Brief Definitive Report

HEAT-SHOCK PROTEIN SYNTHESIS BY HUMAN POLYMORPHONUCLEAR CELLS

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The polymorphonuclear leukocytes (PMNs) arise from stem cells in the bone marrow and are released to the periphery in huge numbers where they survive for ~12 h and function as the major cellular component of the acute inflammatory response. The nuclear chromatin of mature PMNs is coarsely clumped, suggesting that much of the genome is inactive (1). Lacking evidence of a nucleolus and having very little endoplasmic reticulum or ribosomes, these cells are generally thought to be preprogrammed end products capable of functioning without significant biosynthetic activity. This conclusion is consistent with observations that phagocytosis is independent of RNA and protein synthesis (2). However, when placed in short term culture, PMNs have been shown to engage in protein synthesis that is modified by glucocorticoids or Con A (3, 4). On the basis of such data, both protein synthesis and gene activation have been postulated to play a role in the response of PMNs to inflammatory stimuli. While evidence has accumulated that new RNA synthesis is required for the production of leukocyte pyrogen (5), elastase (6), and plasminogen activator (3), the full extent to which the transcriptional activity of PMNs can be modulated is unclear.

Heat-shock proteins (HSPs) constitute an extensively studied set of polypeptides that is induced when cells are exposed to elevated temperatures (7) or to a variety of specific chemical modulators (8). Since HSP induction occurs in all organisms so far examined and is known to depend on enhanced *hsp* gene transcription (7, 9), the ability (or inability) of PMNs to synthesize these proteins in response to heat shock might indicate the extent to which gene activation is possible in this system. Transcription-dependent HSP induction in PMNs and its implications are the subject of this report.

Materials and Methods

Cell Preparation. Human peripheral whole blood from adult volunteers (20 ml) was collected into a syringe containing 500 U of preservative-free sodium heparin (Sigma Chemical Co., St. Louis, MO) and 4 ml of 6% dextran T-500 (Pharmacia Fine Chemicals, Uppsala, Sweden) in normal saline. The mixture was sedimented at 1 *g* for 30 min at room temperature, after which the leukocyte-rich plasma was collected into an equal volume of PBS and centrifuged at 200 *g* for 5 min. The pellet was suspended in PBS, recentrifuged, and then resuspended in serum-free DME. Counting with 0.04% trypan

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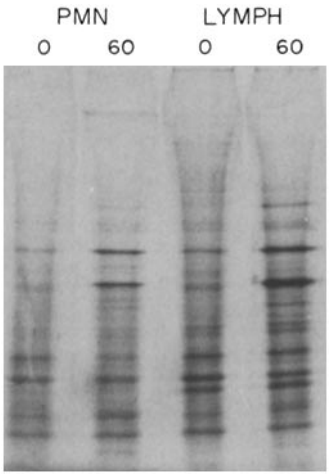

FIGURE 1. SDS-PAGE gradient gel pattern of total [^{35}S]methionine-labeled polypeptides produced by human PMN and lymphocytes (*LYMPH*). Cells were plated in DME and labeled either immediately at 37°C or exposed to 42°C for 60 min and then labeled at 37°C. Labeling of hsp85 and hsp70 is greatly increased after 42°C exposure.

blue showed >97% viable leukocytes. Purified PMNs were prepared by collecting the leukocyte-rich plasma into an equal volume of isotonic HBSS containing 10 mM Hepes (Research Organic, Inc., Cleveland, OH) (HBSS-Hepes). The suspension (3 ml) was layered onto 10 ml of lymphocyte separation medium (Bionetics Laboratory Products, Kensington, MD) and centrifuged at 200 *g* for 40 min at room temperature. Lymphocytes and monocytes were found at the interface while the bottom fraction contained the PMNs and erythrocytes. The pellet and interface cells were collected separately, resuspended in HBSS-HEPES and pelleted by centrifugation for 10 min. The erythrocytes were lysed with cold water for 20 s followed by an equal volume of 2× isotonic HBSS. The PMN preparations (containing 2–3% mononuclear cells, nearly all of which were small lymphocytes) were found to be 97% viable after being alternately washed with isotonic HBSS and centrifuged twice more.

