Brief Definitive Report

RNA AND PROTEIN SYNTHESIS IN HUMAN PERIPHERAL BLOOD POLYMORPHONUCLEAR LEUKOCYTES*

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The polymorphonuclear leukocytes (PMNs) circulating in peripheral blood are derived from granulocytic precursors in the hemopoietic marrow. The cells that are released into the circulation, the mature PMNs, are generally believed to be terminally differentiated end products, devoid of significant biosynthetic capacity. In particular, although a few reports have described some RNA (1, 2) and protein synthesis (3) by these cells, the generally accepted view emphasized in the literature is that PMNs probably synthesize very little, if any, RNA and only little protein (4, 5). This view is consistent with the relative scarcity of ribosomes and endoplasmic reticulum (6), and with the ability of PMNs to ingest particles when RNA and/or protein synthesis are blocked (1). In addition to their endocytic activities, however, PMNs respond to a variety of stimuli, for example, by emerging from the circulation and migrating to sites of inflammation; and there is no evidence that these responses are fully programmed by pre-existing molecular equipment in a manner resembling the phagocytic functions.

While studying the secretion of plasminogen activator by PMNs, we observed that enzyme production was markedly reduced by inhibitors of RNA or protein synthesis (7). These results suggested that RNA and protein synthesis might be necessary for the response of PMNs to inflammatory agents and other effectors, and led us to re-examine macromolecule synthesis in these cells. We have found that the majority of human peripheral blood PMNs synthesize RNA and proteins during short-term cultures; in addition, synthesis can be modulated by low concentrations of either concanavalin A (Con A) or glucocorticoids.

Materials and Methods

Materials. Reagents were obtained as described (7). Puromycin and ribonuclease A (70 U/mg) were from the Sigma Chemical Co., St. Louis, Mo.; Uridine (5,6 ^{3}H) 40 Ci/mmol, leucine (4,5 ^{3}H) 40 Ci/mmol, and algal protein hydrolysate (^{3}H or ^{14}C) 0.1 mCi/ml were all from New England Nuclear, Boston, Mass.

Methods

PREPARATION OF CELLS. Human peripheral blood PMNs were obtained from heparinized (2 U/ml) blood of healthy volunteers, and purified as described (7). Differential counting of cells prepared in this way showed them to be 96–97% neutrophils, 2–3% eosinophils, 0.5% basophils, and 1% or less mononuclear cells in all preparations used for these experiments. More than 95% of the cells were viable, as judged by their capacity to exclude trypan blue.

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INCORPORATION OF RADIOLABELED PRECURSORS INTO ACID-INSOLUBLE MACROMOLECULES. Purified PMNs (1.3×10^6) were plated in 60-mm tissue culture dishes (Falcon 3002, BioQuest, BBL, & Falcon Products, Becton, Dickinson & Co., Cockeysville, Md.) in 3 ml of Eagle's medium supplemented with 2.5% fetal bovine serum (FBS), in presence or absence of inhibitors. After 2 h, some of the cultures received Con A (10^{-7} M) and all the cultures were made 10 μCi/ml in either [^{3}H]uridine or [^{3}H]leucine. The cells were resuspended by gentle pipetting 4 h later, washed twice by centrifugation, (300 *g*, 5 min), and resuspended in phosphate-buffered saline (PBS). An equal volume of cold 10% trichloroacetic acid (TCA) was added, and the resulting precipitate was collected on Whatman GFC filters (Whatman, Inc., Clifton, N. J.). The filters were washed with 5% TCA, dried, and counted in Liquifluor-toluene (New England Nuclear, Boston, Mass.) in a Packard liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.)

