on IL-1β promoter activity stimulated by the essential monocyctic transcription factor Spi.1 but is strongly inhibitory to transcriptional activation by NF-IL6 and to the synergistic activation by NF-IL6 and Spi.1. Because of its ability to bind to specific C/EBP elements in the promoters of multiple genes and its ability to interact with other transcription factors, NF-IL6 is involved in transcriptional regulation of a wide range of genes. Interaction between HSF1 and NF-IL6 could thus be an important mechanism in HSF1 regulation of generalized gene transcription during endotoxin stress.

HSF11 is the transcriptional activating protein of the heat shock genes (2). It plays an essential role in mediating the cellular response to physiological and environmental stresses including elevated temperature, ultra violet radiation, exposure to amino acid analogs, and heavy metal intoxication (2–5). During stress, HSF1 is rapidly converted from a latent monomer to a nuclear active trimeric form. Active HSF1 binds to the promoters of heat shock genes and activates transcription (2, 4). Recent studies have shown that HSF1 is essential in protection against the toxic effects of bacterial endotoxin (6). We and others have demonstrated that HSF1 may carry out this function through transcriptional repression of cytokine genes, including TNFα, and IL-1β, suggesting a role for HSF1 in antagonizing the acute phase response (APR) through transcriptional repression of APR-mediating genes (1, 6, 7). In the current study, we have examined the mechanisms of IL-1β repression by HSF1.

IL-1β is expressed primarily by activated monocytes in response to a variety of stimuli including bacterial lipopolysaccharide (LPS) endotoxin, phosphor myristate acetate (PMA), and other cytokines (8). It is implicated in a series of physiologic and pathologic processes, including the mediation of fever, lymphocyte activation, and the regulation of acute phase genes (9, 10). The expression and function of IL-1β in humans are regulated at a number of different levels. These include modulation of transcription, mRNA stabilization, post-translational proteolytic processing of pro-IL-1β, and inhibition of IL-1β receptor binding by a naturally occurring IL-1β antagonist (1, 8, 11–13). The regulation of IL-1β gene transcription is dependent upon the activity of the myeloid-specific transcription factor Spi-1/PU.1 (14), which binds specifically to multiple elements in proximal IL-1β promoter and activates transcription (11). Spi-1 has also been shown to be a major determinant in myeloid-specific expression of the integrin cell surface receptor CD11b (15), the c-fms proto-oncogene, which codes for the macrophage colony stimulating factor receptor (16), and the macrophage scavenger receptor (17). The transcription factor for IL-6 (NF-IL6) is the other major regulator involved in inactivation of IL-1β transcription (1). NF-IL6 is a bZIP transcription factor of the C/EBP family (18–20) that is constitutively expressed in resting monocytes and immediately activated by agents such as LPS, PMA, and IL-6 (21, 22). NF-IL6 has been shown to activate IL-1β transcription by binding to the promoter at two different sites (21, 22). Previous studies have shown that NF-IL6 is necessary for activation of IL-1β transcription by LPS and is capable of synergistically cooperating with Spi-1, resulting in strong activation of the IL-1β core promoter (1, 12).

Because IL-1β is a cytokine immediately responding to a wide variety of proinflammatory agents and affecting the func-

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‡ The abbreviations used are: HSF, heat shock factor; HSP, heat shock protein; TNF, tumor necrosis factor; IL, interleukin; APR, acute phase response; LPS, lipopolysaccharide; PMA, phosphor myristate acetate; NF-IL6, nuclear factor of interleukin 6; HSE, heat shock element; ET, reverse transcriptase; bZIP, basic zipper; GST, glutathione S-transferase; EMSA, electrophoresis mobility shift assay; PMSF, phenylmethylsulfonyl fluoride; WTH, winged helix turn helix; C/EBP, CCAAT enhancer binding protein.
tion of a wide variety of targets, negative regulation of IL-1β expression is crucial for limiting potentially damaging aspects of inflammation and maintaining balance in the host. At the physiological level, IL-1β expression is subject to feedback inhibition through the release of glucocorticoids following stimulation of the hypothalamic-pituitary-adrenal axis and antagonism of IL-1β expression at the transcriptional and posttranscriptional levels (23–26). The inhibition of the expression of IL-1β and a number of other cytokines, including IL-2, IL-6, granulocyte-macrophage colony-stimulating factor, TNF-α, and interferon γ, has been observed at elevated temperatures in the fever range, suggesting the existence of a thermally regulated feedback inhibitory mechanism (27–29).

