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DIFFERENTIAL INDUCTION OF STRESS PROTEINS AND FUNCTIONAL EFFECTS OF HEAT SHOCK IN HUMAN PHAGOCYTES[1]

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Abstract—Induction of specific heat shock (HS) proteins (HSP) has been described as a response of human monocytes to phagocytosis, and HSP may play protective roles in infection and immunity. Here we compared the stress response in monocytes and polymorphonuclear neutrophils during exposure to the classical inducers of HSP, i.e., HS and cadmium. We also investigated the stress response in these two phagocytic cells after particulate (phagocytosis) and nonparticulate [f-Met-Leu-Phe (FMLP)] activation of the respiratory burst enzyme NADPH oxidase. HS and cadmium induced stress protein synthesis in both cell types. In contrast, phagocytosis induced HSP in monocytes only, while FMLP did so in neutrophils only. This differential regulation of stress proteins might relate to physiological and functional differences between monocytes and neutrophils. With respect to functional effects of HS, we examined, in human monocytes and in neutrophils, the effect of HS on NADPH oxidase-mediated O_2^- generation as well as on phagocytosis, bacterial killing, and superoxide dismutase (SOD) activity. In monocytes, as in neutrophils, NADPH oxidase activity was inhibited by HS, while thermotolerance prevented this inhibition. Phagocytosis and bacterial killing were unaltered by HS. SOD activity transiently increased in monocytes but decreased in neutrophils upon exposure to HS. These observations indicate differential induction of HSP in human phagocytes and differential regulation of phagocytes' functions by HS.

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INTRODUCTION

Exposure of cells to various types of stresses, including elevated temperatures, oxidative injury, heavy metals, and proinflammatory cytokines, results in the transcriptional activation of a specific set of genes, the heat shock (HS) genes and the synthesis of the stress/HS proteins (HSP). HSP are usually classified into families according to their apparent molecular weight, the hsp70 family comprising both constitutive (hsc70) and inducible (hsp70) proteins as well as the 78-kDa glucose-regulated protein, grp78. Oxidation-specific proteins such as heme oxygenase (HO) or superoxide dismutase (SOD) are also part of the stress protein families.

We have proposed that HSP are up-regulated in inflammation and that they modulate the inflammatory process (1, 2). Inflammatory cells such as monocytes–macrophages (mϕ) and neutrophils are characterized by their high phagocytic rate associated with the activation of the respiratory burst enzyme NADPH oxidase and massive generation of superoxide anions (O_2^-) and other reactive oxygen species (ROS) (see reference 3 for review). Activation of NADPH oxidase can occur via both particulate (phagocytic) and nonparticulate stimuli. Among the latter, we investigated the effects of both formyl-methionyl-leucyl-phenylalamine (FMLP) and of phorbol myristate acetate (PMA) (4).

ROS are essential in antibacterial and antiparasitic defenses and are directly involved in bacterial killing (5). Neutrophils are involved in host defenses against acute infections, while monocytes are the hallmark of chronic inflammation. On the other hand, ROS, as well as cytokines and lipid mediators, have also been reported to participate in the regulation of HSP synthesis in phagocytes. We have previously shown that phagocytosis of red blood cells induces in human mϕ the synthesis of the classical HSP (65, 70, 90 and 110 kDa) and of HO (6), whereas bacterial phagocytosis (*Staphylococcus aureus*) only induces hsp70 (7). In this study, we compared the effects of phagocytosis and of FMLP on stress protein synthesis by monocytes and neutrophils, using HS and cadmium, respectively, as positive controls for HSP and HO induction.

HS exerts a number of functional effects on cells, including transient modifications in transcription and translation, cytoskeletal alterations, and inactivation of specific enzyme activities (8, 9). Preexposure of cells to a previous, mild HS, usually renders them thermotolerant toward the alterations induced by a subsequent exposure to thermal or other injuries (10). There is increasing evidence for a role for one or several members of the hsp70 family in antigen processing and presentation (11, 12), whereas hsp65 represents immunodominant antigens in most pathogens (13).

HSP might also contribute to the regulation of the production of inflam-

matory toxic substances such as ROS (14). We have previously reported that in vitro exposure to HS reversibly inhibits NADPH oxidase activation and O_2^- production in neutrophils, (14). In contrast, in murine mϕ, HS has been suggested to increase the respiratory burst (15). We therefore compared the effects of HS on phagocyte cell functions, in particular the activation of NADPH oxidase and the resulting O_2^- production and bacterial killing, in neutrophils and in monocytes. To further determine the role of HSP in the regulation of the respiratory burst activity, we treated monocytes with the 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$]. Indeed, it has been shown that the calcium-regulating, immunomodulatory hormone 1,25-$(OH)_2$-D_3 induces differention of monocytes and increases both oxidative metabolism and HSP synthesis in these cells, along with thermotolerance (16–19).

We found that HS and cadmium, but not phagocytosis, induced a similar stress response in both monocytes and neutrophils. HS inhibited NADPH oxidase in both monocytes and neutrophils, while the generation of O_2^- was maintained in thermotolerant cells. In contrast to the O_2^- generating capacity, phagocytosis and bacterial killing were unaffected by HS. SOD activity was transiently increased in monocytes after HS and phagocytosis of *S. aureus* but decreased in neutrophils exposed to elevated temperature. These results may be of relevance to the differential biologic role of these two major phagocytes.

MATERIALS AND METHODS

Reagents. Cadmium sulfate was from Fluka (Buchs, Switzerland). PMA, lysostaphin, and FMLP were from Sigma (St. Louis, Missouri). 1,25-$(OH)_2D_3$, kindly provided by Drs. Fisher and Kaiser (Hoffmann-La Roche, Basel, Switzerland), was used to induce monocyte differentiation. Electrophoresis purity reagents were from Bio-Rad Laboratories (Richmond, California). Acridine orange and crystal violet were from BioMérieux (Marcy l'Etoile, France). $FeSO_4$ and all other reagents were from Merck (Darmstadt, Germany).

