Induction of heat shock proteins and their possible roles in macrophages during activation by macrophage colony-stimulating factor

Shigetada TESHIMA*, Kazuhiro ROKUTAN*,‡, Masayuki TAKAHASI†, Takeshi NIKAWA* and Kyoichi KISHI*
*Department of Nutrition, School of Medicine, University of Tokushima, Tokushima 770, and †Cellular Technology Institute, Otsuka Pharmaceutical Co., Tokushima 771-01, Japan

INTRODUCTION

The macrophage colony-stimulating factor (M-CSF), first identified as an L cell macrophage growth factor by Stanley and Heard [1], plays a crucial role in survival, proliferation, and differentiation of mononuclear phagocyte lineages [2]. In addition, M-CSF activates tissue macrophages and increases their capacity for killing microbes [3] or tumor cells [4]. In response to appropriate stimuli, macrophages release large amounts of partially reduced oxygen intermediates, including superoxide anion (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$), through the activity of a membrane-associated NADPH oxidase [5]. These oxygen intermediates have been postulated to play a central role in microbicidal and cytotoxic activities. The capacity for O$_2^-$ release is markedly enhanced by bacterial lipopolysaccharide (LPS) [6] and cytokines including γ-interferon [7], interleukin 3, granulocyte-macrophage CSF, and M-CSF [8,9]. During activation of macrophages, certain functional proteins have been reported to be induced [10], and some of them have been identified as a nitric oxide synthase [11], the phagocyte cytochrome b, an essential component of the O$_2^-$-generating oxidase [12], and prostaglandin endoperoxide synthase 2 [13].

In human blood monocytes, exposure to heat, glucose starvation, or oxidants and physiological oxidative stress during phagocytosis induce heat shock proteins (HSPs) [14-17]. In addition, activators of monocyte functions, such as LPS and tumor necrosis factor, and an inducer of differentiation, 1,25-dihydroxyvitamin D$_3$, have been shown to increase the synthesis of HSP70 in these cells [14,16]. These results suggest that HSPs may play a role in the activation and differentiation of monocyte macrophages as well as in autoprotection against various types of insults. On the other hand, it is not well understood how the stress response occurs during activation of tissue macrophages.

In the present experiments, we show that M-CSF induced stress-inducible HSP90, HSP70, HSP60 and a constitutively expressed HSP with a molecular mass of 73 kDa (HSC70) during activation of macrophages in vitro under LPS-free conditions. We also studied the mechanism of inductions of HSPs and their possible roles in macrophages.

MATERIALS AND METHODS

Reagents and media

Recombinant human C-terminal-truncated M-CSF [18] was used in the present experiments. The structure and biological activity of this recombinant M-CSF have already been described [19]. Superoxide dismutase (SOD, from horse heart), ferricytochrome c, phorbol 12-myristate 13-acetate (PMA) and LPS (from Escherichia coli K-235) were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Catalase from bovine liver was purchased from Wako Pure Chemical Industries, Osaka, Japan. Dulbecco's modified Eagle's medium (DMEM) was from Flow Laboratories, McLean, VA, U.S.A. Hanks balanced salt solution without

Abbreviations used: CHX, cycloheximide; DMEM, Dulbecco's modified Eagle's medium; DTT, dithiothreitol; ECL, enhanced chemiluminescence; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HSE, heat shock element; HSF, heat shock transcription factor; IEF, isoelectric focusing; HSP, heat shock protein; HSC, constitutive heat shock protein; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; M-CSF, macrophage colony-stimulating factor; PMA, phorbol 12-myristate 13-acetate; PVDF, poly(vinylidene difluoride); SOD, superoxide dismutase.

