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Research Article

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Expression of Interleukin-1α and β Genes by Human Blood Polymorphonuclear Leukocytes

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Abstract

Expression of IL-1α and β genes was studied in human blood PMN with close monitoring of the effects of contaminating mononuclear leukocytes (MNL). We provide evidence that PMN both transcribe and translate IL-1α and β genes after stimulation with LPS or IL-1α. A combination of mouse thymocyte comitogen proliferation assay, ELISA, and immunocytochemistry was required to establish that IL-1α and β synthesis observed in preparations of PMN could not be accounted for by the low level of contaminating MNL. Synthesis of IL-1β in PMN exceeded that of IL-1α, but little or no IL-1α was released by PMN.

Although increases in IL-1 mRNA after stimulation of PMN and MNL with LPS were similar, PMN were less efficient than MNL in translating IL-1 mRNA. In contrast, PMN and MNL IL-1α and β mRNAs were translated with equal efficiency in rabbit reticulocyte lysates, suggesting that synthesis of IL-1 in PMN is subject to some form of translational control.

We conclude that PMN stimulated with LPS efficiently transcribe but inefficiently translate IL-1 genes relative to MNL. IL-1β transcription and translation predominates over that of IL-1α, and IL-1β is the predominant IL-1 protein released by PMN. IL-1 can induce its own synthesis in PMN. (*J. Clin. Invest.* 1991. 87:1312–1321.) Key words: neutrophils • endotoxin • mononuclear leukocytes

Introduction

Interleukin 1 α and β are 17-kD proteins coded by separate genes. The proteins, which are synthesized by a variety of cell types including activated macrophages (1), keratinocytes (2), stimulated B lymphocytes (3), and fibroblasts (4), are potent mediators of inflammation and immunity (reviewed in 2, 5). Effects of IL-1 during the inflammatory process include production and release of prostaglandins and collagenase by fibroblasts and synovial cells, induction of fever, and production and release of acute-phase reactants in hepatocytes (2). The cloning and expression of the human IL-1α and β genes (1) make it possible to determine other cell types that may produce IL-1 and play a role in the inflammatory process.

Polymorphonuclear neutrophilic leukocytes are fundamental participants in acute inflammation. They have often been characterized as terminally differentiated cells with insignificant RNA or protein synthesis, consistent with their limited quantity of ribosomes and endoplasmic reticulum (6, 7). Recent reports advocate that PMN are capable of RNA and protein synthesis and that gene products of PMN may contribute to the inflammatory process. For example, an increase in fibronectin RNA and protein occurred in synovial PMN preparations obtained from patients with inflammatory joint disorders as compared with peripheral blood PMN from the same patients (8, 9). It has also been reported that preparations of blood PMN synthesize other mRNA and proteins important in inflammation including plasminogen activator (10), elastase (11), heat shock proteins (12), actin, CR1, FcR, CR3 α-chain, MHC class 1 antigen (13), tumor necrosis factor (TNF) α[1], granulocyte–colony-stimulating factor (G-CSF), and macrophage–colony-stimulating factor (M-CSF) (14).

Since Bennett and Beeson originally reported that PMN were the source of endogenous pyrogen (15), the notion that PMN synthesize endogenous pyrogens such as IL-1 has been controversial. Several reports suggest that PMN may synthesize and release the endogenous pyrogen IL-1. Tiku et al. reported that human blood PMN released bioactive IL-1 (16, 17). Goto et al. (18) demonstrated that preparations of mouse and rabbit PMN produced an IL-1-like protein when stimulated with kaolin. Lindemann et al. observed that human blood PMN stimulated with GM-CSF expressed IL-1α and β mRNA and secreted IL-1-like bioactivity into the culture media (19). Although these studies provided provocative information, the possibility that even a minor contamination with mononuclear leukocyte (MNL) cells might account for the results precluded a definite conclusion that PMN express IL-1 genes.