More recently, it has been shown that HSF1 plays an essential function in protection against endotoxemia, and transgenic mice with disrupted Hsf1 genes die rapidly when exposed to endotoxin (6). Our studies suggest that HSF1 functions at least partially through repression of proinflammatory cytokines, and endotoxin (6). Our studies suggest that HSF1 functions at least partially through repression of proinflammatory cytokines, and endotoxin (6). Our studies suggest that HSF1 functions at least partially through repression of proinflammatory cytokines, and endotoxin (6). Our studies suggest that HSF1 functions at least partially through repression of proinflammatory cytokines, and endotoxin (6). Our studies suggest that HSF1 functions at least partially through repression of proinflammatory cytokines, and endotoxin (6). Our studies suggest that HSF1 functions at least partially through repression of proinflammatory cytokines, and endotoxin (6). Our studies suggest that HSF1 functions at least partially through repression of proinflammatory cytokines, and endotoxin (6). Our studies suggest that HSF1 functions at least partially through repression of proinflammatory cytokines, and endotoxin (6). Our studies suggest that HSF1 functions at least partially through repression of proinflammatory cytokines, and endotoxin (6). Our studies suggest that HSF1 functions at least partially through repression of proinflammatory cytokines, and endotoxin (6). Our studies suggest that HSF1 functions at least partially through repression of proinflammatory cytokines, and endotoxin (6). Our studies suggest that HSF1 functions at least partially through repression of proinflammatory cytokines, and endotoxin (6). Our studies suggest that HSF1 functions at least partially through repression of proinflammatory cytokines, and endotoxin (6).
bated for 10 min on ice in 200 μl of CERI solution containing 0.75 mM PMSF, 2.0 μg/ml aprotinin and leupeptin, 20 mM NaF, and 20 mM Na3VO4. 11 μl of CERII solution was then added, and cytoplasmic extracts were collected by centrifugation at 12,000 × g for 5 min. The pellet nuclei were lysed in 100 μl of NER solution containing 2 mM PMSF, 2.0 μg/ml aprotinin and leupeptin. The extracts were then aliquoted and stored at −80 °C.

The oligonucleotide probes were synthesized and labeled by end filling with 32P. The sequences of the oligonucleotides used in EMSA are shown below: 1) consensus HSE from human hsp70A gene, 5′-CACCT-CGGCTGGAATATTCCCGACCTGGCAGCCGA-3′ and 2) IL-1β promoter fragment containing binding elements for PU.1-NF-IL6, 5′-TTTCAATCAATTAAAGGGAAAGGGGAAA-3′.

Each binding mixture (12 μl) for EMSA contained 2.0 μl of nuclear extract or 10 μl of in vitro translated protein, 2.0 μg of bovine serum albumin, 2.0 μg of poly(dI-dC), 0.5–1.0 ng of labeled double-stranded oligonucleotide probe, 12 mM HEPES, 12% glycerol, 0.12 mM EDTA, 0.9 mM MgCl2, 0.6 mM dithiothreitol, 0.6 mM PMSF, and 2.0 μg/ml aprotinin and leupeptin (pH 7.9). Final concentrations of KCl in the binding mixture were defined for optimal binding of each oligonucleotide. The samples were incubated at room temperature for 15 min and then electrophoresed on 4.5% polyacrylamide gels. The results were visualized by autoradiography.

In Vitro Protein Interaction Assay—To produce GST fusion proteins and control GST protein, 250-ml cultures of E. coli DH5α expressing GST/NF-IL6-zip fusion protein, GST/HSF1 fusion protein, or GST control protein were incubated by shaking at 37 °C until A600 reached 0.4–0.6. Isopropyl-β-D-thiogalactopyranoside was then added to the bacterial culture to a final concentration of 0.5 mM to induce GST fusion protein expression. GST proteins were prepared as described previously (32). For each in vitro protein binding reaction, 50 pmol of GST fusion protein or GST control protein was immobilized on glutathione-Sepharose beads and then incubated with 20–25 μl of in vitro translated, 35S-labeled proteins in 500 μl of binding buffer containing 20 mM Tris-Cl (pH 8.0), 100 mM NaCl, 10 mM EDTA, 2.5% Nonidet P-40, 1 mM dithiothreitol, 2.0 mM PMSF, 2.0 μg/ml aprotinin, and 0.5 μg/ml leupeptin. The binding reaction was carried out at 4 °C for 30 min with gentle rocking. The protein-GST beads were washed five times with binding buffer and analyzed on a 10% SDS-PAGE gel. As input controls, 1 μl of in vitro translation samples was run in parallel with relevant binding reactions.