‡ To whom correspondence should be addressed.
Phenol Red (HBSS) was purchased from Nissui Pharmaceutical Co., Tokyo, Japan. Methionine-free RPMI 1640 was from Gibco, Grand Island, NY, U.S.A. 35S-labelled protein labelling mix containing ≥ 77% l-methionine and ≥ 18% l-cysteine (specific radioactivity 1000–1200 Ci/mmol) was obtained from DuPont/New England Nuclear, Boston, MA, U.S.A. Polyclonal antibodies against HSP90 [20], HSC70 [21], HSP70 [22] and HSP60 [23] were gifts from Dr. Hideaki Itoh, Akita University, Japan. Rabbit antiserum raised against heat shock transcription factor 1 (HSF1) was kindly provided by Dr. Akira Nakai and Dr. Kazuhiro Nagata, Kyoto University, Japan. Mouse casein was purchased from Yukijirushi Co., Sapporo, Japan. Poly(dI-dC)-poly(dI-dC) and amphotiles of pH 4.0–6.5, 5.0–8.0 and 3.5–9.5 were obtained from Pharmacia LKB Biotechnology Inc., Sweden. [γ-32P]ATP (> 500 Ci/mmol), [α-32P]dCTP (3000 Ci/mm), T4 polynucleotide kinase, an enhanced chemiluminescence (ECL) Western-blotting detection system, and a random-primer labelling kit were from Amersham Japan, Tokyo, Japan. cDNA probes for human HSP70 (A.T.C.C. 57499) and human glyceraldehyde-3-phosphate dehydrogenase (GAPDH; A.T.C.C. 57091) were purchased from the American Type Culture Collection, Rockville, MD, U.S.A. The human HSC70 cDNA probe was a gift from Dr. Richard I. Morimoto, Northwestern University, Evanston, IL, U.S.A.

Isolation and cultivation of resident peritoneal macrophages

C3H/He-slc mice at 8 to 12 weeks of age were purchased from Shizuoka Laboratory Animal Center, Shizuoka, Japan. Resident peritoneal macrophages were prepared in a LPS-free system as described previously [24]. All reagents to which macrophages were exposed in culture were determined to be free from detectable LPS by the Luminus amoeboocyte lysate assay (Etoxate, Sigma). Peritoneal cells were collected by washing the peritoneal cavity with ice-cold saline. After washing the isolated cells twice with cold saline, they were suspended in DMEM and 1 × 10^6, 3 × 10^6, or 1 × 10^7 cells were placed in each well of a 24-well culture plate (Corning Glass Works, Corning, NY, U.S.A.), a 35-, or a 60-mm-diam. plastic dish (Corning Glass Works, Corning, NY, U.S.A.), or a 35-mm-diam. plastic dish (Corning Glass Works) respectively. After incubation for 2 h at 37 °C in 5% CO₂/95% air, non-adherent cells were removed by vigorously washing three times with ice-cold saline. Macrophages were further cultured overnight in DMEM without any supplement and then were left untreated or were treated with various concentrations of M-CSF for the indicated times. Differential counts of the adherent cells used for experiments were determined microscopically after Giemsa and non-specific esterase staining [24]. Resident preparations were > 95% macrophages.

Assay of O₂ release

O₂ release from macrophages was assayed by measuring the SOD-inhibitable reduction of ferricytochrome c as described previously [24]. The amount of O₂ release was spectrophotometrically determined at 550 nm and expressed in terms of nmol/mg of protein per h. Cell protein was determined by the Lowry method using BSA as a standard.