Cells and Media. Neutrophils and monocytes were isolated from blood of normal human volunteers by gradient centrifugation and monocytes purified by adherence as previously described (4, 6). Cells were maintained in RPMI 1640 (Gibco, Paisley, Scotland) supplemented, for the monocytes only, with 10% fetal calf serum (Gibco). For metabolic labeling experiments, cells were cultured in methionine-free RPMI 1640 (Gibco). Where indicated, monocytes were preincubated with 1,25-$(OH)_2D_3$ (10 ng/ml, 24 h). Sheep erythrocytes (SRBC) (BioMérieux) were opsonised with a rabbit antiserum (kindly provided by Chantal Briottet, Pathology Department, CMU, Geneva) (20). All cell cultures were maintained at 37°C in an humidified atmosphere containing 95% air and 5% CO_2.

Exposure to HS, Cadmium, or Activating Agents. We first established that the temperatures inducing HSP synthesis without altering other cell functions were respectively 44°C (30 min) in monocytes and 43°C (30 min) in neutrophils (not shown). Preexposure to HS (for thermotolerance induction) was thus performed at these temperatures, with a recovery period at 37°C for 2–2.5 h, as previously described (6). Exposure for 20 min to a temperature 1°C above the former, i.e., 45°C for monocytes and 44°C for neutrophils, was used in the experiments aimed at inducing reversible

inhibition in specific cell functions. In all experiments aiming at determining the effects of HS on phagocytosis, bacterial killing, and determination of SOD activity, cells were exposed to one HS only, i.e., 20 min to 45°C (monocytes) or to 44°C (neutrophils). Incubation with cadmium sulfate (20–150 μM) was for 3 h. Incubation with FMLP (1 μM) and PMA (1–10 ng/ml) was for 3 h and 30 min, respectively.

Bacteria. *S. aureus*, strain Wood USA 46 (kindly provided by Dr. P.D. Lew, Division of Infectious Diseases, Geneva), were grown overnight in a Mueller-Hinton broth (Difco Laboratories, Detroit Michigan) at 37°C under aerobic conditions and washed three times with PBS (Gibco), then centrifuged. For protein analysis experiments, bacteria were suspended in PBS 7% NaCl and inactivated at 85°C for 1 h. The bacteria were washed four times and kept at −20°C. For bacterial killing experiments, live *S. aureus* were opsonized with 10% pooled human serum for 30 min at 37°C, with gentle shaking, then washed once before being added to the cells.

Phagocytosis and Bacterial Killing Assay. SRBC were opsonized as described (6). The cells were allowed to phagocytose opsonized SRBC (oSRBC) at a ratio of oSRBC to phagocyte ranging from 40:1 to 600:1 for 1–3 h. Erythrophagocytosis was estimated by counting phagocytic cells using a light microscope (Olympus, Schwerzenbach, Switzerland).

Other cells were incubated with inactivated *S. aureus* at the ratio of 8:1 to 200:1 for 1.5–3 h, with or without $FeSO_4$ (0.5 mM). Opsonized *S. aureus* was added to monocyte suspensions at the ratio of 20:1 for 30 min at 37°C with gentle shaking. Free bacteria were then lysed with 10 units/ml lysostaphin at 37°C. The cells were stained with 0.0005% acridine orange and counterstained with 0.005% crystal violet (21).

Analysis of Protein Synthesis and Expression. After exposure to the various types of stresses, cells were washed twice with PBS and labeled for 90 min at 37°C with 6 μCi/ml L-[^{35}S]methionine (specific activity > 1000 Ci/mmol) (Amersham Laboratories, Buckinghamshire, England). After HS, neutrophils were allowed to recover for 20 min and monocytes for 2 h at 37°C before labeling. After labeling, the cells were washed twice with PBS and lysed in SDS-sample buffer. Proteins were resolved by one-dimensional polyacrylamide gel electrophoresis (SDS-PAGE) (22). Aliquots corresponding to equal cell numbers were loaded on each lane. Labeled proteins were revealed by autoradiography (Kodak Scientific Imaging Film, X-OMAT AR). For immunoblotting analysis, proteins were electrotransferred to nitrocellulose membranes. The membranes were saturated with casein-containing buffer for 2 h and hybridized with a monoclonal anti-human constitutive (N27, a gift from W.J. Welch, San Francisco, California) and inducible hsp70 (StressGen, Victoria, Canada). Specific binding of this antibody was detected as described (7).

Superoxide Production and SOD Activity. Generation of O_2^- was determined by the SOD-inhibitable reduction of ferricytochrone *c* after stimulation for 5 min (neutrophils) and 30 min (monocytes) with 100 ng/ml PMA in serum-free medium. Reduction of ferricytochrome *c* in each sample was read at 550 nm in a double-beam spectrophotometer (UVIKON 930, Kontron, Zürich, Switzerland) as previously described (23).

For determination of SOD activity, monocytes were exposed to HS (44°C, 20 min, 2 h recovery) or cocultured with *S. aureus* for 3 h. The cells were gently rubbed off the bottom of culture dishes and washed three times with PBS. The pellets were then resuspended in a phosphate buffer containing 50 nM $NaPO_4$ and 0.1 mM EDTA (pH 7.8) at a concentration of 10^7 cells/ml, sonicated for 2 × 30 sec at power level 3 in a Branson Sonifier, and centrifuged. Protein content in the supernatant and total protein content for western blot analysis were determined according to Bradford (24). BSA was used as standard. SOD activity was measured in crude extracts using xanthine–xanthine oxidase, an O_2^--generating system as described by Crapo et al. (25).

For analysis of SOD expression, western blotting was performed as described above for HSP characterization, but using a polyclonal anti-manganous SOD antibody (The Binding Site Limited, Birmingham, England).