RESULTS

Heat Shock Represses Transcription of the IL-1β Gene—We have previously demonstrated inhibition of LPS-stimulated IL-1β mRNA expression by heat shock and LPS-stimulated IL-1β promoter activity by HSF1 overexpression (1). Using RT-PCR, we confirmed our earlier observation. IL-1β is not expressed in THP-1 monocytic cells prior to stimulation, but inhibited LPS-induced IL-1β expression from an HSF1 expression vector represses c-fms promoter activity in transfection assays.2 As controls, we show that HSF70 transcription is induced by heat shock in the absence and presence of LPS, whereas transcription of the housekeeping gene β-actin was not affected.

HSF1 Binds to NF-IL6 Directly—Because our previous studies indicated that repression of the IL-1β promoter by heat was a direct effect of HSF1, we next examined potential mechanisms of IL-1β repression by HSF1. Our earlier genetic studies identified NF-IL6 as an important transcription factor involved in IL-1β transcription in response to LPS stimulation (1, 12). Our studies have also suggested the involvement of NF-IL6 in HSF1-mediated repression of IL-1β transcription because mutation of the NF-IL6-binding site adjacent to the HSF1-binding site in the IL-1β promoter abolished transcriptional repression by HSF1 (1). We therefore examined direct interactions between HSF1 and NF-IL6 by co-immunoprecipitation assays. We firstly attempted co-immunoprecipitation using monocytic cell lines treated with LPS and PMA, which activate NF-IL6, and heat shock, which activates HSF1. For reasons not clear to us, we were not able to obtain a NF-IL6 level that was high enough to unambiguously demonstrate HSF1/NF-IL6 association in the nuclear extracts when cells were either treated with

2Xie, Y., Chen, C., Stevenson, M., Hume, D., Avron, P., and Calderwood, S. (2002) Biochem. Biophys. Res. Commun. 291, 1071–1080.
a physiological concentration of LPS and/or PMA or treated with heat shock (data not shown). However, physical interaction of HSF1 and NF-IL6 was strongly suggested from our previous study, which showed that in vitro translated NF-IL6 inhibits the DNA binding activity of HSF1 in CHO K1 cells using EMSA (35). EMSA is a sensitive assay and may detect protein-protein interaction not detectable by immunoprecipitation assay (35). To confirm this observation first made in CHO K1 cells with endogenous proteins in monocytes, we performed EMSA using nuclear extracts from THP-1 treated with heat shock in the absence or presence of physiological concentrations of LPS and PMA. Fig. 2A presents the EMSA results reproducible in three separate experiments. Also shown in the lower panel of Fig. 2A is the PhosphorImager quantification of the HSF1-HSE bands (left side) or NF-IL6-C/EBP complexes (right side). It is evident that heat shock induces the formation of HSF1-HSE complex, which is supershifted by specific anti-HSF1 antibody (Fig. 2A, second and third lanes). Stimulation of the cells by LPS and PMA, which induces NF-IL6 in monocytes, significantly reduces the DNA binding activity of HSF1 (Fig. 2A, fourth lane). Conversely, LPS and PMA induces DNA binding of NF-IL6 to C/EBP element, which was inhibited by specific anti-NF-IL6 antibody (Fig. 2A, seventh and eighth lanes). Heat shock inhibits NF-IL6 DNA binding activity to an extent comparable with specific antibody treatment (Fig. 2A, ninth lane). These data clearly demonstrated that activation of HSF1 by heat shock interferes the DNA binding of NF-IL6, whereas activation of NF-IL6 also inhibits the DNA binding by HSF1. However, these data did not show physical interaction between HSF1 and NF-IL6. To examine the physical interaction between HSF1 and NF-IL6, we have established a model system. CHO K1 cells were stably transfected with NF-IL6 expression vector, and clones with high levels of NF-IL6 expression were selected and used to examine HSF1/NF-IL6 association by co-immunoprecipitation assays. Fig. 2B shows the presence of NF-IL6 with HSF1 in complexes immunoprecipitated by anti-HSF1 antibody (Fig. 2B, upper and lower panels, fourth lane). In the reciprocal experiment, anti-NF-IL6 antibody co-immunoprecipitates both NF-IL6 and HSF1, demonstrating the association of HSF1 and NF-IL6 during heat shock (Fig. 2B, upper and lower panels, eighth lane). Our results therefore indicate that heat shock converts HSF1 into a form that can directly interact with NF-IL6.