We report here a study of IL-1α and β gene expression in human blood PMN in which we closely monitored the effects of contaminating MNL. We provide evidence that PMN both transcribe and translate IL-1α and β genes after stimulation with LPS. Also, IL-1 stimulates its own synthesis in PMN. Transcription of IL-1α and β mRNA is similar in PMN and MNL. However, PMN translation of mRNA in whole cells is less efficient than that of MNL. In contrast, both PMN and MNL mRNAs were translated with equal efficiency in an in vitro rabbit reticulocyte lysate protein synthesis system.

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1. *Abbreviations used in this paper:* MNL, mononuclear leukocytes; PCR, polymerase chain reaction; TNF-α, tumor necrosis factor α; rhIL-1, recombinant human IL-1 (α or β); rmIL-1α, recombinant mouse IL-1α.

Methods

PMN isolation. Heparinized venous blood from adult volunteers was mixed 1:1 with 3% Dextran T500 (Pharmacia Fine Chemicals, Piscataway, NJ) at 22°C for red cell sedimentation. The leukocyte-rich plasma then was removed and centrifuged. Cells were resuspended in HBSS and centrifuged through Isolymph (Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, NY) to separate MNL and PMN. After isolymph centrifugation, preparations containing PMN (henceforth referred to a PMNp) and erythrocytes were pelleted. Contaminating erythrocytes were removed from PMNp by hypotonic lysis. Isolated PMNp were 99% granulocytes (1–3% eosinophils, 97–99% neutrophils), and 1–2% MNL as determined by a modified Wright's stain. Monocytes were uniformly present at < 1%, as measured by nonspecific esterase stain. MNL removed from the interface of the Isolymph and HBSS were routinely 30–40% monocytes and 60–70% lymphocytes as determined by a modified Wright's stain or nonspecific esterase stain. At times monocytes were further separated by adherence to plastic for 12 h in RPMI 1640 media with 10% FCS.

Culture conditions. PMNp or MNL (3×10^6 cells/ml unless indicated) were suspended in sterile polypropylene tubes or tissue culture dishes with RPMI 1640 media with L-glutamine, 10% low endotoxin FCS, and 50 μg/ml gentamicin. Cell suspensions were incubated with or without 10 μg/ml *Escherichia coli* lipopolysaccharide (0111:B4; Sigma Chemical Co., St. Louis, MO) for various times (0–18 h) at 37°C in 5% CO_2 incubator. Other stimuli employed were recombinant mouse IL-1α (Hoffmann LaRoche, Inc., Nutley, NJ), recombinant human (rH)-IL-1α and β (Immunex Corp., Seattle, WA), and recombinant human TNFα (Chiron Corp., Emeryville, CA). Viability was determined at each time point by trypan blue exclusion and was routinely > 90% for up to 18 h. Controls to which no LPS had been added contained < 50 pg/ml of LPS as determined by the limulus lysate assay.

RNA isolation. Total cellular RNA was isolated by guanidine thiocyanate: ethanol precipitation as described (20). Briefly, PMNp or MNL were centrifuged at various times and the cell pellet lysed in 4 M guanidine thiocyanate buffer (4 M guanidine thiocyanate, 50 mM Tris, pH 7.6, 10 mM EDTA, 2% Sarkosyl, and 0.1 M 2-mercaptoethanol). The cells were then disrupted using a Polytron (Brinkmann Instruments, Inc., Westbury, NY), and the RNA precipitated using 0.63 vol of 100% ethanol. Precipitated RNA was separated by centrifugation at 17,000 *g* for 30 min at −10°C. The RNA pellet was resuspended in 4 M guanidine thiocyanate buffer and again precipitated with 0.5 vol of 100% ethanol. The precipitated RNA was again centrifuged and the pellet resuspended in 100 mM NaCl, 50 mM Tris, pH 9.0, 10 mM EDTA, and 0.5% SDS. The RNA was then extracted twice in phenol, twice in chloroform:isoamyl alcohol, and then precipitated in ethanol. Routinely, 100–200 μg of total RNA was isolated from 1×10^9 PMNp and 200–400 μg of total RNA was isolated from 1×10^9 MNL.

