# Induction of Heat Shock Protein 47 Synthesis by TGF-$\beta$ and IL-1$\beta$ Via Enhancement of the Heat Shock Element Binding Activity of Heat Shock Transcription Factor 1

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**With most immunological reactions, tissue fibrosis, collagen overproduction caused by immune cytokines, is inevitably associated. Among the various immune cytokines, heat shock protein 47 (HSP47) is a procollagen-specific molecular chaperon and is essential for secretion of procollagen from cells. Induction of HSP47 by TGF-$\beta$ has been previously reported in rat skeletal myoblasts and mouse osteoblasts, but not in human diploid fibroblasts. As for IL-1$\beta$, its effect on HSP47 has not been elucidated. In the present study, using human embryonic lung fibroblast cells, we first disclosed that both TGF-$\beta$ and IL-1$\beta$ induced HSP47 synthesis. We then revealed that the binding of the heat shock element (HSE) by heat shock transcription factor 1 (HSF1) was enhanced by both cytokines. We further demonstrated that trimer formation of HSF1, which is essential for its binding to HSE, was induced by these cytokines. The enhancement of HSP47 synthesis and their trimer formation of HSF1 were augmented by using a combination of both cytokines. Collectively, TGF- $\beta$ and IL-1$\beta$ were found to induce trimer formation of HSF1 which in turn bound to HSE of HSP47, resulting in the enhancement of HSP47 expression. Thus, HSP47 could well be a good candidate for molecular targeting in controlling tissue fibrosis, given that both principal fibrinogenetic cytokines (TGF-$\beta$, IL-1$\beta$) are commonly involved in its induction through HSF1 trimerization.** *The Journal of Immunology,* **2002, 168: 5178–5183.**

Heat shock protein (HSP)[2] is a general term for proteins induced in cells by changes in the intracellular milieu resulting from a variety of environmental changes including elevated temperatures, cell differentiation, and cell cycle progression (1–4). HSP functions by binding to newly synthesized polypeptides to ensure their appropriate folding, or repairing the polypeptides that have become denatured due to the stresses of transporting them into particular subcellular compartments (1, 2).

A recently discovered HSP, HSP47, unlike other HSPs, is a basic protein with an isoelectric point near 9 and a relatively small molecular mass (47 kDa) (5–7). HSP47 localizes in an endoplasmic reticulum (ER) with the ER retention signal sequence RDEL at its C terminus, where it binds to various types (I–V) of newly synthesized procollagens to transport them from the ER to the Golgi apparatus, facilitating their triple helix formation. During this process, procollagens dissociate from HSP47 as the pH inside ER decreases from 7.0 to 6.3 and are secreted at the cell surface; the freed HSP47 molecules are then cycled back to the ER (6, 8). Accordingly, HSP47 is considered to be a collagen-specific molecular chaperon expressed by collagen-secreting cells such as fibroblasts (6).

With most chronic immunological disturbances, including collagen diseases, autoimmune diseases, chronic infection, allergic reactions, etc., tissue fibrosis is inevitably associated and frequently become a determining factor of clinical course and prognosis of the diseases. In the fibrotic lesions, the expressions of HSP47 mRNA and collagen are reported to correlate (9). For example, increased coexpression of collagen and HSP47 at the fibrotic lesion of rat nephritis induced by antithymocyte serum has also been reported (10). In humans, a close correlation between the expression of HSP47 and that of collagen in the tissues of pulmonary fibrosis or progressive systemic sclerosis has also been demonstrated by immunohistochemical studies (11, 12).

Generally, fibrogenesis is effected by various immune cytokines forming a complex network, with TGF-$\beta$ and IL-1$\beta$ being the principal cytokines (13–17). Both TGF-$\beta$ and IL-1$\beta$ induce synthesis of collagen of fibroblasts. TGF-$\beta$ is known to stimulate the synthesis of mRNA encoding colligin, a rat homologue of HSP47 in rat skeletal myoblasts and that in mouse osteoblasts (18, 19). However, the effect of TGF-$\beta$ on expression of HSP47 in human diploid fibroblast is unknown, and as for IL-1$\beta$, no such investigation has been made.

The mechanism of HSP expression in heat stress involves a heat shock transcription factor (HSF) interacting with a highly conserved sequence in heat shock protein genes termed the heat shock element (HSE) (20, 21). By heat treatment, the HSF monomer is activated by conversion to a trimer that is capable of binding to the HSE. Binding of HSF to the HSE induces transcription of heat shock genes (20, 21).

In previous investigations, we reported that endogenous TNF augmented HSP72 expression via enhancement of the HSE-binding activity of HSF in mouse tumorigenic fibroblasts (L-M cells)

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[2] Abbreviations used in this paper: HSP, heat shock protein; HSF, heat shock factor; HSE, heat shock element; ER, endoplasmic reticulum; HEL, human embryonic lung fibroblast.