We next used in vitro protein binding assays to determine the domains in HSF1 and NF-IL6 involved in HSF1/NF-IL6 interaction. E. coli-expressed full-length HSF1-GST fusion protein was immobilized on glutathione-Sepharose and incubated with in vitro translated and 35S-labeled proteins from two NF-IL6 constructs, full-length NF-IL6 and a truncated NF-IL6 mutant containing primarily the bZIP region. The latter construct was chosen in addition to wild type NF-IL6, because previous studies indicated that NF-IL6 physically interacts through the bZIP region with a series of other proteins, including NF-κB, v-Myb, AP-1, and retinoblastoma protein (36–41). As shown in Fig. 2C, both full-length NF-IL6 and the bZIP region of NF-IL6 bind to the HSF1-GST fusion protein (Fig. 2C, lanes 2 and 5). The binding was specific because no interaction was detected from the incubations with GST control protein (Fig. 2C, lanes 3 and 6). To determine the specificity of HSF1 and NF-IL6 association, in vitro translated NF-IL6 was incubated with GST/HSF1 in parallel with GST/HSF2A (Fig. 2D). Specific binding was observed only between HSF1 and NF-IL6 (Fig. 2D, lane 3) but not between HSF2A and NF-IL6 (Fig. 2D, lane 6). The HSF2A used here was cloned from a human HeLa cell cDNA library and encodes a protein identical in sequence to that encoded by a human HSF2 described previously (42). HSF2 belongs to the HSF protein family and is structurally related to HSF1. The fact that NF-IL6 binds to HSF1 but not HSF2A indicates that the association is highly specific to HSF1. We next performed a reversed binding experiment using, in this case, a GST/NF-IL6-bZIP fusion protein and confirmed the interaction of in vitro translated HSF1 with GST-NF-IL6-bZIP fusion protein (Fig. 2E, lane 3). Next we attempted to identify domains of HSF1 that are involved in binding to NF-IL6 using a series of in vitro translated and 35S-labeled HSF1 mutants with deletions from the C terminus. Fig. 2F shows that HSF1 with C-terminal deletions of 150 and 250 amino acids (pHSF1/1–379 and pHSF1/1–279) bound avidly to the GST-NF-IL6-bZIP fusion protein (Fig. 2F, lanes 6 and 9). However, the binding ability was lost when 350 amino acids were deleted from the C’ terminus of HSF1 (HSF1/1–179) (Fig. 2F, lane 12). These results imply that the amino acid residues from 179 to 279 are necessary for HSF1 to bind to NF-IL6. We cannot, however, conclude from our results that this region binds to NF-IL6 independently of other residues of HSF1. It has been shown that the intact trimerization domain (amino acids 137–211) is important for the association of HSF1 monomers to form trimers (43). This requirement may also be true for the association with NF-IL6. C-terminal deletion of HSF1 to amino acid residue 179 eliminated the last N-terminal leucine zipper and therefore resulted in the disruption of trimerization domain and loss of binding ability to NF-IL6. It is noteworthy that the 1–379 and 1–279 mutants bound to NF-IL6 more effectively than did wild type HSF1 (Fig. 2F). This may be because in vitro translated full-length HSF1 folds into a latent intramolecular coiled coil through the interaction of the N’-terminal leucine zipper domain with the C’-terminal leucine zipper (leucine zipper 4; amino acids 383–415) (44). This structure can be altered by either heat shock or deletion of C-terminal residues involved in intramolecular binding, permitting the formation of intermolecular coiled coil, which produces trimeric HSF1 competent to bind DNA (43–45). Indeed, increased binding of the deletion mutants to NF-IL6 correlated well with their ability to bind to a consensus HSE as determined by EMSA assay (Fig. 2G). These findings as well as the co-immunoprecipitation studies suggest the requirement of heat shock to convert the conformation of HSF1 into a form capable of interacting with NF-IL6 at high affinity (Fig. 2, C–G). Deletion of 100 amino acids to yield 1–429 did not activate HSE binding, whereas deletion of 150 amino acids to yield 1–379 strongly activated DNA binding (Fig. 2G). This latter mutant deletes the C-terminal leucine zipper shown previously to bind the N-terminal trimerization domain and inhibit trimerization and DNA binding in vitro and in vivo (44, 45). HSE binding activity remained strong until deletion of 350 C-terminal amino acid residues to yield 1–179; the latter deletion removes a significant proportion of the N-terminal leucine zipper trimerization domain (46, 47). Thus the regulation of the DNA binding ability of in vitro translated HSF1 resembles the regulation in vivo with, in each case, a requirement for an N-terminal leucine zipper domain and negative regulation by the C-terminal leucine zipper (44, 45). Therefore, the interactions of HSF1 with NF-IL6 and with HSE elements in DNA both appear to require residues between amino acids 179 and 279, including the trimerization domain (Fig. 2, F and G), and to be inhibited by sequences in the region of leucine zipper 4. A series of control experiments were performed to determine the specificity of the HSF1-NF-IL6 interaction. The in vitro trans-
Fig. 2. Interaction of HSF1 and NF-IL6. A, EMSA THP-1 cells were treated with heat shock (42.5 °C, 30 min, second and third lanes), LPS and PMA (100 ng/ml and 10 ng/ml, respectively, seventh and eighth lanes), or both (fourth, fifth, ninth, and tenth lanes). The nuclear extracts were
labeled NF-IL6 or HSF1 did not bind to the GST control protein (Fig. 2, C, lanes 3 and 6; D, lanes 2 and 5; E, lane 4; and F, lanes 2, 5, 8, and 11). The GST/HSF2A fusion protein was not able to bind to in vitro translated NF-IL6 (Fig. 2D, lane 6). Furthermore, no protein binding was observed when GST/NF-IL6b was incubated with an in vitro translation reaction from an empty vector (Fig. 2E, lane 1). Therefore, our data suggest that HSF1 and NF-IL6 are physically associated and that their interaction requires a sequence from HSF1 containing the N’ terminal leucine zipper region (amino acids 137–212) and a portion of the transcriptional regulatory domain (amino acids 215–310) and the bZIP region of NF-IL6.