Northern blot analysis. Between 10 and 15 μg of total RNA were electrophoresed through 1% agarose, 6.6% formaldehyde gels in 1 × 3-(4-morpholino)propane sulfonic acid (MOPS) buffer (0.02 M MOPS, 5 mM sodium acetate, and 1 mM EDTA (21), and electroblotted onto Nytran (Schleicher and Schuell, Keene, NH) as described by the manufacturer. Equal amounts of RNA were loaded onto the gels as determined by ethidium bromide staining of the gel before and after transfer. After transfer, Nytran filters were baked at 80°C in a vacuum oven for 2 h.

The plasmids containing human IL-1α cDNA (pHIL No. 7) or human IL-1β cDNA (pHIL No. 11) (kindly supplied by Peter Lomedico, Hoffmann-LaRoche, Nutley, NJ) were labeled by nick-translation with [α-^{32}P]dCTP (3,000 Ci/mmol, New England Nuclear, Boston, MA) to a sp act of 1×10^8 cpm/μg DNA. Nytran filters were hybridized as described by Schibler et al. (22). Filters were wet in 2 × SSC (1 × SSC contains 0.15 M NaCl, 0.015 M sodium citrate) and prehybridized for 2–3 h at 42°C in 50% formamide, 5 × SSC, 50 mM $NaPO_4$ pH 6.5, 50 μg/ml heparin, and 0.1% SDS. After prehybridization, filters were maintained for 24 h at 42°C in fresh prehybridization solution containing $1–3 \times 10^6$ cpm/ml of denatured nick-translated ^{32}P-labeled cDNA probe. Filters were washed twice in 2 × SSC, 0.2% SDS for 15 min at room temperature and then once in 0.2 × SSC, 0.2% SDS for 15 min at 57°C. Autoradiography of hybridized Nytran membranes was done at −70°C using XAR-5 film (Eastman Kodak Co., Rochester, NY) and Duplex Cronex Lightning Plus intensifying screens (EA, Wilmington, DE). For quantitative analysis, Nytran membranes were exposed to preflashed Kodak XAR-5 film and autoradiographs scanned using a laser densitometer (Ultroscan XL; LKB Instruments, Inc., Bromma, Sweden) (23).

First strand synthesis. First strand DNA was synthesized by dissolving 2 μg of total RNA in DEPC-treated H_2O, 0.1 μg/ml BSA (Promega Biotec, Madison, WI), 1 × HRT buffer (Bethesda Research Laboratories, Gaithersburg, MD), 0.5 mM dNTP (Pharmacia), 20 U RNasin (Promega), 0.01 ng/ml olido $(dT)_{15}$ primer (Promega), 100 U M-MLV reverse transcriptase (Bethesda Research Laboratories) with a final vol of 10 μl. The reaction was incubated for 1 h at 37°C followed by 10 min at 75°C.

Polymerase chain reaction (PCR). The 10-μl first strand cDNA mixture was combined with 4 μl 10 × PCR reaction buffer (buffer conditions determined by Mary Sorci-Thomas, Wake Forest University, Winston-Salem, NC) containing: 670 mM Tris pH 8.8, 160 mM $(NH_4)_2$ SO_4, 67 mM M_9Cl_2, 100 mM 2-mercaptoethanol, 67 μM EDTA. 5′ and 3′ primers for IL-1β were obtained from Clonetech Laboratories, Palo Alto, CA. They consisted of 5′-ATGGCAGAAGTACCTAAGCTC and 3′-TTAGGAAGACACAAATTGCATGGTGAACTCAGT. Each IL-1β primer was added to give a final concentration of 1 mM along with 50 μM dNTP and 2.5 U of *Thermus aquaticus* DNA Polymerase (Perkin-Elmer, Norwalk, CT) with a final vol of 50 μl. The PCR amplification was carried out in a Perkin-Elmer thermal cycler set for 45 cycles. The PCR temperatures used were: 94°C for denaturing; 50°C for annealing; 72°C for primer extension. The PCR products were ethanol precipitated and electrophoresed in a 1.5% agarose gel and diffusion blotted to Nytran.