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(22–24), whereas Yamamura et al. (19) demonstrated that in a mouse osteoblast cell line, two distinct promoters differing from HSE drove the HSP47 gene in response to TGF-$\beta$ treatment.

In the present study, we first used human embryonic lung fibroblast (HEL) to confirm the enhancement of HSP47 expression by TGF-$\beta$ and IL-1$\beta$, and we then demonstrate that HSF1 is also involved in HSP47 induction by these cytokines.

## Materials and Methods

### *Cell lines and materials*

HEL cells, a human embryonic lung fibroblast cell line, were cultured in DMEM supplemented with 10% FBS (Flow Laboratories, North Ryde, Australia) at 37°C under 5% $CO_2$.

### *Cytokines*

Recombinant human TGF-$\beta$ was purchased from AUSTRAL Biologicals (San Ramon, CA) and activated in 5 mM HCl before use. Recombinant human IL-1$\beta$ was purchased from CISTRON Biotechnology (Pine Brook, NJ) and dissolved in conditioned medium for use.

### *Antibodies*

Anti-HSP47, anti-HSF1, and anti-HSF2 Abs and HSP47 cDNA were generously provided by Dr. K. Nagata (Department of Cell Biology, Chest Disease Research Institute, Kyoto University, Japan).

### *Metabolic labeling and immunoprecipitations*

Cells ($1 \times 10^6$) were grown in 35-mm culture dishes (Falcon 3025; BD Biosciences, Mountain View, CA) in DMEM supplemented with 10% FBS and treated under various conditions. Then the cells were washed twice with a methionine-free medium (ICN Biomedicals, Costa Mesa, CA) and labeled with 3.7 MBq/ml [$^{35}$S]methionine (DuPont/NEN Research Products, Boston, MA) for 1 h. Cells were then washed with PBS and solubilized in lysis buffer (1% Nonidet P-40, 0.15 M NaCl, 50 mM Tris-HCl (pH 8.0), 5 mM EDTA, 2 mM *N′*-ethylmaleimide, 2 mM PMSF, 2 $\mu$g/ml leupeptin, and 2 $\mu$g/ml pepstatin) for 30 min at 4°C. After centrifuging lysates at 10,000 $\times$ *g* for 30 min, the supernatant was incubated with the anti-HSP47 mAb or anti-human IgG at 4°C for 18 h and subsequently added to protein A-Sepharose R CL-4B Sepharose beads (Pharmacia Biotech Norden, Sollentuna, Sweden) and allowed to shake at 4°C for 1 h. The immunoprecipitates were washed three times with lysis buffer and eluted by boiling the beads for 3 min in Laemmli's SDS sample buffer. The samples were then analyzed by 10% SDS-PAGE and autoradiography. HSP47 synthesis levels were quantified by densitometry (dual-wave length TLC scanner CS-910; Shimazu, Kyoto, Japan) at 750 nm.

### *Gel mobility shift analysis*

Cells ($5 \times 10^6$) were grown in culture dishes (Falcon 3025; BD Biosciences) in DMEM supplemented with 10% FBS and treated under various conditions. Cells were harvested, centrifuged, and rapidly frozen in liquid $N_2$. The frozen pellets were suspended in a buffer containing 20 mM HEPES (pH 7.9), 5% (v/v) glycerol, 50 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM PMSF, and 0.5 mM DTT and centrifuged at 100,000 $\times$ *g* for 5 min. The supernatants were frozen in liquid $N_2$ and stored at $-80$°C. The protein concentration was estimated with an assay kit (Bio-Rad Laboratories, Hercules, CA). DNA-binding reaction mixtures contained, in a final volume of 10 $\mu$l, 10 $\mu$g protein of cell extracts, 1 $\mu$g poly(dI-dC) (Pharmacia Biotech Norden), 0.25 $\mu$g pUC19, 0.75 ng of an HSE -oligonucleotide (upper strand, 5′-GATCTCGGCTGGAATATTCCCGACCTG-GCAGCCGA-3′) end labeled with $^{32}$P (DuPont/NEN Research Products), and a buffer composed of 10 mM Tris (pH 7.8), 5% glycerol, 50 mM NaCl, 1 mM EDTA, and 0.5 mM DTT. For competition experiments, unlabeled competitor HSE was added to the reaction mixture to a 100-fold molar excess over the labeled probe. Reactions were incubated for 30 min at room temperature and analyzed on 4% polyacrylamide gels. The gels were dried and autoradiographed. The Ab-binding reaction mixtures for supershift analysis contained, in a final volume of 10 $\mu$l, 10 $\mu$g protein of cell extracts, 1/10 diluted anti-HSF 1 or anti-HSF 2 Ab, and PBS. The mixtures were incubated on ice for 15 min before the DNA-binding reaction.