RESULTS

Induction of Stress Proteins in Human Monocytes and Neutrophils: Effects of HS, Cadmium, and Phagocytosis

HS and Cadmium. The induction of stress proteins was compared in human monocytes and neutrophils using SDS-PAGE. As positive controls for HSP induction, monocytes and neutrophils were exposed to HS or cadmium. Both in monocytes and in neutrophils, HS and cadmium induced the synthesis of all classical HSP families (proteins of 60–65, 70, 83–90, and 110 kDa). In addition, cadmium also induced HO (Figure 1A and B).

Phagocytosis. We next compared the ability of these two types of phagocytic cells to synthetize HSP in response to phagocytosis of SRBC, opsonized or not (Figure 2A and B) and *S. aureus* (Figure 3A and B). As previously described, phagocytosis of both nonopsonized and opsonized SRBC led to the induction of HSP and of HO by monocytes (Figure 2A, lanes 2 and 3). In contrast to monocytes, no HSP synthesis was observed in neutrophils (Figure 2B, lanes 3–8). Although phagocytosis of oSRBC was quantitatively less important in neutrophils than in monocytes, the absence of induction of stress proteins in the former cells could not be explained by a lack of phagocytosis (Figure 2C).

In monocytes, phagocytosis of *S. aureus* induced a 78-kDa protein likely related to the glucose regulated protein grp78 (indeed, the calcium ionophore A23187 and EGTA induced an identical 78-kDa doublet in human monocytes; our unpublished data) (Figure 3A, lane 1). In contrast, phagocytosis had no effect in neutrophils (Figure 3B, lanes 2–4). In the monocytes, addition of exogenous iron modulated the stress response, decreasing the induction of grp78 while increasing hsp70 and HO (Figure 3A, lanes 1 and 2), but had no effects in neutrophils (no shown).

Effects of NADPH Oxidase Activation by Nonparticulate Agonists on HSP Synthesis

NADPH oxidase is activated not only during phagocytosis, but also by nonparticulate agonists such as FMLP. We thus compared the effects of FMLP to those of HS on the stress response in monocytes and neutrophils. Incubation of monocytes with FMLP had no effect on hsp70 accumulation, which was only observed with HS (Figure 4A). In contrast, FMLP-mediated stimulation of neutrophils led to an increase in hsp70 expression, which was similar or even more

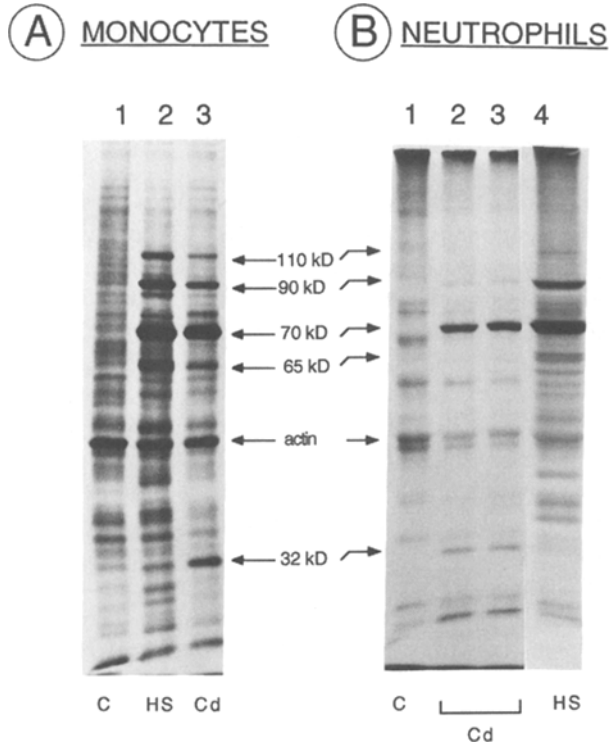

Fig. 1. Effects of heat shock and cadmium on stress protein synthesis in monocytes and neutrophils. The cells were exposed to HS or incubated with different concentrations of cadmium sulfate (Cd) as described in Materials and Methods, then labeled and processed for SDS-PAGE. Exposure of monocytes (A) to Cd (20 μM, lane 3) induced the expression of the classical HSP (110, 90, 70, 65 kDa) in a way similar to HS (lane 2) but also induced HO, which was not the case with HS. HS and Cd (100 and 150 μM, lanes 2 and 3) had similar effects in neutrophils (B).

important than with HS (Figure 4B, lanes 1–3 and 4–6). It is noteworthy that the constitutive expression of hsp70 was higher in monocytes than in neutrophils.

Table 1 summarizes the differential stress/heat shock protein induction in monocytes and neutrophils.

Effect of HS on Phagocytic Functions in Human Monocytes and Neutrophils

Effects of HS on Superoxide Production. We previously reported that HS inhibits O_2^- generation in neutrophils (26). We now compared the effects of HS and recovery after HS on the generation of O_2^- in monocytes and in neutrophils