Southern blot analysis. A 23-bp IL-1β oligonucleotide (a gift from S. Kunkel, University of Michigan, Ann Arbor, MI) contained within the PCR fragment was end labeled as described (24). PCR filters were prehybridized and hybridized at 50°C in 6 × SSC, 1 × Denhardts, 100 μg/ml salmon sperm DNA, 100 μg/ml heparin, 0.1% nonfat dry milk for 24 h. Hybridization conditions contained 2×10^6 cpm/ml end-labeled oligonucleotide. Filters were washed twice in 2 × SSC, 0.1% SDS at room temperature. Autoradiographs were produced and analyzed as described in Northern blot analysis.

Biological assay for IL-1. Suspensions of PMNp or MNL were incubated as described under culture conditions. At various times, the media were harvested and centrifuged to remove cell debris. The media supernatant was then filter sterilized and assayed for IL-1 activity using the C3H/HeJ mouse thymocyte comitogen proliferation assay (25).

ELISA assay for IL-1α and β. IL-1α and IL-1β immunoreactivities were measured with commercially available assay kits. The IL-1α assay (Endogen, Inc., Boston, MA) had a sensitivity of 75 pg; the IL-1β assay (Cistron Technologies, Pine Brook, NJ) had a sensitivity of 25 pg. These assays do not cross-react significantly with other known cytokines.

Immunocytochemistry. Immunocytochemistry was performed essentially as described by Chensue et al. (26). Briefly, PMN or MNL (3×10^6 cells/ml) were plated on Lab-Tek 8 chambered slides (American Scientific Products Div., American Hospital Supply Corp., McGraw Park, IL) and incubated with or without 10 μg/ml of *E. coli* 0111:B4 LPS for 1 h at 37°C in a 5% CO_2 incubator. The PMN were then fixed with 4% paraformaldehyde in PBS for 5 min. The fixed cells could be stored in PBS at 4°C for up to one week. In some experiments PMN were treated with 20 μg/ml of cycloheximide for 1 h before stimulation with LPS.

For staining, the slides were treated with 50% methanol and 3% hydrogen peroxide for 10 min then washed three times with PBS. A 1:50 dilution of normal rabbit serum was added for 15 min at 37°C, the slides were decanted and treated for 15 min at 37°C with a 1:1,000 dilution of goat anti-human IL-1α or β or the same dilution of nonim-

mune goat serum. Preparations were then rinsed three times with PBS and incubated for 10 min at 37°C with a 1:200 dilution of biotinylated rabbit anti-goat IgG (Vector Laboratories, Inc., Burlingame, CA). After washing three times with PBS, the slides were treated with a 1:1,000 dilution of peroxidase-labeled streptavidin (Sigma) at a stock concentration of 0.1 mg/ml in PBS with 1% BSA. The slides were then incubated for 15 min and washed three times with PBS. Cells were then overlaid with 100 μl of equal vol of 3-amino-9-ethylcarbazole 40 mg/ml in *N,N*-dimethylformamide, (Sigma) (40 mg/ml in *N,N*-dimethylformamide), 3% hydrogen peroxide in 0.1 M sodium acetate, and incubated at 37°C for 5 min. Preparations were then washed and counterstained with Mayer's hematoxylin solution (Sigma) and Biomedic crystal mount (Fisher Scientific Co., Allied Corp., Pittsburgh, PA).

In vitro translation of RNA in rabbit reticulocyte lysates. In vitro translation of PMNp or MNL was done using an in vitro translation kit (NEK-001; New England Nuclear). For the assay, 5 μg of total RNA from PMNp or MNL, 10 μl of rabbit reticulocyte lysate, 8 μl of in vitro translation cocktail mix, and 50 μCi of [^{35}S]methionine (800 Ci/mmol) were incubated at 37°C for 1 h. The translations were terminated either by the addition of sample buffer for SDS-polyacrylamide gel or by the addition of immunoprecipitation buffer. Those samples that were diluted with immunoprecipitation buffer were then immunoprecipitated with anti-human IL-1α or β antisera and analyzed on SDS-polyacrylamide gels.

