Enhanced LPS-induced TNFα production in heat-shocked human promonocytic cells: Regulation at the translational/post-translational level

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Abstract

Heat shock proteins (hsp) play an important role in maintaining cellular homeostasis and protecting cells from various insults. Recent evidence also implicates hsp in the regulation of the immune response, particularly the inflammatory process. In the present study, we showed that human promonocytic cells (THP-1) produced elevated levels of tumor necrosis factor alpha (TNFα) after incubation with bacterial lipopolysaccharide (LPS) when cells were pre-stressed by a mild heat shock (HS) of 42°C (1.5 h) followed by recovery at 37°C (3 h) in comparison with non-stressed cells also stimulated with LPS. This enhanced TNFα production was not due to changes in nuclear factor-κB (NF-κB) activation, TNFα transcription rates, or mRNA stability. Thus, an effect at the translational or posttranslational level is likely responsible. Elevated production of TNFα was not observed when cells were stimulated with LPS immediately after stress or when HS temperature was increased to 43°C. This negative effect of HS is likely due to a harmful effect of temperature. Moreover, enhanced LPS-induced TNFα production was not observed after differentiation of promonocytes into macrophage-like cells. Thus, our results show that the stress temperature, recovery period, and differentiation stage of the cell modulate the effect of HS on the inflammatory process.

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1. Introduction

Exposure of cells to elevated temperatures induces a change in the pattern of gene expression leading to expression of heat shock proteins (hsp). Several other physiological stressors have also been shown to induce up-regulation of hsp, including hypoxia, oxidants, UV radiation, sodium arsenite, and amino acid analogues [1–3]. Hsp have been classified according to their molecular weight and sequence similarity. The Hsp70 family is composed of four members: Hsp70, Hsc70, Grp78, and Mt70. Hsc70, Grp78, and Mt70 are constitutively expressed, whereas Hsp70 is induced exclusively after stress. These proteins are present in different sub-cellular compartments and their functions are not completely identical [1]. While stress-induced expression of hsp has been observed in all cells from prokaryotes to higher eukaryotes, the level of hsp expression differs among various species and cell types [4]. Under normal conditions, hsp are involved in several cellular processes including protein folding, translocation of polypeptides across membranes, assembly of protein complexes, and protein degradation. Thus, hsp have been coined molecular chaperones. Following stress, hsp are involved in the repair of cellular damage, the disposal or folding of affected proteins, and the stabilization of cellular processes [1–3].

The presence of hsp induced by a mild stress results in protection against subsequent lethal insults, a phenomenon coinined stress tolerance [1,5]. A large number of studies have correlated the expression of hsp with protection against several clinically relevant conditions including ischemia/ reperfusion injury [6,7], oxidative injury, hemorrhagic shock [8], endotoxemia [9,10], and sepsis [11–13].
Studies using cells in culture have further shown that hsp expression protects against apoptosis [14,15]. Delivery of Hsp70 via virus expression vectors improved myocyte survival after ischemia [16] and lung injury after sepsis [17]. In other studies, transgenic mice expressing Hsp70 were found to be resistant to cardiac [18,19] and cerebral [17] ischemia/reperfusion injury. Lipopolysaccharide (LPS) is a cell wall constituent of Gram-negative bacteria that induces various inflammatory and immunostimulatory factors in different cell types, particularly macrophages and monocytes [22]. LPS activates several signal transduction pathways leading to the transcription of many genes involved in the inflammatory process, such as tumor necrosis factor alpha (TNFα) and interleukin (IL) 6, via nuclear factor-κB (NF-κB). Several immunopathological features of LPS-induced shock can be attributed to the presence of TNFα [23]. Hsps have been found to modulate the expression of several inflammatory mediators, such as TNFα [24], IL-1β [25], and nitric oxide (NO) [26–28]. However, the mechanisms of this altered expression of inflammatory factors by HS treatment are not completely understood. In this study, we found elevated levels of TNFα in human promonocytes (THP-1 cells), but not in macrophage-like differentiated THP-1 cells after HS pretreatment and stimulation with LPS. The mechanisms for the enhanced production of TNFα appear to be at the translational or posttranslational level.