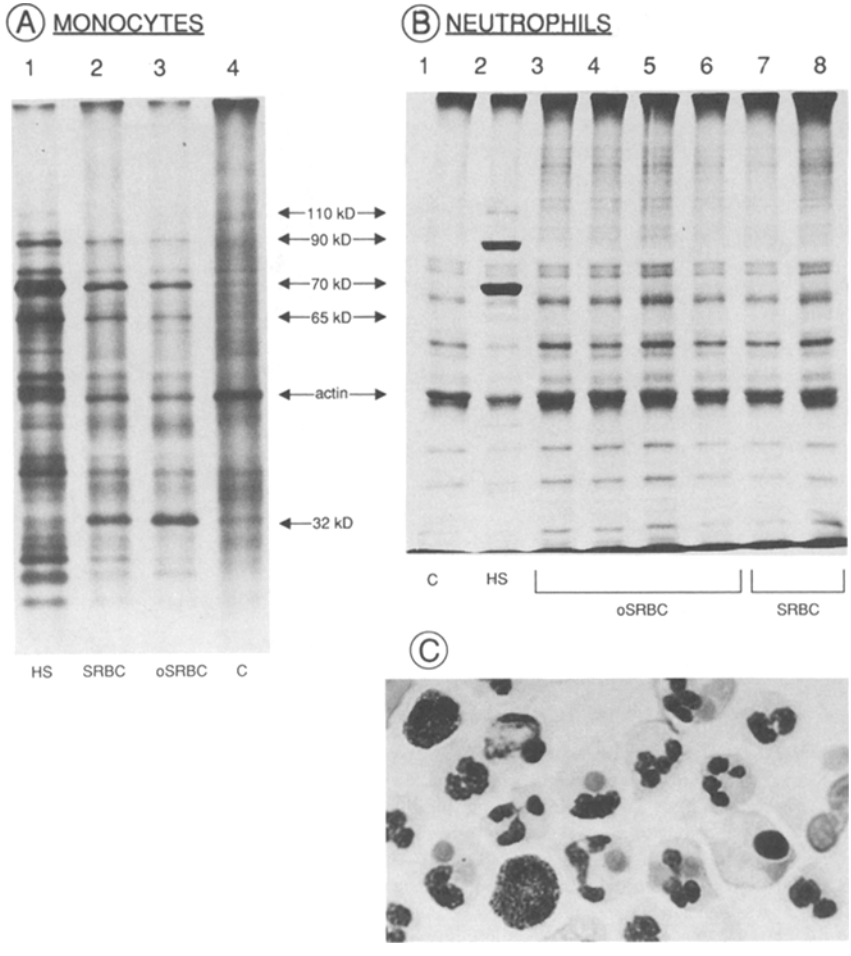

Fig. 2. Effects of heat shock or erythrophagocytosis on stress protein synthesis in monocytes and neutrophils. The cells were either exposed to HS or incubated for 1 h with SRBC or oSRBC, then labeled and processed for SDS-PAGE. Phagocytosis by monocytes (A) of SRBC or oSRBC (lanes 2 and 3, ratio 1:600) induced the expression of the classical HSP and of HO, the latter protein being expressed at high levels with oSRBC. In neutrophils (B) there was no induction of HSP by erythrophagocytosis of either oSRBC (lanes 3–6) or SRBC (lanes 7–8).

(Figure 5). Monocytes were preincubated with (B) or without (A) 1,25-$(OH)_2D_3$, then exposed to HS, and O_2^- generation determined. HS inhibited NADPH oxidase activity by approximately 50%. Preexposure of cells to first a mild HS followed by recovery had a partial thermoprotective effect on NADPH oxidase activity in neutrophils (Figure 5C), which was increased in monocytes (which

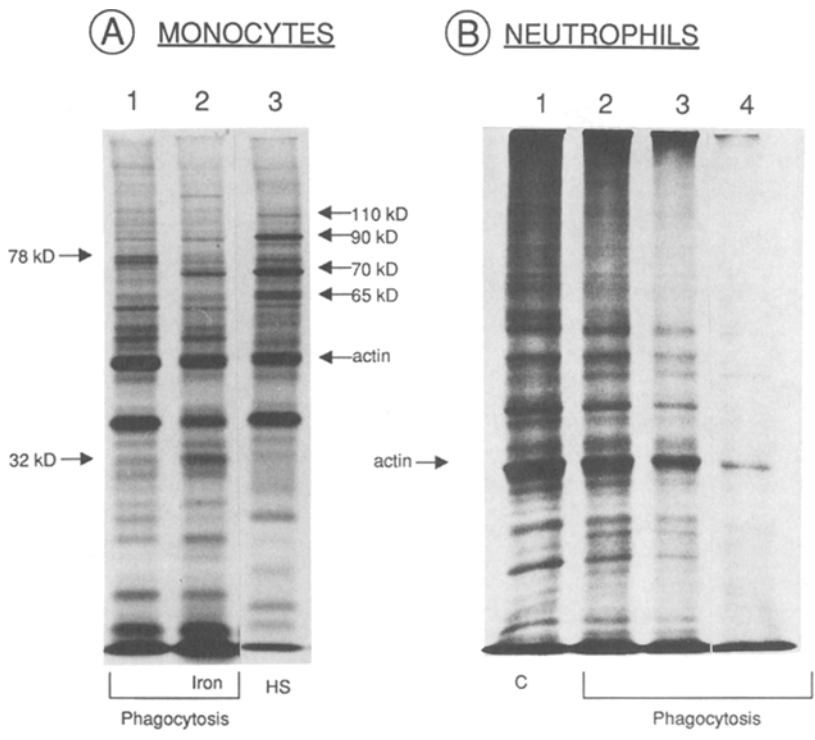

Fig. 3. Effects of phagocytosis of *S. aureus* on stress protein synthesis in monocytes and neutrophils. Monocytes (A) were incubated with *S. aureus* (lanes 1 and 2) at a 1:200 ratio in absence (lane 1) or presence (lane 2) of 0.5 mM $FeSO_4$ (iron) for 3 h. Phagocytosis of *S. aureus* induced a 78-kDa protein (probably related to the glucose regulated protein grp78). Addition of iron altered this response, leading to an increase in hsp7 and HO, while decreasing grp78. In neutrophils (B), phagocytosis of *S. aureus* did not induce a stress response. The observed decrease in normal protein synthesis was dependent upon the ratio cell-*S. aureus* and the time of incubation (lane 2, 1:8, 1.5 h; lane 3, 1:32, 1.5 h; lane 4, 1:32, 3 h).

express more HSP than neutrophils) (Figure 5A). As expected (17–19), these effects were enhanced when monocytes were preincubated with 1,25-$(OH)_2D_3$ (Figure 5B).

Effect of HS on Phagocytosis. We then evaluated the effect of HS and recovery after HS on the cells' capacity to phagocyte oSRBC (Table 2). HS did not significantly alter phagocytosis, although there was a trend to such a decrease. As stated above, phagocytosis rates were lower in neutrophils than in monocytes, but the effects of HS were similar.