The Physical Interaction between HSF1 and NF-IL6 Is Correlated with Functional Competition—Having shown the physical interaction between HSF1 and NF-IL6 in vitro and in vivo, we then examined whether this physical interaction is responsible for the inhibition of IL-1β induction in vivo using transient transfection assays. To eliminate the interference of endogenous monocyctic transcription factors and to access the functional interaction of HSF1 and NF-IL6 under a low background, we have conducted our experiments in a non-monicytic cell line, HeLa S3. These cells are deficient in both Spi.1/PU.1 and NF-IL6, and we have carefully characterized the conditions required for IL-1β transcription in these non-monicytic cells (12). Co-transfection of NF-IL6 expression vector with the IL-1β core promoter reporter gene, pGL3/IL-1DT, induced the promoter activity to about 5-fold (Fig. 3A). Because the Ets family protein Spi.1/PU.1 has been implicated as a crucial transcriptional regulator of IL-1β (11, 12), we next included Spi.1 in the transfection assays to evaluate the effect of HSF1 expression on Spi.1-activated IL-1β transcription. As shown in Fig. 3B, co-expression of Spi.1 with the IL-1β promoter reporter construct induced the promoter activity about 6-fold. However, the combination of NF-IL6 and Spi.1 activated the promoter by ~150-fold, demonstrating the strong synergism between these two factors shown previously (12) (Fig. 3C). These results are in agreement with the study by Yang et al. (12), who identified physical association between the bZIP region of NF-IL6 and the winged helix turn helix (wHTH) domain of Spi.1 and strong functional cooperation between these two factors, which most likely forms the basis for regulation of IL-1β transcription under native condition. To assess the effects of HSF1 in NF-IL6/Spi.1 synergism, HSF1 was simultaneously expressed with NF-IL6, Spi.1, or both factors in the presence of IL-1β reporter plasmid. As shown in Fig. (A and B), the expression of HSF1 abolished the transcriptional activation by NF-IL6 but had little effect on the transcriptional activation by Spi.1. However, expression of HSF1 led to a significant reduction of the promoter activity synergistically activated by NF-IL6 and Spi.1 of 90% (Fig. 3C). The residual IL-1β core promoter activity not repressed by HSF1 may be due to the effect of Spi.1 alone, because HSF1 was not effective in antagonizing its individual effect (Fig. 3B). Our results indicate that the transcriptional repression by HSF1 involves NF-IL6-mediated transactivation and that this effect is likely to result from the physical interaction between HSF1 and NF-IL6.