2. Materials and methods

2.1. Cell culture and heat shock (HS)

The human promonocytic cell line THP-1 (TIB 202, American Type Culture Collection) was maintained in suspension in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 50 U/ml penicillin, and 50 μg/ml streptomycin at 37 °C in 5% CO₂ incubator. Cells were maintained at a density of 3×10⁵ to 1.5×10⁶ cells/ml, and used in log-phase of growth between the third and thirteenth passages. Cells were free of mycoplasm. Promonocytic THP-1 cells were differentiated into macrophage-like cells that adhered to plastic and expressed CD14 by incubation with phorbol 12-myristate 13-acetate (PMA, 10 ng/ml) for 18 h [29]. HS was performed in a 5% CO₂ incubator at 42 °C or 43 °C for 1.5 h, followed by recovery at 37 °C. Cells were stimulated with Escherichia coli LPS serotype 026:B6 (Difco Laboratories, Detroit, MI) at 37 °C.

2.2. Western blot analysis

Cells were lysed with cold RIPA buffer (20 mM HEPES, pH 7.4, 1% Triton X-100, 50 mM NaCl, 1 mM EGTA, 5 mM β-glycerophosphate, 30 mM sodium pyrophosphate, 100 mM sodium orthovanadate, 0.1 mM phenyl-methyl-sulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml pepstatin A) and centrifuged at 250×g for 10 min. Equal amounts of protein from the cell lysates were separated by SDS-PAGE, transferred onto a PVDF membrane filter and quenched with Tris-buffered saline-Tween 20 (TBS-T; 20 mM Tris, 500 mM NaCl, 0.1% Tween 20) containing 5% powdered milk for 1 h at 25 °C. Blots were incubated with a mouse monoclonal antibody against human Hsp70 (1:5000) (Stressgen Biotechnologies Corporation, Victoria, BC, Canada) or monoclonal antibody against β-actin (1:5000) (Sigma, St. Louis, MO), in fresh blocking buffer for 1 h at 4 °C. Then, blots were washed three times with TBS-T and incubated with a horseradish peroxidase-conjugated antibody-IgG antibody (1:50000) (Amersham Pharmacia Biotech, Inc., Piscataway, NJ) in blocking buffer for 1 h at 25 °C. Membranes were washed with TBS-T and the antibody signal was visualized by incubation with SuperSignal (Pierce Biotechnology, Rockford, IL) for 3–5 min and detected using a Chemi Genius system (Gyngene, Syngenic, Inc., Frederick, MD).

2.3. Electrophoretic mobility shift assay

Nuclear extracts were isolated using a modification of the protocol by Dignam et al. [30]. Briefly, cells (4×10⁶) were centrifuged at 250×g for 10 min at 4 °C, resuspended in 400 μl of hypotonic buffer A (HEPES 10 mM pH 7.9, KCl 10 mM, EDTA 0.1 mM, EGTA 0.1 mM, DTT 1 mM, PMSF 0.5 mM, and 10 μg/ml each of leupeptin, aprotonin, and pepstatin) and incubated on ice for 10 min. Nonidet P-40 at a final concentration of 0.6% was added and lyses were vortexed for 4 s. Nuclei were isolated by centrifugation at 2800×g for 10 min at 4 °C and the pellet was resuspended in 15 μl of buffer C (HEPES 20 mM pH 7.9, 1 M NaCl, 5% Glycerol, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, 10 μg/ml each of leupeptin, aprotonin, and pepstatin) and gently agitated on an orbital shaker at 4 °C for 30 min. The suspension was centrifuged at 14000×g for 10 min at 4 °C, and the supernatant was aliquoted and stored at −70 °C. NF-κB DNA binding assay was performed using a double-stranded oligonucleotide containing the consensus binding site for NF-κB (Promega, Madison, WI) and was endlabeled with [γ-32P]ATP (7000 Ci/mmol, NEN Life Science Products, Boston, MA) using T4 polynucleotide kinase (Promega) as described previously [31].