### *Western blot analysis*

Cells (1 $\times$ 106) were grown in culture dishes in DMEM supplemented with 10% FBS and treated under various conditions. Cells were then washed with PBS and solubilized in lysis buffer for 30 min at 4°C. After lysates were centrifuged at 10,000 $\times$ *g* for 30 min, the supernatant was used as whole cell extract. To prepare subcellular fraction, cells were lysed on ice in hypotonic buffer (20 mM Tris-HCl (pH 8.0), 0.5 mM $CaCl_2$, 25 mM NaCl, 2 mM PMSF, 2 $\mu$g/ml leupeptin, and 2 $\mu$g/ml pepstatin), followed by Dounce homogenization (40 strokes with a loose-fitting pestle). The extract was then centrifuged at 10,000 $\times$ *g* for 10 min at 4°C, and the supernatant solution was used as the cytoplasmic extract. The pelleted material was washed with hypotonic buffer and resuspended in lysis buffer. After sonication, the extract was centrifuged at 15,000 $\times$ *g* for 10 min at 4°C, the supernatant solution representing the soluble nuclear fraction was removed. Then each extract was subjected to SDS-PAGE using 7.5% polyacrylamide gels. After electrophoresis, the fractionated protein was transferred to a nylon membrane (Schleicher & Schuell, Dassel, Germany). The membrane was incubated for 60 min at 37°C with a 1/500 dilution of the HSF1 Ab or a 1/2000 dilution of $\beta$-actin Ab (Sigma-Aldrich, St. Louis, MO). The immunoblot was developed using a 1/2000 dilution of HRP-labeled donkey anti rabbit IgG secondary Abs (Amersham, Buckinghamshire, U.K.) and an enhanced by the ECL system (Amersham).

### *Northern blot analysis*

Total RNA was isolated with the guanidinium isothiocyanate method from HEL cells ($1 \times 10^7$). The RNA (5 $\mu$g/lane) was fractionated in a 1.0% formaldehyde agarose gel and then transferred to a nylon filter membrane. The membrane was hybridized with a $^{32}$P-labeled HSP47 cDNA probe or GAPDH cDNA for 24 h at 42°C and autoradiographed. The bands were quantified using densitometry at 750 nm.

### *Cross-linking assay*

Whole cell extracts were prepared by quick-freezing pellets of PBS-washed cells in liquid $N_2$. The pulverized pellet material was thawed and resuspended in ~2 packed-cell volume of buffer A (HEPES (pH 7.9), 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, 2 mg/ml leupeptin, 2 mg/ml pepstatin, and 25% glycerol). After 10 min incubation on ice, the extract was centrifuged at 10,000 $\times$ *g*. For the cross-linking assay experiment shown, 15-$\mu$l aliquots of the supernatant were incubated for 10 min at room temperature with 2 mM glutaraldehyde. Reactions were stopped by the addition of 1 $\mu$l of 2 M lysine, and aliquots were electrophoresed on a 5% polyacrylamide SDS-PAGE gel. Immunoblotting was performed as described above.

## Results

### *Dose-dependent induction of HSP47 protein by TGF-$\beta$, IL-1$\beta$, or a combination of the two*

We first examined the effects of TGF-$\beta$ (0, 0.1, 1.0, 5.0, and 10.0 ng/ml), IL-1$\beta$ (0, 0.1, 1.0, 5.0, and 10.0 ng/ml) and a combination of the two on HSP47 protein expression in HEL cells, using metabolic labeling and immunoprecipitation methods. After 24 h of treatment, expression of HSP 47 was dose dependently induced by TGF-$\beta$ alone and IL-1$\beta$ alone, reaching a plateau at a dose of 5.0 ng/ml (Fig. 1, *a* and *b*). We therefore conducted the following experiments by using 5.0 ng/ml of each cytokine when used for the noncombination experiments. However, when we examined the combined effect of these cytokines, we used a concentration of 1.0 ng/ml for each cytokines, avoiding the plateau effect found with 5.0 ng/ml. As shown in Fig. 1*c*, this 1.0-ng/ml combination did in fact have an apparent augmenting.

### *Time course analysis of HSP47 protein induction by TGF-$\beta$ and IL-1$\beta$*

To study the time course of HSP47 induction, we used methods similar to those in Fig. 1. With both TGF-$\beta$ (5.0 ng/ml) and IL-1$\beta$ (5.0 ng/ml), HSP47 began to increase after 6 h and peaked at 24 h followed by a slight decrement. (Fig. 2).