Effects of HS on Bacterial Killing. Some O_2^- production relates to the capacity of phagocytes to kill bacteria and because HS inhibited NADPH oxidase

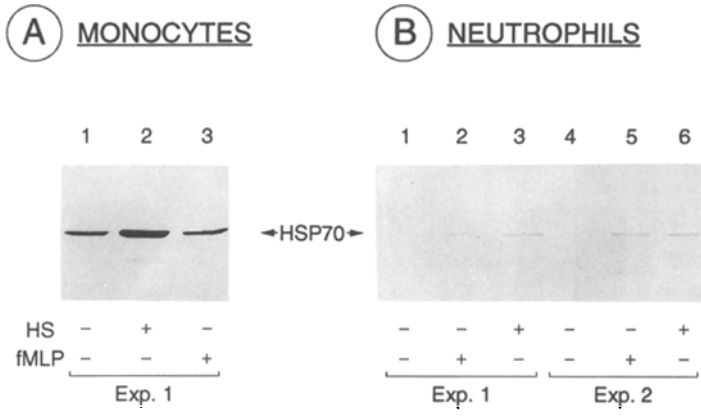

Fig. 4. Effects of FMLP on hsp70 expression in monocytes and neutrophils. In monocytes (A), FMLP (1 μM) did not increase hsp70 expression above basal (compared to HS, lanes 2 and 3, respectively), while in neutrophils (B), basal expression of hsp70 was undetectable and was induced to similar levels by HS and by FMLP.

Table 1. Differential Stress Protein Induction in Monocytes and Neutrophils[a]

	Monocytes	Neutrophils
Heat shock	+++	+++
Cadmium	+++ (HSP and HO)	+++ (HSP and HO)
Erythrophagocytosis	+++ (HSP and HO)	−
Bacterial phagocytosis	+	−
(*S. aureus*)	GRP78? w/o iron	
modulated by iron	HSP and HO with iron	
Other phagocytic stimulus (OZ)	++	−
Respiratory burst stimulation with nonparticulate agonists		
PMA	±	−
PAF	+ (GRP78?)	−
FMLP	−	+ (hsp70)

[a]w/o = without; − = no induction of HSP synthesis; +, ++, +++ = semiquantitative appraisal of HSP synthesis on SDS-PAGE.

activity, we also examined the effects of HS on bacterial killing (Table 3). Bacterial killing of *S. aureus* was determined after staining with acridine orange by analyzing the percentage of phagocytic cells that contained dead bacteria (orange) as compared to live bacteria (green). According to this method, HS did not modify bacterial killing. The time course of bacterial killing (compare 30, 60, and 90 min) showed, for all conditions, a similar increase over time in the number of phagocytic cells that contained killed bacteria (Table 3).

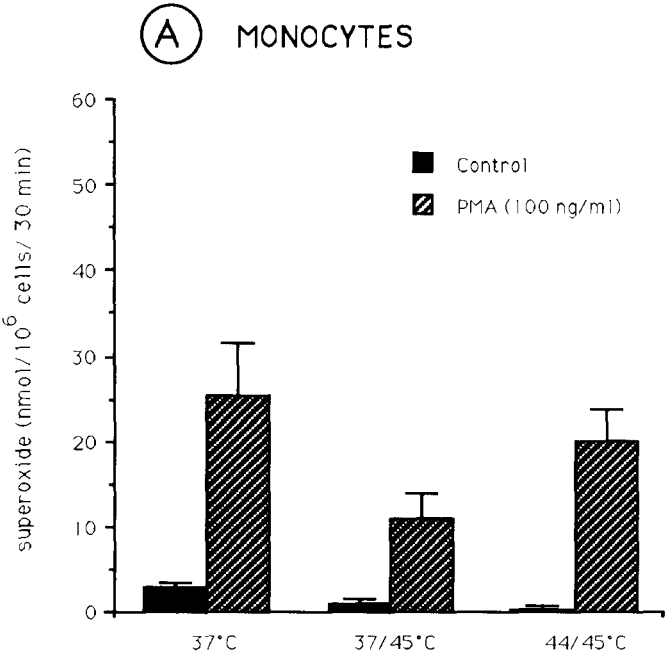

Fig. 5. Superoxide production by monocytes and neutrophils after HS. Monocytes were maintained for 43 h without (A) or with 1,25-$(OH)_2D_3$ (B). The cells were exposed to 45°C or to sequential 44°C, 150 min recovery and 45°C before stimulation with PMA (100 ng/ml, 30 min) for O_2^- production. A similar procedure was used for neutrophils (C) except that temperatures were 43°C and 44°C and stimulation with PMA was for 5 min. Values represents means ± *SEM* for three distinct experiments.

Effects of HS on SOD Activity and Expression in Human Monocytes and Neutrophils

As SOD is a HSP in bacteria and SOD activity has been described to be modulated by HS in mammals (27), we investigated the effect of HS on SOD activity and expression in monocytes and neutrophils. The pooled results of three and two distinct experiments are shown for monocytes and neutrophils, respectively, in Table 4. HS transiently increased SOD activity in monocytes (Table 4). The effects of HS were similar in this respect to those of phagocytosis of *S. aureus* (Table 4). HS did not affect the expression of SOD as assessed by western blotting (not shown). In contrast, HS decreased SOD activity in neutrophils.