Metabolic labelling with [³⁵S]methionine and gel electrophoresis

Macrophages were treated with 1 µg/ml M-CSF for the indicated times. After washing with normal saline prewarmed at 37 °C, they were further cultured for 2 h in methionine-free RPMI 1640 containing 50 µCi [³⁵S]methionine (80 µCi/ml), 1 µg/ml M-CSF and 0.1% mouse autologous serum. Control cells were labelled in the same manner without M-CSF. The labelling was terminated by aspirating the medium and washing three times with ice-cold PBS. A 50 µl aliquot of lysis buffer consisting of 50 mM Tris/HCl (pH 7.4), 6 M urea, 1 mM EDTA, 1 mM EGTA, 1 mM PMSF and 0.33 mM leupeptin was promptly added. The macrophages were scraped with a ‘rubber policeman’ and collected in microcentrufuge tubes. Cell extracts were then passed through a 22-gauge needle 20 times, and soluble proteins, obtained by centrifugation at 11300 g for 20 min at 4 °C, were subjected to SDS/PAGE or two-dimensional PAGE. For SDS/PAGE, samples were mixed with the same volume of double-strength Laemmli’s buffer and boiled for 5 min. The sample containing 5 × 10^6 c.p.m. 35S was resolved in SDS/10% PAGE. The gel was stained with Coomassie Brilliant Blue R-250 and dried for autoradiography. For two-dimensional PAGE, soluble proteins were dissolved in 40 µl of lysis buffer consisting of 0.4 % pH 4.0–6.5 amphotile, 0.8 % pH 5.0–8.0 amphotile, 0.8 % pH 3.5–9.5 amphotile, 5 % (v/v) glycerol, 0.5 % Nonidet P-40, 2 % (v/v) 2-mercaptoethanol and 0.001 % Bromophenol Blue. The mixture containing 1 × 10^6 c.p.m. 35S was subjected to isoelectric focusing (IEF) in disc gels of 4% polyacrylamide containing the above lysis buffer lacking 2-mercaptoethanol and Bromophenol Blue. After IEF, the gels were incubated with Laemmli’s SDS buffer for two 15-min periods. The second electrophoresis was carried out in SDS/10%PAGE. The gels were treated with DMSO/2,5-diphenyloxazole for fluorography, dried and exposed to Fuji RX-R film (Fuji Photo Film, Tokyo, Japan). The radioactivity of labelled proteins precipitated with 5 % (v/v) trichloroacetic acid was counted using a liquid scintillation counter, and the results were expressed as c.p.m./mg of protein.

Western-blot analysis

Cell extracts (40 µg of protein) were subjected to SDS/10%PAGE and transferred to a poly(vinylidene difluoride) (PVDF) filter. After blocking with 4 % (w/v) milk casein for 30 min, the filter was incubated for 1 h at room temperature with a 1:1000 dilution of polyclonal antibody against HSP90, HSP70, HSC70, or HSP60. Bound antibodies were detected using an ECL system.

Assay of H₂O₂-induced cell damage

Cell viability after exposure to 1 mM H₂O₂ for 1 h was assessed by measuring the percentage release of lactate dehydrogenase (LDH) as described previously [25]. Macrophages cultured in 24-well culture plates (1 × 10⁶ cells/well) were left untreated or treated with 1 µg/ml M-CSF for 8 h, washed three times with HBSS, then exposed to 1 mM H₂O₂ in HBSS for 1 h.

Northern-blot analysis

Total RNA was isolated with an acid guanidinium thiocyanate–phenol–chloroform mixture [26]. A sample (15 µg) of total RNA was subjected to electrophoresis in 1 % agarose gel containing 0.6 M formaldehyde and transferred to Hybond N-plus filters (Amersham). The filters were prehybridized in hybridization solution containing 50 % formamide, 5 × SSC (1 × SSC; 0.15 M NaCl, 0.015 M sodium citrate), 5 × Denhardt’s solution and 0.1 % SDS for 4 h at 42 °C. The filters were hybridized for 16 h at 42 °C by incubating with the cDNA probe specific for human HSP70, human HSC70, or human GAPDH in the presence of 100 µg/ml heat-denatured salmon sperm DNA and 10 % (w/v) dextran sulphate. These probes were labelled with [α-³²P]dCTP to a specific radioactivity of > 1 × 10⁶ c.p.m. using a random-primer kit. The filters were washed twice with 2 × SSC containing 0.1 % SDS at 50 °C and then three times with 0.2 × SSC.
containing 0.1% SDS at 65 °C. Bound probes were detected using a Fujix Bio-Analyzer BAS-2000 (Fuji Photo Film) and autoradiographed by exposure of the filters to Fuji RX-R films for 2 or 3 days at −80 °C.