Results

IL-α and IL-1-β mRNA synthesis by LPS-stimulated PMNp. To determine levels of IL-1α or β mRNA in PMNp, total RNA isolated from PMNp incubated with or without 10 μg/ml LPS for various times was analyzed by Northern blot hybridizations. Fig. 1 is a representative autoradiograph of three such time course experiments in which panels *A* and *B* were probed with ^{32}P-labeled IL-1α or β cDNA. There was no IL-1α mRNA detected in PMNp in the basal state (panel *A*, lane *1*) and IL-1α mRNA was not seen in PMNp after a 2–3 h incubation with LPS (panel *A*, lanes *2* and *3*). However, the 2.2-kb IL-1α mRNA was observed in PMNp after 4 h stimulation with LPS and it became most abundant after 9 h LPS stimulation (panel *A*, lanes *4* and *5*). PMNp, although 90% viable, had no detectable IL-1α mRNA after stimulation with LPS for 18 h (panel *A*, lane *6*).

IL-1β mRNA was also detected in LPS-stimulated PMNp and it was present at higher levels than IL-1α mRNA (panel *B*, lanes *2–6*). IL-1β mRNA was not detected in unstimulated cells and levels peaked between 4 and 9 h after stimulation with LPS.

Since MNL (including monocytes and lymphocytes) are also capable of transcribing the IL-1 genes when stimulated (1), we wished to eliminate the possibility that MNL contamination might be responsible for the IL-1α and β mRNA signal observed with PMNp. As previously indicated, our PMNp preparations were routinely contaminated with ~ 1% MNL. Thus, total RNA from suspensions of MNL that were equivalent to either 10%, 5%, 2.5%, or 1% of the total cell number of PMN was probed after 9 h with IL-1α or β cDNA. MNL stimulated with LPS for 9 h had no detectable IL-1α mRNA at any cell concentration used, and PMN IL-1β mRNA levels were eight times higher than the IL-1β mRNA observed at the equivalent 10% MNL contamination (data not shown). To further compare PMNp and MNL we performed PCR on equal amounts of RNA isolated from control PMNp and MNL, and PMNp and MNL stimulated by LPS. One experiment was performed after 3 h of LPS stimulation, and another at 6 h. As shown in Fig. 2, levels of IL-1β mRNA in MNL were somewhat greater (2.4 times as determined by scanning) than PMN when stimulated with LPS for 3 h; no appreciable differences between PMNp and MNL were seen at 6 h, and no mRNA was detected by PCR in unstimulated PMNp or MNL.