### *Time course analysis of HSP47 mRNA induction by TGF-$\beta$ or IL-1$\beta$*

We then examined the effects of TGF-$\beta$ or IL-1$\beta$ on expression of HSP47 mRNA in HEL cells by Northern blotting. The intensity of the bands corresponding to HSP47 mRNA began to increase after

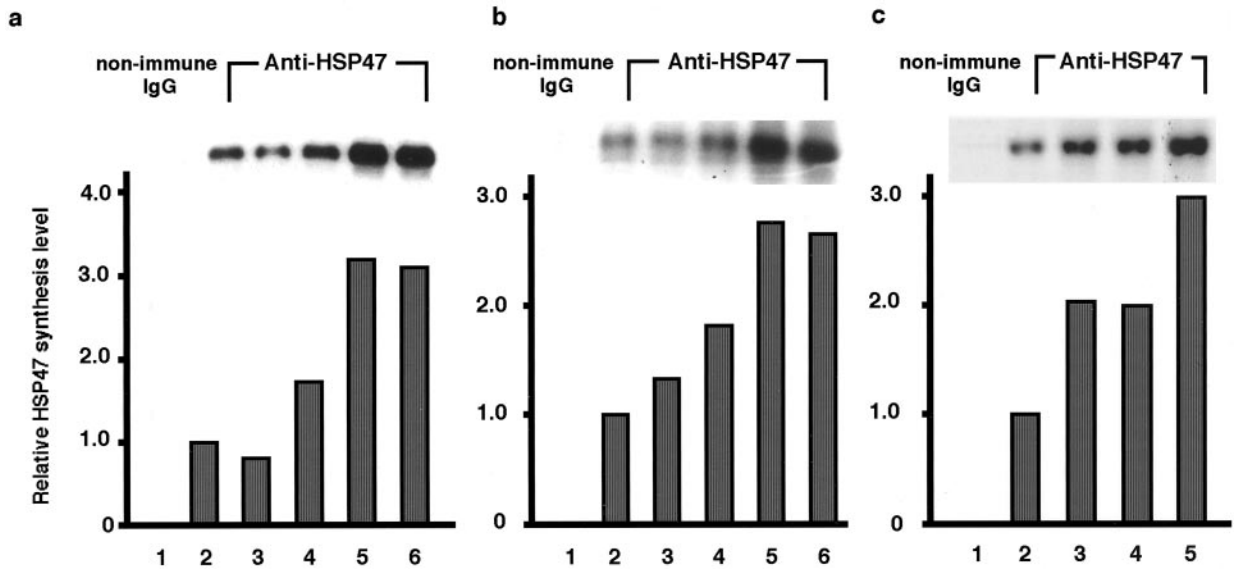


**FIGURE 1.** Dose-dependent induction of HSP47 protein by TGF-β, IL-1β, and their combination. Cells were incubated with various concentrations of TGF-β for 24 h at 37°C. Cells then were labeled with [$^{35}$S]methionine for 1 h. Cell lysates were incubated with anti-HSP47 mAb or nonimmune IgG at 4°C for 18 h and subsequently mixed with CL-4B Sepharose beads. Immunoprecipitates were then analyzed by 10% SDS-PAGE and autoradiography. *a,* Newly synthesized HSP47 after treatment with TGF-β. The relative intensity of each band was estimated densitometrically by normalizing the values for treated HEL cells to those from cells with no treatment (relative density, 1). The concentrations of TGF-β were 5.0 ng/ml (*lanes 1* and *5*), 0 ng/ml (*lane 2*), 0.1 ng/ml (*lane 3*), 1.0 ng/ml (*lane 4*), and 10.0 ng/ml (*lane 6*). The lysates were precipitated with nonimmune IgG (*lane 1*) and anti-HSP47 mAb (*lanes 2–6*). *b,* Newly synthesized HSP47 after treatment with IL-1β. The concentrations of IL-1β were 5.0 ng/ml (*lanes 1* and *5*), 0 ng/ml (*lane 2*), 0.1 ng/ml (*lane 3*), 1.0 ng/ml (*lane 4*), and 10.0 ng/ml (*lane 6*). The lysates were precipitated with nonimmune IgG (*lane 1*) and anti-HSP47 mAb (*lanes 2–6*). *c,* Newly synthesized HSP47 in combination with TGF-β and IL-1β. The concentrations of TGF-β and IL-1β were 1 ng/ml TGF-β and IL-1β (*lane 1* and *5*), no treatment (*lane2*), 1 ng/ml TGF-β (*lane 3*), and 1 ng/ml IL-1β (*lane 4*).

3 h of treatment and reached a plateau at 12 h (Fig. 3), which precedes the peaking time (24 h) of HSP47 induction (Fig. 2).

*Stimulation of HSF1 binding to HSE*

To determine whether the HSF binding to HSE is mediated in the activation of HSP47 gene transcription by TGF-β or IL-1β, we performed a gel-mobility shift analysis using a 5′-$^{32}$P-labeled synthetic oligonucleotide containing the human HSE sequence (Fig. 4). The intensity of the band corresponding to the HSE-HSF complex was substantially greater in HEL cells treated for 6 h with 5.0 ng/ml TGF-β or IL-1β than in that of untreated cells. Then, to elucidate which type of HSFs (HSF1 or HSF2) is involved in enhancement of HSP47 gene expression, we conducted a supershift assay using anti-HSF1 and anti-HSF2 Abs. The band intensity of the HSE-HSF Ab complex showed an apparent dose-dependent increase in the fractions treated with the anti-HSF1 Ab but not in those with the anti-HSF2 Ab.