Cell Culture. PMNs and lymphocytes were plated at a density of 1.5×10^7/25-mm plastic polystyrene tissue culture dish containing 2 ml of serum-free DME and maintained at 37°C in an atmosphere of 10% CO_2. L929 cells (Mycoplasma-free; MA Bioproducts, Inc., Walkersville, MD) were maintained in DME supplemented with 10% FCS and plated at 2×10^6 under similar conditions. Preliminary experiments showed that these cell densities were optimal for subsequent labeling.

Heat Shock and Labeling. Cells were placed on an aluminum plate in an incubator maintained at the desired temperature. We have shown that equilibration occurs within 10 min, so the duration of heat shock is not usually corrected for equilibration time. At the termination of heat exposure, cultures were washed twice with PBS and labeled at 37°C for 1.5 h in methionine-free DME containing 20 μCi/ml [^{35}S]methionine (Amersham Corp., Arlington Heights, IL). After labeling, cultures were washed with PBS and cells were lysed wth 1% SDS/1% 2-ME and heated at 100°C for 1 min. Actinomycin D (20 μg/ml) was added to some cultures before and immediately after heat shock.

Polyacrylamide Gel Electrophoresis. 5–15% SDS-polyacrylamide gradient slab gels are prepared as previously described (10). Equal counts per minute were loaded per gel lane. Gels were dried immediately after the completion of the run and autoradiographed using Kodak XAR-5 film. Autoradiograms were scanned densitometrically to determine the relative proportion of [^{35}S]methionine incorporated into the HSP bands.

Results

Exposure to heat easily induces human PMNs to synthesize proteins with molecular weights of 70,000 and 85,000, as shown by the SDS-PAGE (Fig. 1). These correspond to hsp70 and hsp85 induced under similar conditions in L929 cells (11) and human lymphocytes (12), two other mammalian systems in which

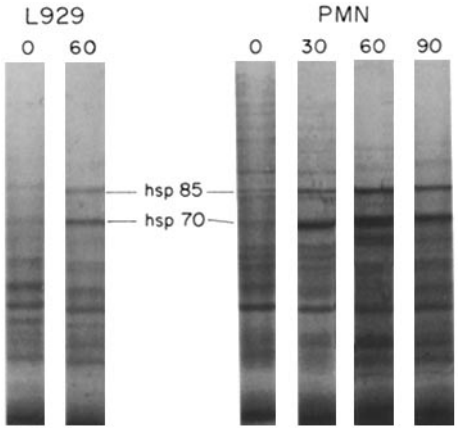

FIGURE 2. SDS-PAGE pattern of total [^{35}S]methionine-labeled polypeptides synthesized by L929 cells (*L929*) and human neutrophils (*PMN*). Cells were plated in DME and labeled either immediately at 37°C or exposed to 42°C for 30, 60, or 90 min and then labeled at 37°C.

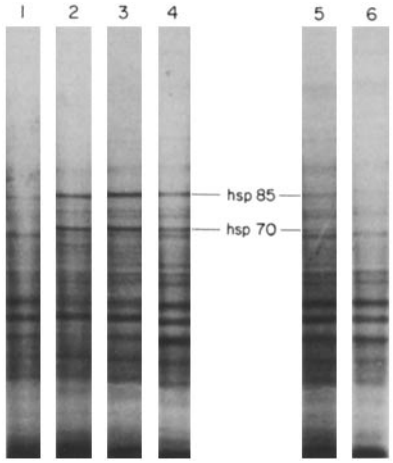

FIGURE 3. SDS-PAGE pattern of total [^{35}S]methionine-labeled polypeptides synthesized by human PMNs. Cells were plated in DME and labeled either immediately at 37°C (*1*) or exposed to 42°C for 1 h (*2*), 3 h (*3*), or 5 h (*4*), and then were labeled at 37°C. (*5* and *6*) Patterns of proteins synthesized when cells were heat shocked at 42°C for 1 h, returned to 37°C, and labeled after 3 h or 5 h, respectively.

HSP induction is well documented. Considering the relative rates of [^{35}S]methionine incorporation and HSP synthesis, contaminating lymphocytes could have contributed no more than 5% of the HSP synthesis exhibited by control PMN preparations at 37°C and no more than 10% of HSP synthesis following heat shock.