HSF-1 Binds to NF-IL6 and Blocks NF-IL6/Spi.1 Synergism—A number of studies have shown that the bZIP region of NF-IL6 and the WHTH domain of Spi.1 and other ETS family proteins directly interact with essential transcription factors and play a key role in functional cooperativity (36–41, 48–51). Particularly, the synergistic activation of the IL-1β core promoter by NF-IL6 and Spi.1 is mediated by such a protein-protein interaction (12). Because we have shown that HSF1 binds directly to NF-IL6, we wanted to determine whether HSF1/NF-IL6 association led to the inhibition of NF-IL6/Spi.1 interaction. Fig. 4A shows the results of competitive GST fusion protein pull-down assay, in which GST fusion protein containing the Spi.1 WHTH domain was incubated with in vitro translated and 35S-labeled NF-IL6 in the absence or presence of increasing amount of in vitro translated HSF1. As shown in Fig. 4A, GST/Spi.1 wHTH binds NF-IL6, and the addition of HSF1 causes a decreased NF-IL6/Spi.1 association in a dose-

![Fig. 3. The effects of NF-IL6, Spi.1, and HSF1 on the IL-1β promoter.](image)

**Note:** The figure legend is not provided in the text. The figure shows the effects of NF-IL6, Spi.1, and HSF1 on the IL-1β promoter. The IL-1β core promoter (~589 + 12) luciferase reporter gene was transfected into HeLa S3 cells along with vectors expressing NF-IL6, NF-IL6 plus HSF1 (A); Spi.1, Spi.1 plus HSF1 (B); NF-IL6, Spi.1, NF-IL6 plus Spi.1, or NF-IL6 plus Spi.1, and HSF1 (C), and the relative transcriptional activities were determined. The luciferase activities were normalized to β-galactosidase activities expressed by co-transfected pCMV. βGal expression vector. Plasmid DNA of empty expression vector was added to achieve equal amounts of total DNA in each transfection. The luciferase activities of the IL-1β reporter gene in cells co-transfected with empty expression vector were used as controls and set to 1. The data represent the means and standard deviations of three separate experiments containing triplicates for each sample.
HSF1, respectively. The reaction in the seventh – the input control. To control for the effects of adding an increasing down assay using 25 A
action. To further examine the effect of HSF1 on NF-IL6/Spi.1 amounts of bovine serum albumin had no effect on the inter-

dependent manner. As a control, incubation with increasing amounts of bovine serum albumin had no effect on the interaction. To further examine the effect of HSF1 on NF-IL6/Spi.1 interaction, we performed EMSA with an oligonucleotide from
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physical interaction between HSF1 and NF-IL6 correlates with the functional antagonism of the two factors and repression of the IL-1β core promoter (Fig. 3A). We have also shown that HSF1 inhibits the cooperative interaction between two essential transactivators required for IL-1β transcription in mono-
cytes, NF-IL6 and Spi.1 (Fig. 3C). This inhibition appears to be the result of competition by HSF1 with Spi.1 for binding to NF-IL6 (Fig. 4). The finding of a direct physical interaction between HSF1 and NF-IL6 in heat-shocked cells suggests that such protein-protein interaction may contribute to repression in vivo (Figs. 1 and 2).