*Effect of TGF-β and IL-1β on HSF1 expression*

To examine the effect of TGF-β or IL-1β on HSF1 expression, we treated HEL cells for 6 h with 5.0 ng/ml TGF-β or IL-1β and analyzed HSF1 by Western blotting. As shown in Fig. 5, the expression level of HSF1 in whole cell extracts was not altered but became lower in cytosolic fraction and higher in nuclear fraction upon cytokine stimulation.

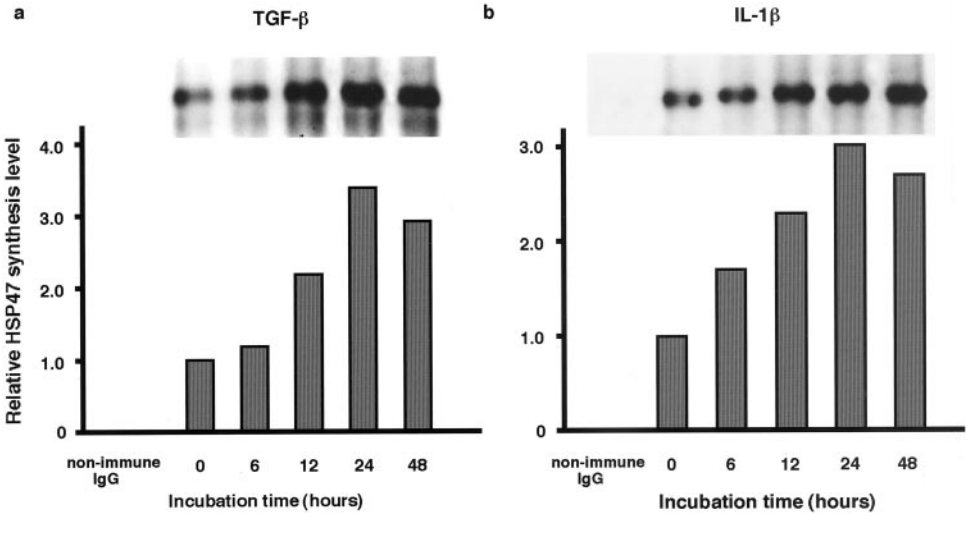


**FIGURE 2.** Time course analysis of HSP 47 protein induction by TGF-β, IL-1β. Cells were incubated with TGF-β or IL-1β at 5.0 ng/ml for 0 h (*lane 2*), 6 h (*lane 3*), 12 h (*lane 4*), 24 h (*lanes 1* and *5*), and 48 h (*lane 6*) at 37°C. The lysates were precipitated with nonimmune IgG (*lane 1*) and anti-HSP47 mAb (*lanes 2–6*). Immunoprecipitation and densitometric analysis were performed as described for Fig. 1. *a,* Newly synthesized HSP47 following treatment with TGF-β. *b,* Newly synthesized HSP47 following treatment with IL-1β.