Preferential HSP synthesis begins as early as 30 min after exposure to 42°C (Fig. 2) and continues for prolonged periods of time. Response to various temperatures, as well as the time course of induction were assessed. Exposure to 40°C for 1–5 h resulted in no HSP synthesis, Exposure to 41°C for 1 h resulted in moderate HSP synthesis, which was maintained after 2 h of continuous exposure, but was reduced to base-line levels after 3 h at the elevated temperature. Maximal HSP synthesis was induced when cells were exposed to 42–43°C. Fig. 3 shows that HSP synthesis induced by exposure to 42°C was maintained after at least 5 h of continuous exposure, but was greatly reduced after 10 h (data not shown). On the other hand, when the cells were exposed to 42°C for 1 h and returned to 37°C, HSP synthesis persisted for 3 h, but was greatly reduced by 5 h after heat shock (Fig. 3, lanes *5* and *6*).

The dependence of HSP induction on RNA synthesis is shown in Fig. 4 where

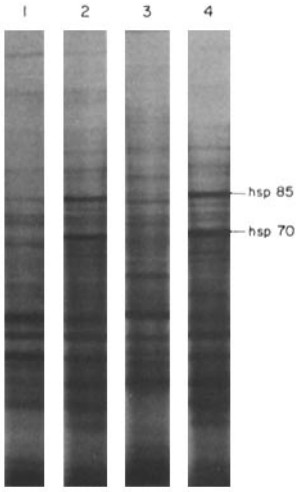

FIGURE 4. SDS-PAGE pattern of total [^{35}S]methionine-labeled polypeptides synthesized by human neutrophils. Cells were incubated in DME and labeled either immediately at 37°C (*1*) or after heat shock at 42°C for 1 h (*2*). Actinomycin D (20 μg) was added to the culture medium before heat shock (*3*), and after heat shock for 1 h at 42°C (*4*). In both instances, labeling was performed at 37°C.

it can be seen that actinomycin D added to cultures before heat shock completely blocked induction of HSP synthesis. No alteration in the extent of induction was apparent if actinomycin D was added immediately after heat exposure.

Discussion

We show that PMNs synthesize the usual major mammalian heat shock proteins, i.e., hsp70 and hsp85 (13), after heat exposure. Although translational mechanisms can lead to preferential HSP synthesis during exposure at high temperatures (see reference 14), when synthesis is assessed at 37°C, as in the present study, such translational control is obviated and induction of the major HSPs can be taken to reflect increased mRNA levels (15). HSP induction was also blocked by actinomycin D, a conventional RNA synthesis inhibitor, thus providing additional evidence for transcriptional regulation. Based on observations that plasminogen activator and leukocyte pyrogen production are dependent on RNA synthesis, an argument has been developed that gene activation can occur in PMNs. Since transcriptional regulation of these genes has not been rigorously demonstrated, the present findings make that argument much more compelling.

Since HSPs are not known to be involved in PMN function, induction of HSP synthesis can be interpreted in two ways: either HSPs are, in fact, essential for PMN function or some, perhaps many, gene products that do not play a direct functional role can be activated in PMNs. Since the function of HSPs is still unknown, it is possible that they play an essential role in PMNs. This question will be resolved as HSP functions are identified. Analysis of PMN gene regulation has naturally focused on gene products that these cells might be expected to produce, e.g., plasminogen activator and leukocyte pyrogen. Although reasonable, this approach has led to the assumption that other genes cannot be activated. Now that *hsp* genes are known to be inducible, the possibility that other genes may also be susceptible to activation under appropriate inducing conditions deserves serious consideration.

Summary

Mature human neutrophils from peripheral blood are known to be capable of limited protein biosynthetic activity. We now show that these cells are inducible for heat-shock protein synthesis when placed in short-term culture. Synthesis of hsp70 and hsp85 as well as the response to various temperatures and the time course of induction were typical for mammalian cell systems. This heat-shock response was blocked by actinomycin D added before heat exposure. This demonstration that *hsp* genes can be activated by heat exposure of terminally differentiated neutrophils supports the hypothesis that gene activation can serve a physiological role in these cells and opens up the possibility that synthesis of other gene products is similarly inducible.

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