AUTORADIOGRAPHY OF CELLS. Purified PMNs (3×10^5) were plated in flat-bottomed glass tubes containing 12-mm diameter glass coverslips, in 1 ml Eagle's medium (for [^{3}H]uridine incorporation) or 1 ml Eagle's medium prepared with 1/10 of the regular amino acids (for ^{3}H-amino acids incorporation), supplemented with 2.5% of FBS. The cells were incubated for 4 h in presence of 5 μCi/ml [^{3}H]uridine or [^{3}H]reconstituted protein hydrolysate, with or without the various inhibitors tested. At the end of the incubation period the tubes were centrifuged at 300 *g* for 5 min, the medium was removed, and the coverslips were fixed in Carnoy's fluid for 30 min at 0°C. In one experiment the [^{3}H]uridine-labeled cultures were incubated after fixation for 2 h at 37°C in PBS containing 10 μg/ml bovine pancreatic ribonuclease. After fixation and washing, the coverslips were processed for autoradiography (L4 Ilford emulsion), developed after 3 wk, and stained with Giemsa.

POLYACRYLAMIDE GEL ELECTROPHORESIS. Purified PMNs (1×10^7) were plated in 1 ml Eagle's medium prepared with 1/10 of the regular concentration of amino acids and supplemented with 0.2% of plasminogen-depleted FBS (7), in presence of 10 μCi/ml of ^{14}C-reconstituted protein hydrolysate. Dexamethasone (10^{-7} M) or Con A (10^{-7} M) were added to parallel cultures. After 10 h of incubation the cells were resuspended by gentle pipetting, and sedimented at 300 *g* for 5 min. More than 85–90% of the cells remained viable at the end of the culture, as estimated by trypan blue exclusion. The supernates were collected, and the pellets resuspended in 1 ml PBS; both supernates and resuspended pellets were made 10% in cold TCA, and allowed to precipitate for 1 h at 0°C. The precipitates were collected by centrifugation (800 *g*, 10 min), redissolved in 1 ml 0.1 M Tris base, and reprecipitated by addition of 1 ml 20% TCA. The final pellets were resuspended in 50 μl of 15% sucrose, 0.1 M Tris base, 5% sodium dodecyl sulfate (SDS) and 0.01 M dithiothreitol (DTT), and boiled for 2 min. Aliquots of the samples were electrophoresed in 8–15% SDS-polyacrylamide slab gels, and the fixed gels were processed for fluorography (8).

Results

Purified human PMNs, cultured in the presence of either [^{3}H]uridine or [^{3}H]-leucine, incorporated radiolabel into acid-precipitable macromolecules (Table I), and the extent of incorporation was proportional both to cell number and to time of incubation (data not shown). Incorporation of [^{3}H]uridine was increased two to fivefold in the presence of Con A; it was inhibited >90% by actinomycin D, an inhibitor of RNA synthesis, and by the glucocorticoid, dexamethasone (Table I). Low concentrations of dexamethasone were effective: [^{3}H]uridine incorporation was reduced by 70% at 10^{-7} M, and by 15% at 10^{-9} M dexamethasone. Incorporation of [^{3}H]leucine into acid-precipitable proteins was only slightly affected by Con A or dexamethasone, but was decreased at least 60% in presence of the protein synthesis inhibitors cycloheximide or puromycin (Table I).

These results indicated clearly that, on the average, cultured PMNs were synthesizing RNA and protein, but they gave no indication of the biosynthetically active fraction of the cell population. To establish the size of the active fraction, cultures that had been exposed to [^{3}H]uridine or ^{3}H-reconstituted protein hydrolysate were fixed, washed free of nonincorporated radiolabeled precursor, and analyzed by

TABLE I

RNA and Protein Synthesis by Cultured Human PMNs

	[^{3}H]uridine (cpm × 10^{-3})		[^{3}H]leucine (cpm × 10^{-3})	
	—	Con A	—	Con A
—	10.0	41.0	5.7	7.9
Actinomycin D, 5 μg/ml	0.7	1.8		
Cycloheximide, 10 μg/ml			2.2	2.3
Puromycin, 10 μg/ml			1.8	1.8
Dexamethasone, 10^{-6} M	1.2	2.3	5.1	6.2