2.4. RNA extraction and Northern blot analysis

Cells (4×10⁶) were centrifuged, the cell pellet was resuspended in 1 ml of TRIZOL (Invitrogen Life Technology, Carlsbad, CA), and RNA was extracted following the manufacturer’s instructions. Total RNA (12 μg) was separated in a formaldehyde-agarose gel and transferred onto nylon membrane. A probe for TNFα (cDNA) was radiolabeled by the random primer method [32] using [α-32P]dCTP and [α-32P]dATP (5 μCi each—3000 mCi/
mmol, NEN Life Science Products) as previously described [33]. Hybridization was performed at 42 °C for 16 h and filters were washed three times with 50 mM Tris, pH 8.6, 1 M NaCl, 2 mM EDTA, and 1% SDS at 42 °C. The signal was detected using a PhosphorImager system (Molecular Dynamics, Amersham Pharmacia Biotech). Blots were stained prior to hybridization with methylene blue (0.03%) in 3 M NaOAc, pH 5.2. The signal corresponding to TNFα was normalized to the respective intensity of the 18S rRNA signal detected by methylene blue staining. The half-life of TNFα was obtained after inhibition of transcription with actinomycin D (10 μg/ml) and calculated by the formula 

\[ k = \frac{2.303}{t_{1/2}} = \frac{\log X_1}{X_2} \]

where \( t \) and \( X \) represent time and RNA concentrations, respectively.

2.5. Nuclear run-off

The procedure was carried out as previously described [34]. Briefly, cells (5×10⁷) were lysed in 10 mM Tris, pH 7.4, 10 mM NaCl, 3 mM MgCl₂, and 0.5% Nonident P-40. The nuclei were isolated by centrifugation at 2800 g for 10 min and the pellet was resuspended in 150 μl of storage buffer (50 mM Tris, pH 8.5, 5 mM MgCl₂, 40% glycerol). Nuclear run-off reaction was performed by mixing the nuclei sample with an equal volume of 50 mM HEPES, pH 7.4, 5 mM MgCl₂, 5 mM dithiothreitol, 150 mM KCl, 10% glycerol, 0.7 mM ATP, GTP and CTP, 0.8 μM UTP, and 0.1 μCi of [α-³²P]UTP and incubated at 30 °C for 30 min. The reaction was stopped by addition of DNase I (10 units) at 30 °C for 10 min, followed by the addition of 4 volumes of 10 mM Tris, pH 8, 0.35 M LiCl, 1 mM EDTA, 7 M urea and 2% SDS, and proteinase K (500 μg/ml), and incubation at 45 °C for 1 h. The sample was combined with 15 μg of tRNA and precipitated with trichloroacetic acid (10%). The pellet was resuspended in 10 mM Tris pH 8, 1 mM EDTA, and 0.5% SDS and hybridized to DNA target (pBluescript SK⁺, TNFα, and human 28 S rRNA) for 4 days at 42 °C. Membranes were washed (6× SSC, 1% SDS and 2× SSC, 0.1% SDS) and the signal was detected using the PhosphorImager system.

2.6. Cytokine quantification

The extracellular medium was centrifuged at 250×g for 10 min and the supernatant was stored at −20 °C. For determination of intracellular TNFα content, cells were lysed, centrifuged and the supernatant was stored at −20 °C. TNFα was analyzed using a commercial ELISA kit (BIOSOURCE, Camarillo, CA).

2.7. Data analysis

Statistical significance was determined by one-way analysis of variance (ANOVA) with Fischer’s post-hoc correction. A value of \( P<0.05 \) was considered significant. Data are expressed as mean±standard error (S.E.).