Preparation of whole-cell extracts and the gel mobility shift assay
Preparation of whole-cell extracts and gel mobility shift assays were performed by the method of Mosser et al. [27]. Cells (1 × 10^6) in 60-mm-diam. culture dishes were left untreated or treated with 1 µg/ml M-CSF for the indicated times. Cells were harvested and collected in microcentrifuge tubes. After washing with PBS by centrifugation at 1200 g for 5 s in a microcentrifuge, pelleted cells were rapidly frozen in liquid nitrogen. The frozen pellets were suspended in lysis buffer containing 20 mM Hepes (pH 7.9), 0.2 mM EDTA, 0.5 mM PMSF, 0.5 mM leupeptin and 0.5 mM dithiothreitol (DTT). The mixture was gently pipetted using a Pasteur pipette and allowed to stand on ice for 15 min. Whole-cell extracts, obtained by ultracentrifugation at 100,000 g for 5 min at 4 °C, were dialysed against binding reaction buffer consisting of 10 mM Tris/HCl (pH 7.8), 50 mM NaCl, 1 mM EDTA, 0.5% (v/v) glycerol, 0.5 mM DTT and 0.5 mM PMSF, and stored at −80 °C.

The heat shock elements (HSEs) in the mouse and human hsp70 promoters are composed of five inverted pentamers (5'-nGAAn-3'), and the first, third and fourth repeats are the same consensus motif [28]. Therefore, a synthetic double-stranded HSE oligonucleotide encoding nucleotides −73 to −107 of the HSE (−GGAGGCGAAACCCCTGGAATATTCCCGACCT-GGCA-3') located in the human hsp70 promoter [29] was used to detect the activation of the heat shock transcription factor (HSF). The HSE oligonucleotide was radiolabelled with [γ-³²P]ATP by 5'-end labelling one strand with T4 polynucleotide kinase. The radiolabelled oligonucleotide was annealed to the complementary strand. Whole-cell extract (5 µg of protein) was mixed with 0.1 ng of ³²P-labelled oligonucleotide and 1 µg of poly(dI-dC)-poly(dI-dC) in the binding reaction buffer. Binding reaction was carried out at 4 °C for 20 min. The HSE-containing complexes were separated in 4%, non-denaturing polyacrylamide gels, and electrophoresis was done with 0.5 × Tris–borate–EDTA at 150 V for 3.5–4 h at 4 °C. Antibody perturbation experiments were performed as described by Sarge et al. [30]; 1 µl of 100-fold diluted rabbit antiserum specific for HSF1 was preincubated with whole-cell extracts at 4 °C for 20 min prior to the binding reaction.

RESULTS

Effect of M-CSF on PMA-stimulated O₂ release by macrophages
Resident macrophages, isolated and cultured overnight under LPS-free conditions, released 56 ± 8 nmol of O₂/mg of protein per h (mean ± S.D., n = 10) with 0.5 µg/ml PMA and 6.6 ± 0.8 (n = 6) without any stimulant. The amounts of PMA-stimulated O₂ release after treatment overnight with different concentrations of M-CSF are shown in Figure 1(A). M-CSF at 1 µg/ml or higher increased the release of the untreated control cells about 3-fold. Macrophages cultured overnight were treated with 1 µg/ml M-CSF for the indicated times, and the amounts of O₂ release without or with 0.5 µg/ml PMA were measured (Figure 1B). M-CSF did not change the spontaneous production of O₂, but PMA-stimulated release began to increase 6 h after addition of 1 µg/ml M-CSF and reached a maximum at 8 h. The time required for activation by M-CSF was similar to that by LPS [6], which has been shown to induce several functional proteins during activation of macrophages [10].