Our data suggest that the interaction between HSF1 and NF-IL6 involves the trimerization and regulatory domains of HSF1 (amino acids 179–279) and the bZIP region of NF-IL6 (Fig. 2). In vitro protein binding studies using HSF1 deletion mutants show that a region between amino acids 179 and 279 encompassing the bulk of the trimerization domain and the transcriptional regulatory domain is involved in the interaction with NF-IL6 (Fig. 2F). It was also found that deletion of the region containing leucine zipper 4 enhances the interaction of HSF1 with NF-IL6 in vitro (Fig. 2F) (43, 44). Leucine zipper 4 is implicated in binding to the N-terminal leucine zipper trimerization domains and, through the formation of an intramo-
lecular coiled-coil, in masking important sites for transcriptional activation (43, 44). Similar processes may also be in-

DISCUSSION

FIG. 4. HSF1 interfere with NF-IL6 and Spi.1/PU.1 interaction. A, the binding of NF-IL6 and Spi.1 was examined by GST pull-
down assay using 25 μl of in vitro translated, 35S-labeled NF-IL6 and 50 pmol of GST fusion protein containing Spi.1 wHTH domain (amino acids 171–272) in the absence (control) or presence of increasing amount (1–15 μl) of in vitro translated, unlabeled HSF1. 1 μl of the in vitro translated, 35S-labeled NF-IL6 was loaded on the SDS-PAGE as the input control. To control for the effects of adding an increasing amount of protein to the incubation, 2–20 μg of bovine serum albumin was used as the competitor in a separated set of experiments shown below. B, a IL-1β promoter fragment containing PU.1/NF-IL6 elements was subjected to EMSA with 10 μl of in vitro translated NF-IL6 and Spi.1 in the absence or presence of HSF1. The identities of NF-IL6 and Spi.1 were confirmed by supershift using specific antibodies against NF-IL6 or Spi.1 (second and fourth lanes). The reactions in the sixth, seventh, and eighth lanes contain 2, 5, and 15 μl of in vitro translated HSF1, respectively. The reaction in the ninth lane contains 5 μl of HSF1 that was preincubated with anti-HSF1 antibody.

dependent manner. As a control, incubation with increasing amounts of bovine serum albumin had no effect on the interaction. To further examine the effect of HSF1 on NF-IL6/Spi.1 interaction, we performed EMSA with an oligonucleotide from the IL-1β promoter, which contains NF-IL6- and Spi.1-binding sites. NF-IL6 and Spi.1 proteins prepared by in vitro transcription and translation each bound to this probe (Fig. 4B, first and third lanes). A mixture of both proteins yielded independent binding of NF-IL6 and Spi.1 as well as an additional complex of slower mobility (Fig. 4B, fifth lane). The addition of HSF1 in the mixture caused the inhibition of this complex in a dose-depen-
dent fashion, whereas preincubation with anti-HSF1 abol-
ished the inhibition by HSF1 (Fig. 4B, sixth through ninth lanes). Our data therefore suggest that the binding of HSF1 to NF-IL6 represses transcriptional activation of the IL-1β promoter through competition with the physical interaction be-
tween NF-IL6 and Spi.1 essential for promoter function and thus leads to an inhibition of the functional cooperation be-
tween the two factors.

Physical Interaction between HSF1 and NF-IL6 Correlates with Transcriptional Repression in Vivo—Our studies show that heat shock is an effective inhibitor of monocyte-specific transcription (Fig. 1). Our data also suggest a mechanism for IL-1β repression during heat shock or fever during the activa-
tion of HSF1 that is able to interact directly with activated NF-IL6 on the IL-1β promoter. We have demonstrated that the physical interaction between HSF1 and NF-IL6 correlates with the functional antagonism of the two factors and repression of the IL-1β core promoter (Fig. 3A). We have also shown that HSF1 inhibits the cooperative interaction between two essential transactivators required for IL-1β transcription in mono-
cytes, NF-IL6 and Spi.1 (Fig. 3C). This inhibition appears to be the result of competition by HSF1 with Spi.1 for binding to NF-IL6 (Fig. 4). The finding of a direct physical interaction between HSF1 and NF-IL6 in heat-shocked cells suggests that such protein-protein interaction may contribute to repression in vivo (Figs. 1 and 2).