3. Results

3.1. Characterization of Hsp70 expression

The kinetics of Hsp70 expression was evaluated in promonocyte THP-1 cells, as well as in macrophage-like (Mφ-THP-1) THP-1 cells that were differentiated by incubation with PMA. HS was induced at 42 or 43 °C for 1.5 h and cells were allowed to recover at 37 °C for 0, 1, 3, 6, 18, and 24 h. At the end of each time point, cells were lysed and the presence of Hsp70 or β-actin was determined by Western blotting. A similar pattern of Hsp70 expression was observed in both the THP-1 and Mφ-THP-1 cell types. Higher levels of Hsp70 were observed after HS at 43 °C in comparison with 42 °C (Fig. 1A and B). No expression of Hsp70 was detected in non-stressed (naïve) cells.

![Fig. 1](image-url) Expression of Hsp70 in THP-1 cells. Non-differentiated THP-1 or PMA differentiated macrophage-like THP-1 cells (Mφ-THP-1) were subjected to HS at either 42 or 43 °C for 1.5 h and allowed to recover at 37 °C for 0, 1, 3, 6, 18, and 24 h. At the end of the recovery period, cells were lysed and equal amounts of protein (50 μg) were separated by SDS-PAGE, transferred onto PVDF, and the presence of Hsp70 or β-actin was detected. Naïve cells (N) correspond to cells maintained at 37 °C (A) Representative Western blot; (B) densitometric analysis of Hsp70 levels.
3.2. LPS-induced TNFα production is increased after HS

We evaluated the effect of HS on TNFα production after incubation with LPS. THP-1 cells were incubated at 42 °C for 1.5 h and recovered at 37 °C for 18 h, whereas naïve cells were maintained at 37 °C. Both HS and naïve cells were then stimulated with different concentrations of LPS (10 to 1000 ng/ml) for 24 h. A concentration-dependent increase in LPS-induced TNFα release was observed in naïve and HS cells. However, TNFα levels were higher in HS-THP-1 cells than in naïve cells. No released TNFα was detected in heat-shocked or naïve cells in the absence of LPS stimulation (Fig. 2A). To investigate whether the effect of HS was dependent on the recovery time after the stress, THP-1 cells were incubated at 42 °C for 1.5 h, then shifted to 37 °C for different time periods (0, 1, 3, 6, and 18 h). At the end of each recovery period, cells were stimulated with LPS (100 ng/ml) for 24 h. Stimulation with LPS immediately after stress or after a 1-h recovery period showed TNFα levels in the extracellular medium that were similar to naïve cells. In contrast, a significant increase in TNFα release was detected in HS cells stimulated with LPS after 3 or 6 h of recovery in comparison with naïve cells (Fig. 2B). The increase in TNFα release correlated with the accumulation of Hsp70 within the cells during recovery time after HS. To further substantiate these findings, cells were heat-shocked at 42 °C for 1.5 h and incubated with LPS (100 ng/ml) at 37 °C for different time periods (1, 3, 4, 18, and 24 h) immediately after stress (Fig. 2C) or after 3 h of recovery (Fig. 2D). No significant differences in TNFα levels were observed between naïve cells and HS cells stimulated with LPS immediately after the stress. In contrast, elevated levels of TNFα were observed in HS cells incubated with LPS 3 h post-stress in comparison with naïve cells (Fig. 2D).

The effect of a higher HS temperature was also evaluated. THP-1 cells were heat-shocked at 43 °C for 1.5 h and incubated with LPS (for 24 h) immediately after HS or after 24 h of recovery. In this case, a reduction of TNFα levels was observed in HS cells with or without recovery after stress in comparison with naïve cells (Fig. 3). In addition, THP-1 cells were differentiated into macrophage-like cells upon incubation with PMA for 18 h at 37 °C. The differentiated cells were heat-shocked at 42 °C for 1.5 h and incubated with LPS (24 h) either immediately after HS or after 3 and 24 h of recovery at 37 °C (Fig. 4). Naïve cells were stimulated with LPS for the same time period. TNFα production was found to decrease in HS cells as compared with naïve cells when LPS stimulation was performed immediately after the stress. This effect was reversed as recovery time increased. Thus, TNFα levels were reduced 37%±6.7% in HS cells as compared to naïve cells after LPS stimulation at 3 h of recovery. However, no significant