Figure 1 shows the effect of the protein synthesis inhibitor, cycloheximide (CHX), on PMA-stimulated O₂ release. In macrophages, a half-maximum inhibition of protein synthesis, estimated by [³⁵S]methionine incorporation into protein precipitated...
Effect of M-CSF on protein synthesis

Resident peritoneal macrophages were incubated with 1 µg/ml M-CSF for 2 or 6 h and then metabolically labelled with L-[³⁵S]methionine for 2 h in the presence of 1 µg/ml M-CSF. Whole-cell proteins were separated by SDS/PAGE or two-dimensional PAGE, and labelled proteins were analysed by autoradiography (Figures 3 and 4). Several proteins including 73, 67, 57, 56, 54, 43, 38 and 30 kDa proteins were induced within 2 to 4 h of addition of M-CSF (Figure 3, lane 2), at which time macrophages did not yet increase the capacity for O₂⁻ release (Figure 1B). Macrophages acquired the maximum activity of O₂⁻ release 8 h after treatment with M-CSF, at which time several other proteins including 90, 72, 60, 52, 48 and 40 kDa proteins were additionally induced.

Autoradiograms of two-dimensional PAGE show the marked changes in protein synthesis during activation by M-CSF (Figure 4). Inductions of low-molecular-mass proteins of less than 30 kDa were more clearly detected by two-dimensional PAGE than SDS/PAGE. At the same time, the autoradiograms revealed that M-CSF decreased the syntheses of certain proteins indicated by open arrows in Figure 4(A).

Detection of HSPs by immunoblotting

The molecular masses and pl values of several proteins, indicated by arrows in Figure 4(B), were compatible with those of known HSPs. Therefore, Western-blot analyses with anti-HSP antibodies were carried out to confirm this, and the results are shown

with trichloroacetic acid, was achieved at 200 ng/ml CHX. CHX at concentrations over 5 µg/ml inhibited 94% of total protein synthesis. As shown in Figure 2, 100 ng/ml CHX completely inhibited the M-CSF-induced increase in PMA-stimulated O₂⁻ release, suggesting that M-CSF induces certain functional proteins responsible for the enhanced capacity for O₂⁻ release.
Induction of heat shock proteins by macrophage colony-stimulating factor

Figure 5 Detection of HSPs by immunoblotting

Whole-cell proteins were extracted from cells freshly isolated and cultured for 2 h (lane 1, 01) or overnight (lane 2, 02) as described in the Materials and methods section. Macrophages were then treated with 1 µg/ml M-CSF for the indicated times (h) (lanes 3–7). Macrophages were also cultured for 8 h with no supplement (lane 8), 1 µg/ml M-CSF (lane 9), or 1 µg/ml M-CSF plus 300 units/ml SOD and 3500 I.U./ml catalase (lane 10). An equal amount of extracted cell proteins (40 µg of protein per lane) was subjected to 10% SDS/PAGE and transferred to a PVDF membrane. Immunoblot analysis with antibody against HSP90, HSP70, HSP60, or HSC70 was carried out as described in the Materials and methods section. Similar results were obtained in three separate experiments.

Figure 6 Time-dependent changes in the amounts of HSPs, PMA-stimulated O$_2^-$ release and [35S]methionine incorporation into cell proteins

The level of each HSP was quantified by densitometry and standardized by the level of respective protein in lane 01 (A). Before or after treatment of cells with 1 µg/ml M-CSF for 0, 2, 4, or 6 h, cells were incubated for 2 h with 20 µM i-[35S]methionine and 1 µg/ml M-CSF. Time-dependent changes in the incorporation of [35S] into cell proteins were determined as described in the Materials and methods section and compared with the time course of the PMA-stimulated O$_2^-$ release (B). Values are mean ± S.D., n = 10.

HSC70 began to increase 1 h after addition of M-CSF, and the levels continued to increase until 8 h. In contrast, M-CSF increased the levels of HSP90 and HSP70 at 6 h, and HSP60 was first detected at this time. M-CSF did not increase spontaneous O$_2^-$ release from macrophages (Figure 1B), but it still up-regulated HSP synthesis in the presence of SOD and catalase (Figure 5, lane 10), suggesting that spontaneously produced oxidants, which were scavenged by SOD and catalase, did not participate in the up-regulation of HSP synthesis by M-CSF.