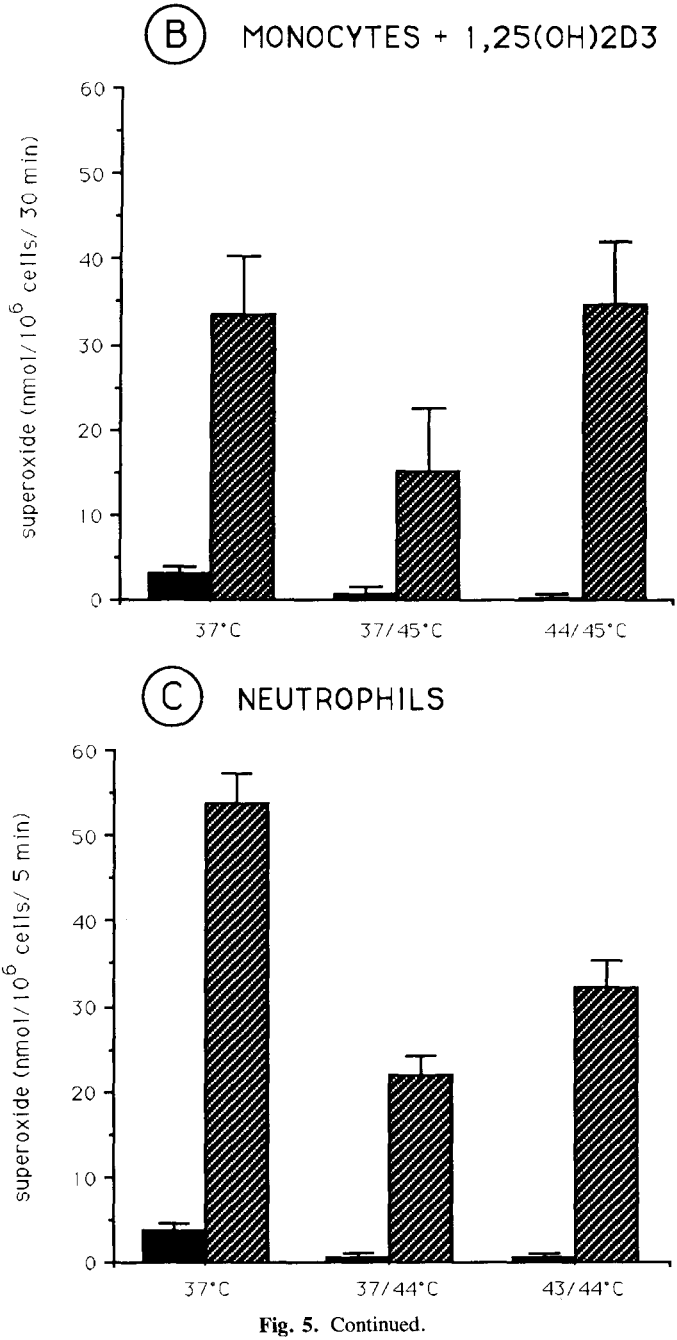

Fig. 5. Continued.

Table 2. Effects of HS on Erythrophagocytosis by Neutrophils and Monocytes[a]

Conditions	Phagocytic neutrophils (%)	Phagocytic monocytes (%)
37°C	31.9 ± 6.5	94.4 ± 2.5
HS	21.6 ± 3.1	74.6 ± 15.2
HS + 2h	23.7 ± 7.7	92.6 ± 2.3

[a]Neutrophils and monocytes were exposed to 43°C and 44°C for 20 min. respectively, or maintained at 37°C (control). Phagocytosis was started immediately after HS or after 2 h recovery at 37°C (HS + 2 h). Values represent means ± *SEM* of percentage of phagocytic cells of four experiments (neutrophils) or three experiments (monocytes); 200 cells per condition were counted.

Table 3. Effects of HS on Bacterial Killing by Monocytes[a]

Time (min)	Control (37°C)	HS	HS + 2 h
30	23.1 ± 6.1	38.8 ± 18.0	30.0 ± 10.2
90	36.7 ± 13.7	35.0 ± 13.5	33.4 ± 12.9
150	47.1 ± 14.7	43.0 ± 10.6	46.2 ± 12.0

[a]Monocytes were cultured for 48 h and maintained at 37°C or exposed to HS (44°C). *S. aureus* were added to monocytes either after HS or after 2 h recovery at 37°C (HS + 2 h). Bacterial killing was measured at the indicated time points. Values represent means ± *SEM* of percentage of killed bacteria of three experiments; 25 cells per condition were counted.

Table 4. Effects of HS and Bacterial Phagocytosis on SOD Activity in Monocytes[a]

	SOD activity (units/mg protein)			
	Control (37°C)	HS	HS + 2 h	Phagocytosis (*S. aureus*)
Monocytes	149.9 ± 21.4	208.0 ± 17.4	178.7 ± 5.5	200.6 ± 18.7
Neutrophils	63.1	54.4	46.8	—

[a]Monocytes were exposed to 45°C and neutrophils to 44°C for 20 min (HS: no recovery; HS + 2 h: 2 h recovery at 37°C). Phagocytosis of *S. aureus* was for 3 h. SOD activity was measured in triplicate in all experiments. Values represent means (± *SEM* for monocytes) of three and two experiments for monocytes and neutrophils, respectively.

DISCUSSION

We report here that the stress response subsequent to the activation of the respiratory burst enzyme NADPH oxidase is differentially regulated in monocytes and in neutrophils, while the classical HSP inducers, HS and cadmium, similarly induced HSP (as for HS) and HO (as for cadmium) in both cell types.

Phagocytic stimuli such as erythrocytes or bacteria (*S. aureus*) only induced a stress response in monocytes. This cannot be explained either by the lower phagocytosis of neutrophils as compared to monocytes or by the lack of NADPH oxidase activation. Indeed, monocytes, even when phagocytosing as few erythrocytes as neutrophils (which may be obtained by decreasing the ratio of bacteria to monocytes) still synthesize HSP. Furthermore, phagocytosis activates the generation of O_2^- in neutrophils as efficiently as in monocytes, if not more so. We thus propose that the observed differences between monocytes and neutrophils rather relate to specific differences in cell functions and physiology. Converging evidence from our laboratory and others suggest that hydroxyl radicals are a key oxygen metabolite for the induction of HSP synthesis. One important differing feature is that neutrophils, in contrast to monocytes, are unable to produce hydroxyl radicals because they contain lactoferrin, which chelates iron (28). In the monocytes, both endogenous—such as released from phagocytosed oSRBC during phagocytosis—and exogenous iron may contribute to the generation of hydroxyl radicals via the Fenton reaction. A role for hydroxyl radicals in HSP induction is further supported by: (1) the lack of induction of HSP synthesis in mϕ phagocytosing opsonized erythrocytes ghosts (ghosts lack hemoglobin from which the iron used in the Fenton reaction is derived) (6, 29) and (2) by the increase in hsp70 observed in monocytes when phagocytosis of *S. aureus* is performed in the presence of iron (7).