![Fig. 2. Effect of HS on LPS-induced TNFα release.](image-url)

- **A**: THP-1 cells were HS at 42 °C for 1.5 h and then returned to 37 °C for 18 h (HS, broken line, open square) or maintained at 37 °C (naïve, solid line, filled square). Cells were stimulated with different concentration of LPS (from 10 to 1000 ng/ml) for 24 h (n=2).
- **B**: Cells were HS at 42 °C for 1.5 h and recovered in the 37 °C incubator for 0, 1, 3, 6, and 18 h (HS, broken line, open square) or maintained at 37 °C for the same time periods (naïve, solid line, filled square). Cells were incubated in the presence or absence (Basal and HS) of LPS (100 ng/ml) for 24 h at 37 °C (n=2).
- **C**: THP-1 cells were subjected to HS at 42 °C for 1.5 h (HS, broken line, open square), and recovered at 37 °C for 0 h (C) or 3 h (D) and incubated with LPS (100 ng/ml) for 1, 3, 4, 18, and 24 h at 37 °C. Naïve cells (solid line, filled square) were maintained at 37 °C and stimulated with LPS as well. TNFα in the extracellular medium was determined by ELISA. The data are presented as the increase of TNFα in the extracellular medium with respect to LPS stimulation in naïve cells at 24 h (*P<0.05, HS+LPS vs. LPS, n=3–5).
The difference in LPS-induced TNFα levels was observed between HS and naïve cells after 24 h of recovery (Fig. 4).

3.3. Heat shock does not alter NF-κB DNA binding activity

We next investigated the possible mechanism for the elevated levels of TNFα after recovery from 42 °C HS. A key component of TNFα expression is the activation of NF-κB [35]. Consequently, we examined whether this effect was due to increased NF-κB DNA binding activity in heat-shocked versus naïve cells. Naïve or heat-shocked cells (after 3 h of post-stress recovery) were stimulated with LPS (100 ng/ml) for 1 h and nuclear protein extracts were prepared to determine NF-κB DNA binding activity by the gel shift assay. No difference in NF-κB DNA binding activity was observed between HS and naïve cells after LPS stimulation. Low NF-κB DNA binding activity was detected in the absence of LPS stimulation or after HS alone (Fig. 5).

3.4. Neither TNFα transcription rate nor mRNA stability is affected after HS

The elevated levels of LPS-induced TNFα in HS cells in comparison with naïve cells could be due to an increase in TNFα transcription rates in HS cells. Transcription rates were measured by the nuclear runoff technique. Naïve or heat-shocked cells (after 3 h of recovery) were stimulated with LPS for 30, 80, and 140 min, cells were harvested, and nuclei were isolated and used for the nuclear run-off reaction. Similar rates of TNFα transcription were observed for heat-shocked and naïve cells after stimulation with LPS (Fig. 6A and B). In addition, we evaluated whether there were differences in TNFα mRNA steady-state levels after stimulation with LPS in HS and naïve cells. Cells were heat-shocked at 42 °C for 1.5 h and stimulated with LPS after 3 h of recovery from HS, whereas naïve cells were maintained at 37 °C and then stimulated with LPS. Total RNA was extracted and TNFα levels detected by Northern blot analysis. Similar steady-state levels of TNFα levels were observed in heat-shocked and naïve cells. No detectable TNFα steady-state mRNA levels were observed in the absence of LPS incubation or after HS alone (Fig. 7). Thus, the elevated levels of TNFα in the extracellular medium could not be correlated with an increase in transcription or increased TNFα mRNA steady-state levels. To address whether the degradation of TNFα mRNA is affected by HS,
THP-1 cells were heat-shocked for 1.5 h at 42 °C and allowed to recover for 3 h. Heat-shocked and naïve cells were stimulated with LPS for 2 h, treated with actinomycin D (10 μg/ml) for 0, 10, 15, 25, 30, 40, and 50 min, and total RNA was extracted. No differences in the disappearance of TNFα mRNA in presence of the transcriptional inhibitor were detected by Northern blot analysis (Fig. 8). Densitometry analysis of TNFα mRNA showed that the half-life was 32 and 30 min for HS and naïve cells, respectively (Fig. 8B).