The levels of the HSPs were quantified by densitometry (Figure 6A), and their changes were compared with both time-dependent changes in [35S]methionine incorporation into cell proteins and PMA-stimulated O$_2^-$ release (Figure 6B). The increase of the capacity for O$_2^-$ release coincided with the inductions of the stress-inducible HSPs. On the other hand, HSC70 protein was more rapidly induced than the stress-inducible HSPs, and its time-dependent accumulation preferentially correlated with the M-CSF-induced increase in protein synthesis (Figure 6B).

Macrophages treated with M-CSF for 8 h were exposed to 1 mM H$_2$O$_2$ for 1 h, and cell viability was estimated by measuring the percentage LDH release. M-CSF-untreated cells released
21.1 ± 1.3% of the total LDH activity (mean ± S.D., n = 6), while the percentage release of M-CSF–treated cells decreased to 8.0 ± 0.7% (mean ± S.D., n = 6). Thus, the activated macrophages significantly acquired resistance to H_2O_2–induced damage (P < 0.001 by Student’s t-test). The acquisition of the resistance with M-CSF was completely blocked by a protein synthesis inhibitor (CHX); macrophages cultured for 8 h with 100 ng/ml M-CSF and 10 μg/ml CHX released 24.6 ± 4.0% (n = 6) of the total LDH activity upon exposure to 1 mM H_2O_2 for 1 h, and M-CSF did not significantly suppress the release (20.0 ± 3.5%, n = 6).

**Detection of HSP70, HSC70, or GAPDH mRNA**

Northern-blot analysis was performed to detect the accumulations of HSP70, HSC70 and GAPDH mRNAs during activation of macrophages by M-CSF (Figure 7A), and the levels of these mRNAs were standardized with the amount of the 18 S ribosomal RNA shown in the bottom of (A). These results are representative of those obtained in four separate experiments.

**Activation of HSF by M-CSF**

The stress response mainly occurs via transcriptional induction of heat shock genes mediated by activation of HSF. HSF binds to the HSE, which consists of contiguous arrays of the alternately oriented pentanucleotide unit 5'-nGAAn-3' found in the promoter of heat shock genes [32].
provide a unique opportunity to study the induction of HSPs, since activation requires inductions of many functional proteins and, at the same time, macromolecules must properly function even under enhanced oxidative stress due to oxygen radicals released by themselves. However, the induction of HSPs in tissue macrophages has not been well documented, since it is difficult to keep macrophages quiescent; trace amounts of contaminated LPS are enough to activate macrophages and adherence to plastic plates can stimulate O$_2^\cdot$ release that may induce HSPs, as shown in blood monocytes [14].

When mouse peritoneal macrophages are isolated and cultured in LPS-free conditions, the amount of O$_2^\cdot$ stimulated by PMA is reported to be less than 100 nmol/mg of protein per h [6]. Resident peritoneal macrophages, prepared and cultured overnight in our LPS-free system, released 56 ± 8 nmol of O$_2^\cdot$/mg of protein per h (mean ± S.D., n = 10) with PMA. Treatment of cells with M-CSF enhanced this capacity about 3-fold. Treatment for at least 8 h was necessary to induce the maximum capacity for O$_2^\cdot$ production. Using this system, we examined in detail the relationship between induction of the major HSPs and activity of the respiratory burst. Western-blot analyses showed that resident macrophages, freshly isolated and cultured overnight under LPS-free conditions, contained small amounts of HSP90 and HSP70 as well as HSC70. Resident macrophages expressed both HSP70 and HSC70 mRNAs. These expressions might be due to the environmental stress during isolation and cultivation. On the other hand, it was reported that human peripheral blood myelomonocytic cells, but not lymphocytes, constitutively expressed a heat-inducible hsp70 gene, and that activation of monocytes by LPS, bacteria, phorbol ester and tumour necrosis factor augmented the expression of HSP70 mRNA [14]. In addition, an inducer of monocyte differentiation, 1,25-dehydroxyvitamin D$_3$, is known to up-regulate the expression of HSP70 [16]. These findings suggest a possible role for the hsp70 gene in leucocyte differentiation. However, Fincato et al. showed that granulocyte-macrophage CSF and M-CSF did not increase HSP70 mRNA levels in monocytes [14].