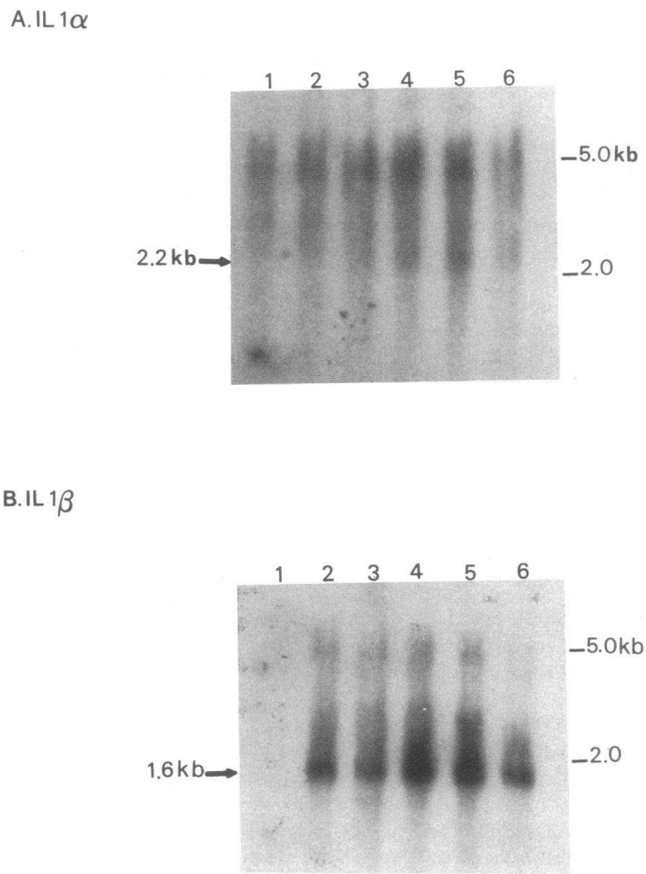

Figure 1. Northern blot analysis of IL-1α and IL-1β RNA production in PMNp. 15 μg of total RNA from PMNp stimulated for 0 (lane *1*), 2 (lane *2*), 3 (lane *3*), 4 (lane *4*), 9 (lane *5*), or 18 h (lane *6*) with 10 μg/ml of LPS were electrophoresed on formaldehyde gels, blotted, and probed with ^{32}P-labeled IL-1α cDNA (*A*) or IL-1β cDNA (*B*).

Monocytes enriched by adhesion to plastic and then stimulated with LPS for 9 h were also examined by Northern blot hybridizations. Even at the equivalent of 10% monocyte contamination, no IL-1α and a faint signal of β mRNA were detectable for up to 9 h (data not shown).

Thus, PMNp were capable of expressing both IL-1α and β mRNA and the IL-1 mRNA was not due to MNL contamination. The enhancement of IL-1 mRNAs in PMNp by LPS was not a generalized effect since we have found *c-ras* mRNA is constitutively expressed in PMNp, but levels of *c-ras* mRNA do not increase in PMNp after stimulation with LPS (Lord, P. C. W., L. M. G. Wilmoth, and C. McCall, unpublished observations).

Synthesis and secretion of IL-1α and β proteins. Although both IL-1α and β mRNA increased in LPS-stimulated PMNp, it was necessary to determine if PMNp could efficiently translate the mRNA. Initially, cell-free supernatants from unstimulated PMNp or PMNp stimulated with LPS were tested in the mouse thymocyte comitogen proliferation assay to measure