Purified PMNs were incubated in presence or absence of Con A (10^{-7} M) and/or various compounds, in medium containing 10 μCi/ml [^{3}H]uridine or [^{3}H]leucine, and processed for determination of incorporation of radiolabel into acid-insoluble macromolecules, as described in Methods. The results are expressed as acid-precipitable cpm [^{3}H]uridine or [^{3}H]leucine incorporated by 1.3 × 10^6 PMNs during 4 h of incubation. Values presented are from a single experiment (triplicate cultures); comparable results were obtained in five separate experiments. For comparison, the incorporation of radiolabel into mononuclear cells (the cells present at the Ficoll-Hypaque plasma interface) was analyzed in parallel. In the experiment presented here 1.3 × 10^6 untreated mononuclear cells incorporated 6.3 [^{3}H]uridine cpm × 10^4 and 4.7 [^{3}H]leucine cpm × 10^4 into acid insoluble macromolecules during 4 h of incubation.

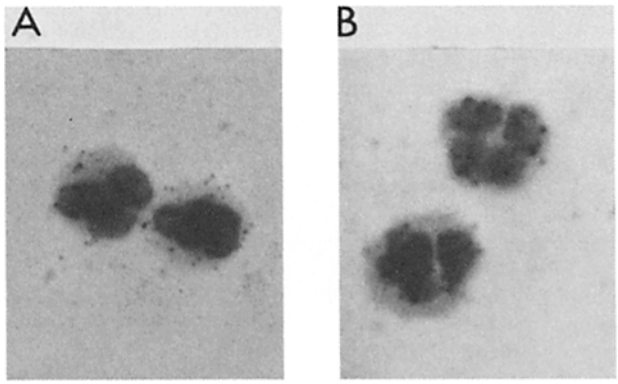

FIG. 1. Autoradiographs of PMNs that had been incubated with ^{3}H-reconstituted protein hydrolysate (A) or [^{3}H]uridine (B).

autoradiography. More than 60% of cells incubated with [^{3}H]uridine were labeled, and the majority of the grains were localized over the nucleus (Fig. 1 B); very few grains were detected in fixed cell preparations incubated with ribonuclease before autoradiography, confirming that the radiolabel was incorporated into macromolecular RNA. Actinomycin D and dexamethasone (as in Table I) decreased the average cellular grain count by 80%. Incorporation of ^{3}H-amino acids was detected in 73% of the cells, and most of the grains were found over the cytoplasm (Fig. 1 A).

Thus, the capacity for macromolecule synthesis appeared to be a property of the PMN population in general, and it was therefore of interest to characterize grossly the spectrum of proteins being formed. Fig. 2 presents autoradiograms of SDS-polyacrylamide gels after electrophoresis of aliquots of cell lysates and culture media; many different proteins were synthesized, some of which were secreted, because they could be found both in the culture medium (Fig. 2 a–c), and in cell lysates (Fig. 2 A–C). The presence of Con A (Fig. 2 b and B) or dexamethasone in the medium (Fig. 2 c and C) produced changes in the pattern of radiolabeled proteins, particularly affecting the secreted proteins suggesting that changes in secretion of some newly