3.5. The intracellular content of TNFα is also elevated in HS cells

The preceding observations suggest that the elevated levels of TNFα observed in HS cells could be due to more efficient translation of TNFα mRNA. If this assumption is correct, HS cells would be expected to have higher intracellular levels of TNFα than naïve cells. Naïve and heat-shocked THP-1 cells (after 3 h of recovery) were incubated with LPS for 140 min, lysed, and then TNFα content was measured by ELISA. The intracellular content

Fig. 7. Steady-state levels of LPS-induced TNFα mRNA after HS. THP-1 cells were heat-shocked at 42 °C for 1.5 h and recovered at 37 °C for 3 h. Naïve cells were maintained at 37 °C. Cells were stimulated with LPS (100 ng/ml) for 0, 1, 2, and 3 h. Total mRNA was extracted and analyzed by Northern blotting as described in Materials and methods. The TNFα mRNA signal was detected using a PhosphorImager system (top). Methylene blue staining for 18 rRNA (bottom). Northern blot representative of four independent analyses.

Fig. 8. Changes in TNFα mRNA stability after HS. THP-1 cells were heat-shocked at 42 °C for 1.5 h (HS) and recovered at 37 °C for 3 h. Naïve cells (N) were maintained at 37 °C. Cells were stimulated with LPS for 2 h, then actinomycin D (Act D, 10 μg/ml) was added and cells were further incubated for 0, 10, 15, 25, 30, 40, and 50 min. Total RNA was isolated and the levels of TNFα mRNA detected by Northern blotting (A). The TNFα mRNA signal was quantitated for HS (broken line-open square) and naïve (solid line-filled square) and plotted versus the incubation time with Act D. \( R^2=0.977 \) with \( t_{1/2}=32 \) min for HS and \( R^2=0.939 \) with \( t_{1/2}=30 \) min for naïve cells. Representative experiment of three independent determinations.
of TNFα was higher in HS cells compared with naïve cells (Fig. 9).

4. Discussion

Recently, hsps have been shown to play an important role in immunomodulation. Hsps participate as immune adjuvants by delivering priming antigen peptides to antigen presenting cells [36,37]. Moreover, hsps present in the extracellular medium appear to activate the expression of cytokines in macrophages [38]. HS treatment also alters cytokine production depending on the stress conditions and cell type. In the present study, we showed that the influence of HS pretreatment on the LPS response in THP-1 cells varied depending on the magnitude of the stress, the recovery time, and the differentiation stage of the cells. Exposure of promonocytes in suspension to a temperature of 43 °C led to inhibition of LPS-induced TNFα expression, whereas HS at 42 °C resulted in increased TNFα production after LPS stimulation with respect to non-stressed cells. This enhanced response to LPS was absent if the cells were differentiated into macrophage-like cells. In addition, the recovery time following the stress was critical to the effect of HS on LPS-induced TNFα production. Thus, the effect of HS depends on temperature, recovery time, and stage of cell differentiation. These apparently conflicting observations could explain the controversial reports in the literature regarding the effect of HS on the inflammatory process. For example, peritoneal macrophages subjected to HS at 45 °C exhibited a decrease in TNFα release when cells were stimulated for 2 to 6 h after stress [24]. Down-regulation of LPS-induced TNFα production after HS (40 °C) was observed in the macrophagic line Raw 264.7. In contrast, mice subjected to hyperthermia (39.5–40 °C) exhibited an increase in LPS-induced TNFα in plasma and liver samples [39]. In another study, injection of LPS into mice resulted in similar TNFα plasma levels for thermally stressed (42 °C) and sham-stressed mice [40]. Whole blood cells isolated from healthy individuals showed an increase in LPS-induced TNFα release after hyperthermia as compared to no-heat pretreatment cells [41]. HS treatment at 43 °C inhibited TNFα-induced nuclear translocation of NF-κB in a human lung epithelium cell line [42]. Similarly, LPS stimulation of peritoneal macrophages and RAW264.7 cells resulted in a decrease in IL-12 p40 mRNA and NF-κB after HS pretreatment at 43 °C [43]. Activity of the inducible NO synthase was found to decrease after LPS stimulation following HS at 43 °C in glial cells [26], whereas incubation of macrophages at 40 °C led to enhanced NO production after LPS stimulation [44]. Bellmann et al. [28] showed that stimulation of WEHI cells with LPS and interferon gamma resulted in increased NO production after 24 h of recovery from HS or in cells constitutively expressing Hsp70.