Our data suggest that the interaction between HSF1 and NF-IL6 involves the trimerization and regulatory domains of HSF1 (amino acids 179–279) and the bZIP region of NF-IL6 (Fig. 2). In vitro protein binding studies using HSF1 deletion mutants show that a region between amino acids 179 and 279 encompassing the bulk of the trimerization domain and the transcriptional regulatory domain is involved in the interaction with NF-IL6 (Fig. 2F). It was also found that deletion of the region containing leucine zipper 4 enhances the interaction of HSF1 with NF-IL6 in vitro (Fig. 2F) (43, 44). Leucine zipper 4 is implicated in binding to the N-terminal leucine zipper trimerization domains and, through the formation of an intramo-
lecular coiled-coil, in masking important sites for transcriptional activation (43, 44). Similar processes may also be in-

Physiological Role of HSF1/NF-IL6 Interaction—The present studies show a coordinate repression of IL-1β, TNF-α, and c-fms genes during heat shock, suggesting a broad role for gene repression in the stress response (Fig. 1). Inhibition of transcrip-
tion is potentially beneficial to cell survival during stress by decreasing the accumulation of novel transcripts and nasc-
ent proteins that may be aberrantly spliced or denatured during heat shock (52, 53). This possibility is supported by a previous study showing that Droso-
phila HSF becomes associated with many chromosomal loci in addition to well charac-
terized HSP genes, including developmental loci that are evi-
dently repressed during heat shock (54). Our observation implies a role for HSF1 in transcriptional regulation of non-
heat shock genes under stress condition. At the physiological level, the higher vertebrates respond to microbial infection with fever, one of the few conditions in which homeotherms experience heat shock. HSF1 is activated by hyperthermia at fever temperature range and represses cytokine genes (7). Other studies have also shown that HSF1 plays an essential role in protecting against the lethal effects of endotoxin shock (6). LPS from bacteria stimulates fever and endotoxic shock through production of proinflammatory cytokines, IL-1β, TNF-α, and IL-6 by activated monocytes and macrophages (55), whereas HSF1 represses the genes encoding these proteins.
Previous studies have shown that heat shock represses function to protect the host from overactivation of the APR through production of proinflammatory cytokines and NF-IL6-activated transcription of the c-fms gene, which plays an important role in monocyte/macrophage proliferation and differentiation (7). Powerful activation of monocytes/macrophages and lymphocytes often leads to the activation of the APR through production of proinflammatory cytokines such as IL-1β and TNF-α that are potentially lethal to the host (27). Therefore, cytokine gene repression by HSF1 may play a regulatory anti-inflammatory role in monocyte/macrophage function to protect the host from overactivation of the APR during infection and fever (6, 28, 29, 59–61). We have also demonstrated that non-steroidal anti-inflammatory drugs activate the DNA binding of HSF1 and repress IL-1β and TNF-α (62), further suggesting an involvement of HSFI in negative regulation of proinflammatory and NF-IL6-activated transcription of genes important for macrophage differentiation and function (65, 66). In addition, C/EBP family proteins are actively involved in the transcriptional activation of APR, developmental, immediate early, and viral genes in other cell types and could thus be the targets of HSFI in regulation of these responses (66, 67). Recent studies demonstrated the activation of C/EBPα and C/EBPβ proteins during hyperthermia, supporting a regulatory role of C/EBP proteins in feedback regulation of heat shock responses (68). The interaction between HSFI and NF-IL6 may provide a clue to how fever-activated HSFI is involved in the negative regulation of genes that are important in the host responses to infection. It is evident that the heat shock response has a complex role in the function of monocytes, because extracellular HSPs such as HSP60 and HSP70 can activate cytokine production, whereas intracellular HSFI and HSP70 act as repressors (69–71). Extracellular HSPs bind to pattern recognition receptors and stimulate inflammation, whereas intracellular components of the heat shock response target the promoters of proinflammatory genes and inhibit septic shock (1, 6, 71).

We propose a model for monocyte gene repression at elevated temperatures based on the demonstration that HSFI antagonizes the functional cooperation between NF-IL6 and Spi.1 through a competition of binding to NF-IL6 (Fig. 5). Physical interactions between NF-IL6 and HSFI as well as between NF-IL6 and Spi.1 provide the basis of our model. In this model NF-IL6 and Spi.1 bind to the IL-1β promoter at the adjacent location. The two factors interact physically and functionally, leading to a strong activation of the IL-1β promoter. Under heat shock stress condition, HSFI becomes activated and competes with Spi.1 to bind to the NF-IL6 bZIP region, resulting in the inhibition of the IL-1β promoter (Fig. 5).

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