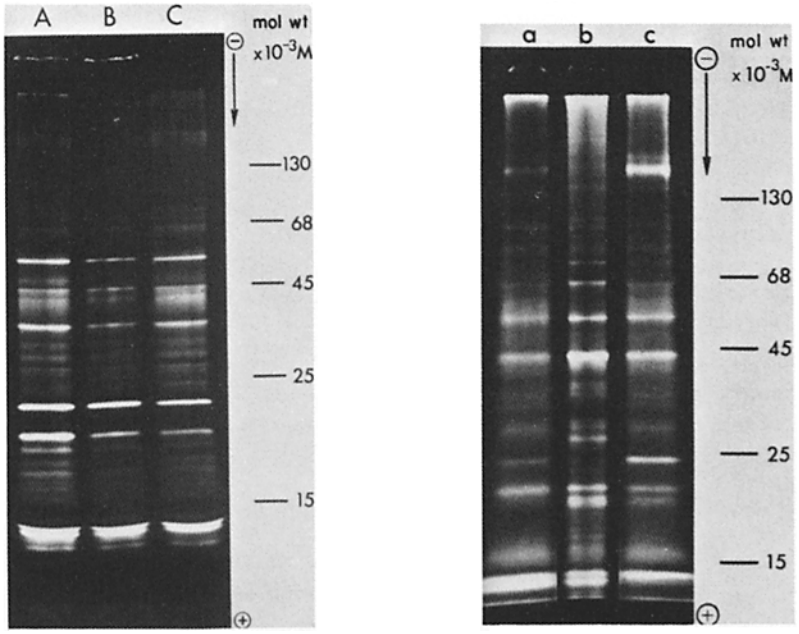

FIG. 2. Autoradiographs of SDS-polyacrylamide gels of supernates (a,b,c) and cell lysates (A,B,C) from cultures of PMNs incubated with ^{14}C-reconstituted protein hydrolysate. a,A: control cultures; b,B: cultures treated with Con A (10^{-7} M); c,C: cultures treated with dexamethasone (10^{-7} M).

synthesized proteins might form a part of the PMNs response to Con A and to glucocorticoids. (Because culture of PMNs for 10 h is accompanied by a small but detectable decrease in cell viability, it is possible that some of the radiolabeled proteins in the medium were released upon cell death rather than actively secreted. The differences in the autoradiographic patterns of cell lysates and culture media and the different responses of these patterns to Con A, and dexamethasone imply that leakage of intracellular material can account for no more than a small fraction of the radiolabeled proteins in the medium.)

Discussion

The capacity to synthesize RNA and proteins, and the significance of such newly synthesized macromolecules in the biological responses of mature PMNs appear to have received little attention. Many of the data in the literature were obtained in studies of macromolecule synthesis using unfractionated leukocytes, and the contribution of cells other than PMNs, such as lymphocytes and monocytes, cannot be accurately evaluated. The availability of separation methods allowing the recovery of highly purified populations of viable PMNs should promote a reinvestigation of RNA and protein synthesis in these cells. Although the rates of precursor incorporation in our experiments were low in comparison with many other eukaryotic cells, we found that the radiolabeling of macromolecular RNA and proteins was readily demonstrable in cultured PMNs purified from peripheral blood. In addition, autoradiographic studies showed that RNA and protein synthesis were detectable in the majority of PMNs, and not merely in some minor subpopulation.

Several lines of evidence suggest that newly synthesized macromolecules are likely to be important for PMN function: (*a*) the production of pyrogen is blocked by RNA and protein synthesis inhibitors (9) and radiolabeled pyrogen has been obtained from leukocytes exposed to radioactive precursor amino acids (10). (*b*) The production of plasminogen activator by human PMNs is blocked by inhibitors of RNA and protein synthesis (7). (*c*) More than 30 biosynthetically labeled polypeptide chains can be separated by SDS-polyacrylamide gel electrophoresis. (*d*) The rate of [^{3}H]uridine incorporation into RNA as well as the electrophoretic patterns of newly synthesized proteins can be pharmacologically modulated by Con A and glucocorticoids (see also reference 11); this suggests that the response of PMNs to such inflammatory and antiinflammatory agents is mediated, at least in part, through effects on RNA and protein synthesis. Taken together, all of these observations suggest that further studies of RNA and protein synthesis in circulating PMNs may yield new insights about the biology of these cells, particularly about mechanisms activated in response to agents that modulate their participation in inflammatory reactions.

Summary

Polymorphonuclear leukocytes purified from human peripheral blood synthesized RNA and proteins when placed in cell culture. Autoradiography of the cultured cells revealed that a majority of mature PMNs were engaged in macromolecule synthesis, and an analysis of newly synthesized proteins by SDS-polyacrylamide gel electrophoresis showed that many different polypeptide chains were synthesized by these cells. The rate of [^{3}H]uridine incorporation and the pattern of newly synthesized proteins were modulated by Con A and glucocorticoids. These results suggest that in spite of their short lifetime and a large preformed enzymatic apparatus, mature PMNs retain a substantial capacity for RNA and protein synthesis; and, further, that modulation of macromolecule synthesis forms part of the mechanism by which PMNs respond to inflammatory and anti-inflammatory stimuli.

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