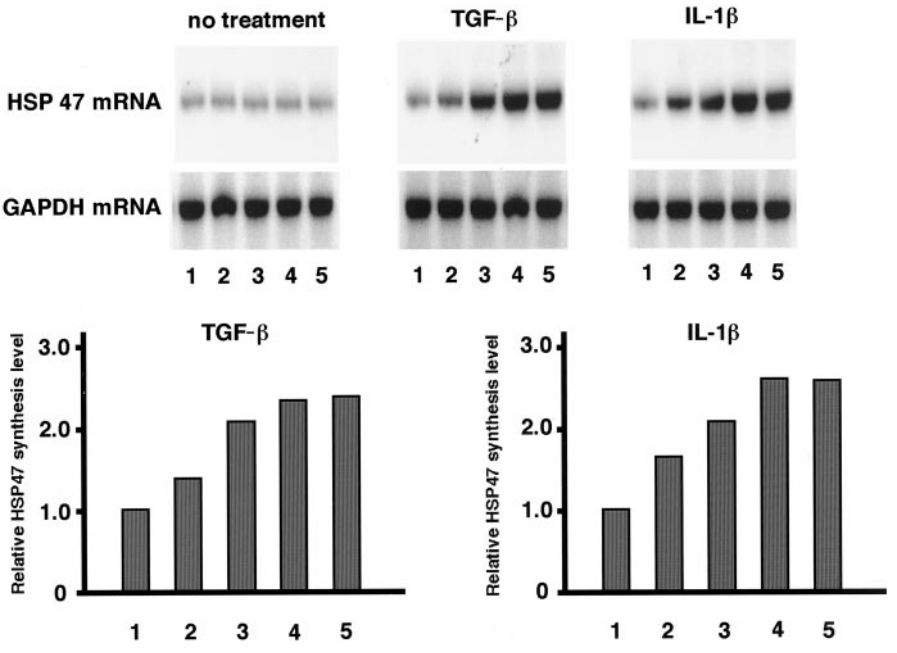


**FIGURE 3.** Time course analysis of HSP 47 mRNA induction by TGF-$\beta$ and IL-1$\beta$. Northern blot analysis of HSP47 mRNA was performed in HEL cells treated with IL-1$\beta$ at 5 ng/ml or TGF-$\beta$ at 5 ng/ml for 0 h (*lane 1*), 3 h (*lane 2*), 6 h (*lane 3*), 12 h (*lane 4*), and 24 h (*lane 5*) at 37°C as described in *Materials and Methods*. The same filter was rehybridized with a GAPDH probe. HSP47 mRNA bands were identified in each lane.

*Trimerization of HSF1 in response to TGF-β and IL-1β, and their combination*

To explore monomer to trimer conversion of HSF1 by TGF-$\beta$, IL-1$\beta$, or their combination, we cross-linked whole cell extracts with glutaraldehyde and subjected them to Western analysis using the anti-HSF1 Ab. Trimer formation became evident after 1 h, peaking at 3 h for both TGF-$\beta$ and IL-1$\beta$ treatments with a reciprocal change of monomers (Fig. 6*a*). When the cytokines were combined, a more prominent monomer-trimer conversion was observed (Fig. 6*b*).

## Discussion

In this study, we first confirmed that HSP47 protein synthesis is inducible in human fibroblasts by TGF-$\beta$ and IL-1$\beta$, principal fibrosis-causing cytokines. That is to say, HSP47 expression in HEL was maximally enhanced by stimulation with 5.0 ng/ml TGF-$\beta$ or

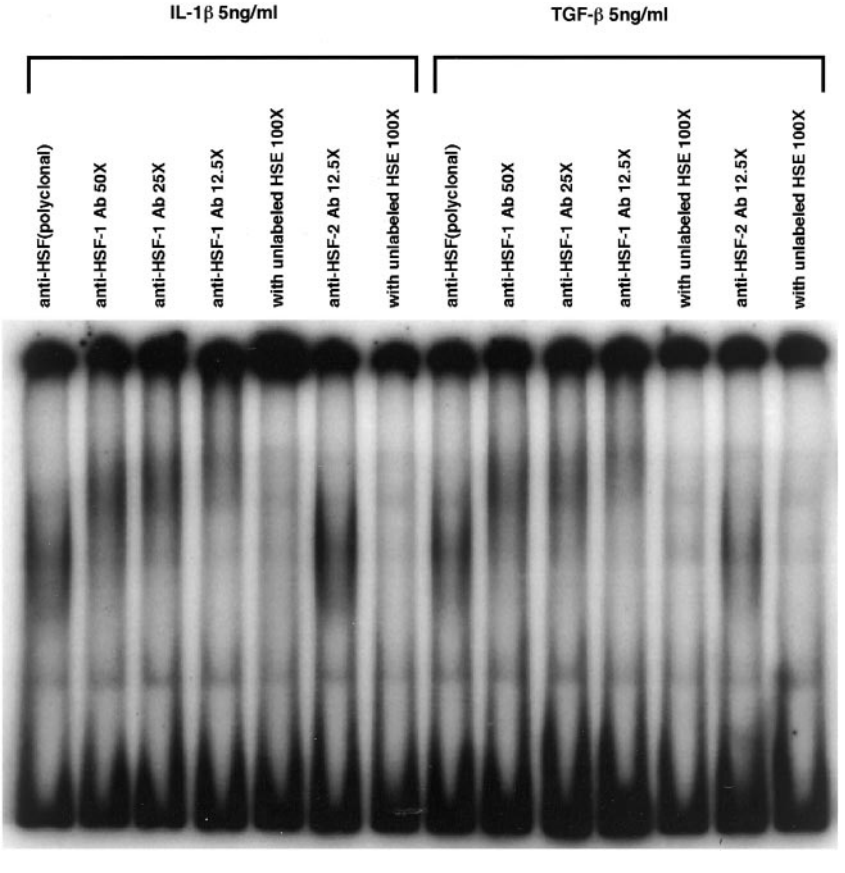


**FIGURE 4.** Detection of a HSF in whole cell extracts prepared from HEL cells. A gel mobility shift assay was performed with a $^{32}$P-labeled HSE oligonucleotide and whole cell extracts prepared from HEL cells incubated with conditioned medium or treated with IL-1$\beta$ at 5 ng/ml or TGF-$\beta$ at 5 ng/ml for 6 h at 37°C. The competition assay was performed with unlabeled HSE. For supershift analysis, protein from cell extracts (20 mg) was incubated with or without various diluted anti-HSF1 or anti-HSF2 Ab. After the mixtures were incubated with $^{32}$P-labeled HSE oligonucleotide, a supershift assay was performed.