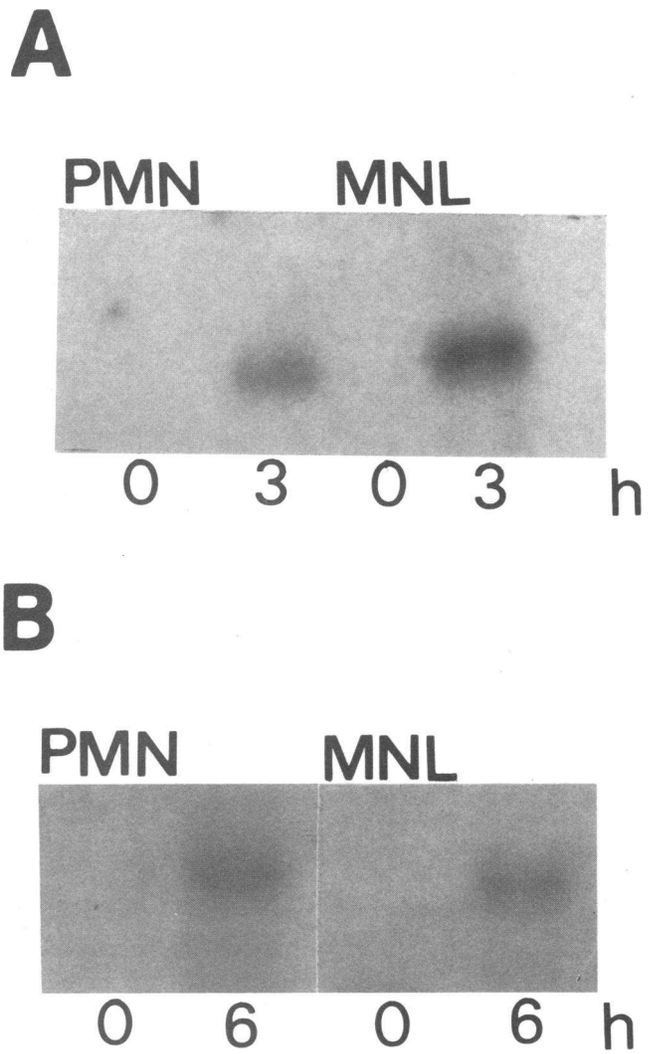

Figure 2. PCR analysis of mRNA of PMNp and MNL stimulated with LPS. Equal amounts of RNA from PMNp and MNL were used for PCR reactions and products probed with the IL-1β oligonucleotide probe as described in Methods. Results from two experiments are shown: one performed at 0 time and 3 h (*A*) and the other at 0 time and 6 h (*B*).

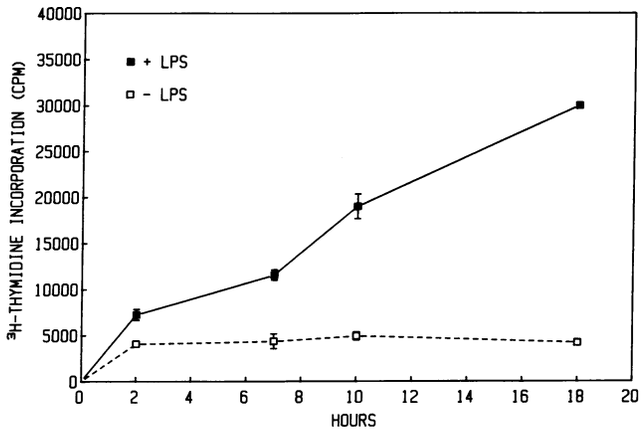

Figure 3. IL-1 bioactivity in culture media from PMNp as measured by mouse thymocyte comitogen proliferation. PMNp were incubated with (■) or without (□) 10 μg/ml LPS for various times. Culture media were removed and IL-1 activity measured by increased [^{3}H]thymidine uptake by thymocytes of the C3H/He2 strain of mice.

released or secreted IL-1-like activity. Fig. 3 shows that PMNp incubated without LPS for up to 18 h had little or no detectable IL-1 bioactivity in the extracellular media. In contrast, IL-1 activity appeared in the media of PMNp stimulated with LPS by 8 h and it continued to increase over an 18-h incubation with LPS. IL-1α and β antisera were employed in an attempt to inhibit thymocyte proliferation induced by PMNp supernatant (Table I). IL-1β antisera inhibited bioactivity by > 80% at 1:500 and 1:100 dilutions of antibody; IL-1α antisera only blocked 50% proliferation at 1:100 dilution of antibody. These data suggested that LPS-stimulated PMNp had predominantly released IL-1β, and little or no IL-1α. This observation was subsequently confirmed by ELISA studies of the release of immunoreactive IL-1α and β.

We then determined if the equivalent of 1% contamination of MNL could account for the release of total IL-1 bioactivity in PMNp. Fourfold dilutions of supernatants from either PMNp, or the equivalent contamination with MNL, were incubated for 18 h with LPS and tested for their ability to induce thymocyte proliferation. As seen in Table II, stimulating activity of cell-free supernatant from 3×10^4 MNL was only slightly less than that of 3×10^6 PMNp. The thymocyte comitogen stimulation by MNL supernatants could be inhibited by IL-1β antisera, but not IL-1α antisera (data not shown). Thus, we could not exclude from these experiments that as little as 1% MNL contamination could have accounted for the total IL-1 bioactivity released by the PMNp, or that an inhibitor of IL-1 had been released by PMNp.