When resident macrophages were treated with M-CSF, it apparently induced the stress-inducible HSP90, HSP70 and HSP60, and a cognate HSC70. After cultivation overnight, macrophages spontaneously released 6.6 ± 0.8 (mean ± S.D., n = 6) nmol of O$_2^\cdot$/mg of protein per h without any stimulant. M-CSF did not change this spontaneous release and it also up-regulated the synthesis of HSPs even in the presence of SOD and catalase, suggesting that oxidative stress due to oxidants spontaneously released by macrophages may not participate in the induction of HSPs by M-CSF. The timing of induction of the stress-inducible HSPs coincided with that of increase in the capacity for O$_2^\cdot$ release, and the extents of their inductions were paralleled to the capacity for O$_2^\cdot$ production. Northern-blot analysis with the cDNA probe for human HSP70 mRNA showed that the HSP70 mRNA level also began to increase at 6 h. The macrophages activated by M-CSF acquired resistance to H$_2$O$_2$-induced cell damage, estimated by measuring the percentage LDH release, supporting the idea that the stress response may play an autoprotective function in macrophages.

M-CSF also stimulated the synthesis of constitutively expressed cognate HSC70, but in this case the level of HSC70 protein started to increase within 1 h, when PMA-stimulated O$_2^\cdot$ release was not yet enhanced. Northern-blot analysis showed that the accumulation of HSC70 mRNA started within 1 h after addition of M-CSF, suggesting that HSC70 may have a functional role during the pre-activation period. After addition of M-CSF, the rate of total protein synthesis measured by incorporation of l-[35S]methionine into cell proteins started to increase within 2 h, and autoradiograms of gel electrophoreses demonstrated that inductions of several specific proteins were already detected within 4 h. It has been suggested that the constitutive HSC70 and stress-inducible HSPs exhibit functional similarities and interact with newly synthesized protein. However, nascent peptides have been shown to preferentially interact with pre-existing HSC70 [37]. The mechanism of induction of HSC70 remains unsolved; however, our results suggest that HSC70 may be induced to interact with newly synthesized proteins during the pre-activation period.

The stress response in mammalian cells is suggested to occur mainly via transcriptional induction of heat shock genes mediated by activation of a pre-existing pool of HSF that binds to HSE [32]. Multiple, distinct HSF genes have been cloned; two from human and mice, three from chickens [38–40]. The differential activation of distinct HSF family members has been suggested to be attributed to the complexity of transcriptional regulation of heat shock genes [41]. It has been suggested that HSF1 is the mediator of stress-induced heat shock genes [41], whereas HSF2 DNA-binding activity is induced during haemin treatment of human K562 cells [42]. Gel mobility shift assays showed that treatment with M-CSF rapidly induced the HSE-binding activity within 15 min, and the activity persisted during the experimental period. This protein–DNA complex was supershifted with anti-serum raised against HSF1, suggesting that M-CSF may activate HSF1. HSEs in both human hsp70 and hsc70 promoters contained three perfect inverted nGAAn repeats [29,43]. Therefore, the rapid activation of HSF may reflect the transcriptional induction of the hsc70 gene.

The results described here show that M-CSF induced both the constitutive and stress-inducible HSPs, at least in part, at the transcriptional level during activation of macrophages in vitro, and suggest that these HSPs as well as HSC70 may play an essential role in the priming of macrophages for enhanced capacity for O$_2^\cdot$ production by functioning as molecular chaperones and by protecting the macrophage against the auto-oxidative damage associated with the respiratory burst.

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