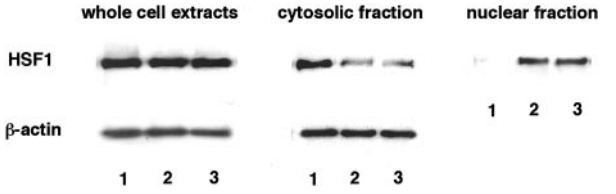


**FIGURE 5.** Effect of TGF-β and IL-1β on HSF1 expression in cytoplasm and nucleus. Western blot analysis of HSP47 protein was performed in HEL cells treated with conditioned medium (*lane 1*), TGF-β at 5 ng/ml (*lane 2*), and IL-1β at 5 ng/ml (*lane 3*) as described in *Materials and Methods*.

IL-1β to almost the same degree, 3 times that in nonstimulated cells. This concentration, 5.0 ng/ml for both TGF-β and IL-1β, is fairly close to those detected in patients with tissue fibrosis. For example, in patients with liver cirrhosis and with pulmonary fibrosis, plasma concentrations of TGF-β and IL-1β were reported to be 3.7 ± 2.1 and 0.1–2.0 ng/ml, respectively (25, 26).

In this regard, 1.0 ng/ml of each these two cytokines, which is within the range of actual plasma concentrations of the above mentioned pathological conditions, synergistically and substantially enhanced HSP47 synthesis when they were combined (Fig. 1*c*). In other words, the synergistic effect suggests that TGF-β and IL-1β may cooperatively and readily evoke fibrosis because they are quite often simultaneously secreted from immune competent cells in inflammatory lesions, even if each concentration is not high enough for either by itself to induce HSP47 (25, 26). We then proved that induction of HSP47 was brought about by the binding of HSF1 to HSE of the HSP47 gene.

Possible mechanisms by which the HSE-binding activity of HSF1 could be enhanced by TGF-β and IL-1β include the following: 1) TGF-β or IL-1β may enhance HSF1 synthesis; 2) TGF-β or IL-1β may induce a conformational change (trimer formation) of HSF1 as seen with heat treatment. The first possibility is highly doubtful because no difference was demonstrated in the HSF1 levels of whole cell extracts between TGF-β- or IL-1β-treated cells and untreated cells by Western analysis. Furthermore, the fact that most HSF1 shifted from cytoplasmic fraction to nuclear fraction by cytokine stimulation favored the second possibility because a similar phenomenon is observed with heat treatment (20, 21) To examine the second possibility, we performed a cross-linking assay using the anti-HSF1 Ab and found that HSF1 is converted from a monomeric form to a trimeric form. The time course analysis in response to treatment with TGF-β or IL-1β showed an increment of HSF1 trimer formation, which peaked at 3 h and decreased at 6 h. Thus, HSF1 is initially converted from a monomer to a trimer with TGF-β or IL-1β treatment, then synthesis of HSP47 mRNA starts by interaction between HSF1 and HSE. The amount of HSP47 mRNA gradually increased by accumulation of newly synthesized HSP47 mRNA. Incidentally, the concept of a synergistic effect of cytokine combination on trimer formation (Fig. 1*c*) is compatible with the results of HSP47 induction (Fig. 6*b*). An apparent inconsistency regarding the effect of various cytokine concentrations that evoked the synergistic effect in Fig. 1*c* (1 ng/ml for each cytokines) and Fig. 6*b* (5 ng/ml for each cytokines) may be interpreted by assuming that there is a maximum amount of active HSF1 (trimer form) that can interact with HSE to induce HSP47 mRNA because the level of HSP47 induced by combined 5 ng/ml TGF-β and 5 ng/ml IL-1β was almost the same as that induced by each cytokine of the same dose. Nonetheless, the fact that the effect and TGF-β or IL-1β was synergistic but not antagonistic suggests the possibility that there may be dissimilar mechanisms (signal routes) to induce HSF1 trimer for each cytokine.

The mechanism of trimer formation of HSF1 hitherto described is hyperphosphorylation of serine/threonine residues of HSF1 by heat treatment to undergo conformational change (20, 21). Hyperphosphorylation prolongs the effective half-life of the HSF1 trimer, which facilitates HSF1 binding activity (27). This mechanism is most likely true for IL-1β because phosphorylation of HSF1 was indeed proved to occur in HEL cells treated with this cytokine (data not shown). These results were consistent with the previous findings by Schett et al. (28) that IL-1 induced activation of HSF1 with phosphorylation and augmented HSP70 expression in synovial tissue from rheumatoid arthritis.