Another hypothesis that might explain the differences between the two types of phagocytic cells is that neutrophils are short-lived phagocytes and have been described as professional "kamikaze." The phagocytosed material may be degraded so rapidly that it is unable to initiate the processes leading to protein synthesis. Alternatively, the short-lived neutrophils may not require additional protective mechanisms during phagocytosis and, in contrast to monocytes, would thus not be programmed to synthesize HSP in processes relating to inflammation.

On the other hand, it is remarkable that in response to FMLP, a nonparticular activator of NADPH oxidase that induces a higher respiratory burst in neutrophils than in monocytes (30), hsp70 expression is induced in neutrophils but not in monocytes. FLMP is one of the strongest activators of neutrophils and, besides to the production of O_2^-, also induces a phospholipase C-mediated inositol phosphate metabolism, as well as generating diacylglycerol, which in turn leads to activation of protein kinase C (PKC) (30). We recently demon-

strated that other activators of PKC, such as PMA, induce a selective up-regulation of hsp90 and hsc70, which relates to PKC activation rather than to O_2^- production (31, Jacquier Sarlin et al., in revision). The conjunction, in the neutrophils, of a selective activation of a number of distinct second messagers and activation pathways together with O_2^- production might explain these differences.

Our results on the effect of HS on different phagocytic functions indicate that HS selectively modulates enzyme activities (i.e., NADPH oxidase and SOD), without altering other phagocytic functions, including phagocytosis and bacterial killing. Interestingly, HS inhibited NADPH oxidase activity but increased SOD activity, both of which could lead to a decrease ability of the host cell to kill intracellular bacteria. We found, however, no correlation between the cells' ability to produce O_2^- and bacterial killing. This indicates that there might be additional mechanisms, besides O_2^- production, which allow phagocytic cells to kill bacteria. H_2O_2, for example, which enters the cells through the plasma membrane more easily than O_2^-, might contribute to such defense mechanisms, and this even more so because the increase in SOD may stimulate the conversion of O_2^- to H_2O_2. On the other hand, the inhibition of NADPH oxidase by HS was incomplete and the remaining amount of O_2^- produced may be sufficient for bacterial killing, a possibility supported by the clinical observations that as little as 10% of NADPH oxidase activity is sufficient to prevent the symptoms associated with chronic granulomatous disease (32).

Our findings also underline the cell specificity of the functional effects of HS, which may explain previous apparently contradictory findings (14, 15). Furthermore, the differential effects of HS on enzyme activities in various cell types may relate to the constitutive expression of HSP, since neutrophils, which express less constitutive hsp70, are more sensitive to the inhibitory effects of HS on enzymatic activities.

The inhibitory effect of HS on NADPH oxidase activity and thus O_2^- generation and the protection of the enzyme by a previous mild HS was similar in monocytes and in neutrophils. In contrast to the transient inhibition of O_2^- generation after HS, which is independent of the synthesis of HSP in neutrophils, and probably relates to HS-induced cytoskeletal alterations, the protection of NADPH oxidase activity by a previous mild HS is dependent on the increased expression of HSP (26). Actinomycin D, which, under determined experimental conditions, selectively inhibits the transcriptional activation of HS genes, abolished the thermoprotective effects obtained by the first mild HS, while 1,25-$(OH)_2D_3$, which in monocytes increases HSP synthesis, increased thermotolerance as well. In 1,25-$(OH)_2D_3$-treated monocytes, there was indeed 100% cellular protection by preexposure to HS.

Our observations on the differential induction of stress proteins and effects of HS on phagocytic functions provide further insights into the behavior of

phagocytic cells upon exposure to different forms of stress, including HS, which might simulate natural situations such as burns, inflammation, or fever. HSP synthesis is subtly regulated, probably to limit damage of the phagocytic cells by the stress and to protect phagocytic functions. Several of the phagocytes' functions appear to be relatively insensible to HS: other mechanisms than HSP synthesis may be involved in the maintenance and protection of these important phagocytic functions.