The mechanism of altered expression of inflammatory factors by HS has been elusive. Our data suggest that the increase in TNFα released by promonocyte cells after HS pretreatment (42 °C) and LPS stimulation is at the translational or posttranslational level. Several observations support this assumption. Neither activation of NF-κB, TNFα transcription rate nor change in TNFα mRNA stability was affected by the HS pretreatment. In contrast, the extracellular and intracellular levels of TNFα were higher in HS than naïve cells, although TNFα mRNA levels were identical. The distribution of TNFα mRNA within polysomes was very similar for heat-shocked and non-stressed cells, suggesting that the levels of initiation are similar between the two cell groups. An increase in the elongation rate, initial folding or translocation of TNFα into the endoplasmic reticulum is likely enhanced in heat-shocked (42 °C) cells. This possibility is consistent with the chaperone function of hsps [1–3]. Moreover, Hsp70 has been previously found to be associated with translating ribosomes. It was hypothesized that this protein was within ribosomes waiting for the nascent polypeptide to be elongated [33]. Thus, it is possible that folding and translocation of TNFα into the secretory pathway may be enhanced by the presence of hsps, in particular Hsp70.

The question that emerges is whether the increase in TNFα production is protective or detrimental. The role of cytokines during inflammation is controversial. On one hand, cytokines are part of the protective mechanisms that combat infection and control injury. However, exaggerated production of cytokines results in pathologies, such as body mass waste or cachexia. Moreover, a poorly controlled inflammatory process is a key factor in the development of septic shock, which is mediated in part by the presence of LPS. Thus, it is possible that hsps can modulate the inflammatory process as part of a general protective mechanism. In fact, prior studies have shown that induction of hsp expression, in particular Hsp70, results in protection from endotoxemia [9], sepsis [17,45], ischemia/reperfusion injury [6,7], and oxidative injury following hemorrhage [8].

Another interesting observation is that the response of promonocytes and macrophages differed. Specifically, promonocytes showed enhanced production of TNFα after HS at 42 °C, whereas differentiated cells (macrophage-like)
displayed an opposite pattern under the identical stress conditions. Consequently, different cells of myeloid lineage could respond differently to stress. Prior studies have shown that the levels of Hsp70 in different organs from the same rat after thermal stress differed significantly [4]. This observation was reproduced in cell lines of different origins under identical conditions of HS [4,46]. Oehler et al. [47] found that HS treatment of whole blood induced Hsp70 depending on the temperature and cell type. Therefore a strong induction of Hsp70 was already seen at 39 °C in monocytes, while in lymphocytes and polymorphonuclear leukocytes Hsp70 was only slightly increased at 41 °C. The amount of recovery time is also crucial to the effect of HS treatment on cytokine production. Thus, short recovery times after the stress result in inhibition or no effect on cytokine production, most likely due to a harmful effect of temperature. On the other hand, longer recovery times, which allow massive production of hsps, result in an enhancement of cytokine production, likely due to the protection of different cellular pathways. In fact, stabilization of translation is likely the mechanism responsible for enhanced LPS-induced TNFα production after 42 °C HS pretreatment in promonocytic cells. In summary, our results illustrate that the effect of HS on cytokine production differs depending on the environmental conditions and cell type. An understanding of the process of hsp protection during inflammation may help in the development of therapies to ameliorate conditions of exaggerated inflammation such as sepsis, cancer, and cardiovascular disease.

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References