Further, stress-activated protein kinase/c-Jun N-terminal kinase, a mitogen-activated protein kinase that phosphorylates HSF1 after heat stress, is also activated by IL-1 (29, 30), suggesting the

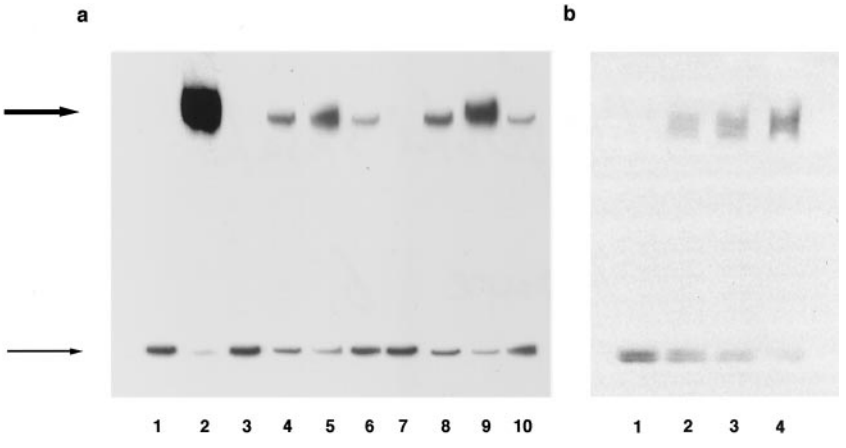


**FIGURE 6.** Trimerization of HSF1 in response to TGF-β, IL-1β, and their combination. *a*, Cells were incubated with conditioned medium (*lane 1*) or treated with heat at 43°C for 2 h (*lane 2*), incubated with TGF-β at 5 ng/ml for 0 h (*lane 3*), 1 h (*lane 4*), 3 h (*lane 5*), 6 h (*lane 6*), or IL-1β at 5 ng/ml for 0 h (*lane 7*), 1 h (*lane 8*), 3 h (*lane 9*), and 6 h (*lane 10*) at 37°C. Whole cell extracts in the presence of the cross-linking reagent glutaraldehyde were subjected to 5% SDS-PAGE and transferred to a nylon filter membrane. The filter was incubated with anti-HSF1 Ab and autoradiographed. The wide arrow indicates the trimer form of HSF1. The narrow arrow indicates the monomer form of HSF1. *b*, Cells were incubated with conditioned medium (*lane 1*), with TGF-β at 5 ng/ml for 3 h (*lane 2*), with IL-1β at 5 ng/ml for 3 h (*lane 3*), or with 5 ng/ml of TGF-β and IL-1β for 3 h (*lane 4*) at 37°C. The method was described in *Materials and Methods.*

involvement of stress-activated protein kinase/c-Jun N-terminal kinase in the phosphorylation of HSF1.

In contrast, phosphorylation of HSF1 was not observed in the cells treated with TGF-$\beta$. In this context, the previous assumption of distinct mechanisms for TGF-$\beta$ and IL-1$\beta$ may be plausible. To explore an alternative mechanism, we investigated the interaction of HSF1 with Smad3, Smad4, and TAK1, well-known mediators of TGF-$\beta$ signaling (31, 32), by a cross-linking assay; however, none of these transducers bound to HSF1 (data not shown). Elucidation of the mechanism for trimer formation of HSF1 by TGF-$\beta$ treatment is needed in the future.

HSF1 is a rather ubiquitous transcription factor for various HSPs other than HSP47 (20, 21, 28). Therefore, it was assumed that TGF-$\beta$ may induce, in addition to HSP47, other types of HSP. However, when we examined the expression of HSP72 in HEL cells treated with TGF-$\beta$, no increment was observed, suggesting some counteracting factors against the induction of HSP72. Further elucidation is needed.

Nevertheless, taken together the present results suggest that both TGF-$\beta$ and IL-1$\beta$ activate HSF1, which in turn stimulates the transcription of HSP47 mRNA, resulting in increased expression of HSP47 protein.

Tissue fibrosis, as the result of various chronic immunological disturbances, modifies the outcome of disorders (9, 13–15). Therefore, development of the modalities to inhibit fibrosis has been long desired. In this regard, the present results suggest that HSP47, a molecule commonly induced by both cytokines, may be an appropriate candidate target to be inhibited rather than the molecules unique for each signal transduced by TGF-$\beta$ or IL-1$\beta$. Further, because HSP47 acts as a universal molecular chaperone for all types of collagen (6), inhibition of HSP47 is thought to interfere with the secretion of various collagens simultaneously. In fact, we have recently confirmed that transfection of HEL cells with HSP47-specific ribozyme brought about almost complete inhibition of collagen secretion from these cells. Therefore, a modality to attenuate the expression of HSP47 could be a promising approach to preventing fibrosis.

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