REFERENCES

1. POLLA, B. S. 1988. A role for heat shock proteins in inflammation? *Immunol. Today* **9**:134–137.
2. POLLA, B. S., and S. KANTENGWA. 1991. Heat shock proteins and inflammation. *Curr. Topics Microbiol. Immunol.* **167**:93–105.
3. BELLAVITE, P. 1988. The superoxide-forming enzymatic system of phagocytes. *Free Radic. Biol. Med.* **4**:225–261.
4. MARIDONNEAU-PARINI, I., S. M. TRINGALE, and A. I. TAUBER. 1986. Identification of distinct activation pathways of the human neutrophil NADPH oxidase. *J. Immunol.* **137**:2925–2930.
5. BEAMAN, L., and B. L. BEAMAN. 1984. The role of oxygen and its derivates in microbial pathogenesis and host defense. *Annu. Rev. Microbiol.* **38**:27–48.
6. CLERGET, M., and B. S. POLLA. 1990. Erythrophagocytosis induces heat shock proteins synthesis in human monocytes-macrophages. *Proc. Natl. Acad. Sci. U.S.A.* **87**:1081–1085.
7. KANTENGWA, S., and B. S. POLLA. 1993. Phagocytosis of *Staphylococcus aureus* induces a selective stress response in human monocytes-macrophages: Modulation by macrophage differentiation and by iron. *Infect. Immun.* **61**:1281–1287.
8. BENSAUDE, O., M. PINTO, M. F. DUBOIS, V. T. NGUYEN, and M. MORANGE. 1990. Protein denaturation during heat-shock and related stress. *In* Stress Proteins. Induction and function. M. J. Schlesinger, G. Santoro, and E. Garaci, editors. Springer Verlag, Berlin. 89–99.
9. LINQUIST, S. 1986. The heat-shock response. *Annu. Rev. Biochem.* **55**:1151–1191.
10. WELCH, W. J., and L. A. MIZZEN. 1988. Characterization of the thermotolerant cell. Effects on the intracellular distribution of heat-shock protein 70, intermediate filaments, and small nuclear riboprotein complexes. *J. Cell Biol.* **106**:1117–1130.
11. POLLA, B. S., and E. MARIETHOZ. 1992. More evidence for a case of chaperones in antigen processing. *Immunol. Today* **13**:421–422.
12. MARIETHOZ, E., F. TACCHINI-COTTIER, M. R. JACQUIER-SARLIN, F. SINCLAIR, and B. S. POLLA. 1994. Exposure of monocytes to heat shock does not increase class II expression but modulates antigen-dependent T cell responses. *Int. Immunol.* **6**:925–930.
13. YOUNG, D. B. 1992. Heat-shock proteins: Immunity and autoimmunity. *Curr. Opin. Immunol.* **4**:396–400.
14. MARIDONNEAU-PARINI, I., J. CLERC, and B. S. POLLA. 1988. Heat shock inhibits NADPH oxidase in human neutrophils. *Biochem. Biophys. Res. Commun.* **154**:179–186.
15. REDDY, M. V., and P. R. J. GANGADHARAM. 1992. Heat shock treatment of macrophages causes increased released of superoxide anion. *Infect. Immun.* **60**:2386–2390.
16. COHEN, M. S., D. E. MESLER, R. G. SNIPES, and T. K. GRAY. 1986. 1,25-dihydroxyvitamin D_3 activates secretion of hydrogen proxide by human monocytes. *J. Immunol.* **136**:1049–1053.
17. POLLA, B. S., A. M. HEALY, M. BYRNE, and S. M. KRANE. 1987. Hormone 1α,25-dihydroxy-

vitamin D_3 modulates heat shock response in monocytes. *Am. J. Physiol. (Cell Physiol. 21)* **252:**C640–C649.

18. Polla, B. S., A. M. Healy, E. P. Amento, and S. M. Krane. 1986. 1,25-dihydroxyvitamin D_3 maintains adherence of human monocytes and protects them from thermal injury. *J. Clin. Invest.* **77:**1332–1339.
19. Polla, B. S., J. V. Bonventre, and S. M. Krane. 1988. 1,25-dihydroxyvitamin D_3 increases the toxicity of hydrogen peroxide in the human monocytic line U937: The role of calcium and heat shock. *J. Cell. Biol.* **107:**373–380.
20. Montesano, R., A. Mossaz, P. Vassali, and L. Orci. 1983. Specialization of the macrophage plasma membrane at sites of interaction with opsonized erythrocytes. *J. Cell Biol.* **96:**1227–1233.
21. Pruzanski, W., S. Saito, and D. W. Nitzan. 1983. The influence of lysostaphin on phagocytosis, intracellular bactericidal activity, and chemotaxis of human polymorphonuclear cells. *J. Lab. Clin. Med.* **102:**298–305.
22. Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head bacteriophage T_4. *Nature* **227:**680–685.
23. Johnston, R. B., Jr. 1984. Measurement of O_2^- secreted by monocytes and macrophages. *Methods Enzymol.* **105:**365–369.
24. Bradford, M. M. 1976. A rapid and sensitive method for quantitation of micrograms quantities of proteins utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:**248–254.
25. Crapo, J. D., J. M. McCord, and I. Fridovich. 1978. Preparation and assay of superoxide dismutases. *Methods Enzymol.* **53:**382–393.
26. Maridonneau-Parini, I., S. E. Malawista, H. Stubbe, F. Russo-Marie, and B. S. Polla. 1993. Heat shock in human neutrophils: Superoxide generation is inhibited by a mechanism distinct from heat-denaturation of NADPH oxidase and is protected by heat shock proteins in thermotolerant cells. *J. Cell Physiol.* **156:**204–211.
27. Loven, D. P., D. B. Leeper, and L. W. Oberley. 1985. Superoxide dismutase levels in chinese hamster ovary cells and ovarian carcinoma cells after hyperthermia or exposure to cycloheximide. *Cancer Res.* **45:**3029–3033.
28. Wang-Iverson, L., K. B. Pryzwanski, J. K. Spitznagel, and M. H. Cooney. Bactericidal capacity of phorbol myristate acetate-treated human polymorphonuclear leukocytes. *Infect. Immun.* **22:**945–955.
29. Donati, Y. R. A., S. Kantengwa, and B. S. Polla. 1991. Phagocytosis and heat shock response in human monocytes-macrophages. *Pathobiology* **59:**156–161.
30. Polla, B. S., G. Werlen, M. Clerget, D. Pittet, M. F. Rossier, and A. M. Capponi. 1989. 1,25-dihydroxyvitamin D_3 induces responsiveness to the chemotactic peptide f-Met-Leu-Phe in the human monocytic line U937: Dissociation between calcium and oxidative metabolic responses. *J. Leukocyte Biol.* **45;**381–388.
31. Malter, J. S., and Y. Hong. 1991. A redox switch and phosphorylation are involved in the post-translational up-regulation of the adenosine-uridine binding factor by phorbol ester and ionophore. *J. Biol. Chem.* **266:**13167–3171.
32. Malech, H. L., and J. F. Gallin. 1987. Neutrophils in human disease. *N. Engl. J. Med.* **317:**687–694.