Since our experiments using bioactivity did not prove that PMNp having translated IL-1α and β mRNA could release "mature" IL-1, we quantified by ELISA assays IL-1α or β immunoreactivity that was released by cells and that which remained cell associated. In these experiments shown in Fig. 4, we used the equivalent of 1% MNL contamination to compare MNL with PMNp. In some experiments we used up to 10% MNL (not shown). IL-1β immunoreactivity was observed in both supernatants and lysates of PMNp by 4–8 h and levels peaked by 8–18 h. In contrast to IL-1β, measurable levels of IL-1α activity were detected only in cell lysates after 10–18 h, and the quantity of cell-associated IL-1α measured was much less than that of IL-1β. It was clear that MNL could not account for the total synthesis of IL-1α or β observed in PMNp, although MNL were more efficient (about seven times) than PMNp in synthesizing IL-1.

*Table I. Effect of IL-1 α or β Antisera on the Mouse Thymocyte Proliferating Capacity of Supernatant from PMNp (3×10^6 cells/ml) Stimulated with 10 μg/ml E. coli LPS for 18 h**

	IL-1 α antibody		IL-1 β antibody	
No antisera	1:500	1:100	1:500	1:100
48±9	52±14	29	9±1	6±7

* Expressed in triplicate as mean cpm $\times 10^3$±SD of [^{3}H]thymidine from three experiments, each performed.

*Table II. Comparison of IL-1 Bioactivity in Culture Media from PMNp and MNL Stimulated with LPS for 18 h**

Dilutions of culture media	PMNp	MNL
	3 × 10⁶ cells/ml	*3 × 10⁴ cells/ml*
1:4	88±7	40±6
1:16	47±1	40±.3
1:64	21±2	17±.9
1:256	13	10

* Expressed as mean cpm × 10^3±SD (n = 3) of [^{3}H]thymidine incorporated into C3H/HeJ mouse thymocytes.

We next determined whether IL-1 could stimulate its own synthesis in PMNp. Fig. 5 shows the effects of LPS and recombinant mouse (rmIL)-1α on synthesis of IL-1β. The rmIL-1α at a concentration of 100 U/ml stimulated production of IL-1β although less efficiently than LPS at 10 μg/ml. The values shown in the figure represent total IL-1β (both supernatant and cell lysate values). These experiments also showed that 10 μg/ml of actinomycin D blocked IL-1β synthesis in response to LPS, confirming that IL-1 synthesis was under transcriptional control. Although we routinely used 10 μg/ml of LPS in our various assays, we determined by IL-1β ELISA that concentrations of LPS as low as 1–10 ng/ml were effective in stimulating IL-1β synthesis in PMNp (data not shown).

Identification of cell-associated IL-1α and β by immunocytochemistry. Immunocytochemistry proved to be a highly sensitive technique for detecting IL-1α or β in specific cell types. Fig. 6, *B* and *C*, shows positive immunoperoxidase staining for IL-1α or IL-1β in PMN stimulated with 10 μg/ml of LPS for 1 h; virtually 100% of the PMN were positive. Panel *A* is a preimmune serum control. Using this technique it was also found that rmIL-1α induced synthesis of IL-1β in PMN (not shown). Monocytes were also positive for IL-1α or β, but only a rare lymphocyte stained positive for IL-1α or β (not shown). Also, we noted that eosinophils stained falsely positive for IL-1 in control preparations, probably reflecting inefficient inactivation of eosinophil myeloperoxidase by the 3% H_2O_2 used in the prestaining step of the assay. The finding that cells (both PMN and monocytes) stained positive for IL-1α or β by 60 min of LPS stimulation was a consistent and reproducible finding. Although immunocytochemistry was a sensitive way to demonstrate IL-1α and β in specific cells, we could not quantify differences between IL-1α or β levels in PMN and MNL by this technique. It is interesting that the process of cell adherence (in the absence of LPS) resulted in positive staining of both PMN and monocytes (not shown). Induction of IL-1 synthesis after adherence also has been reported for blood monocytes (27).

We considered the possibility that the IL-1α and β observed in PMN by immunocytochemistry might represent IL-1 that

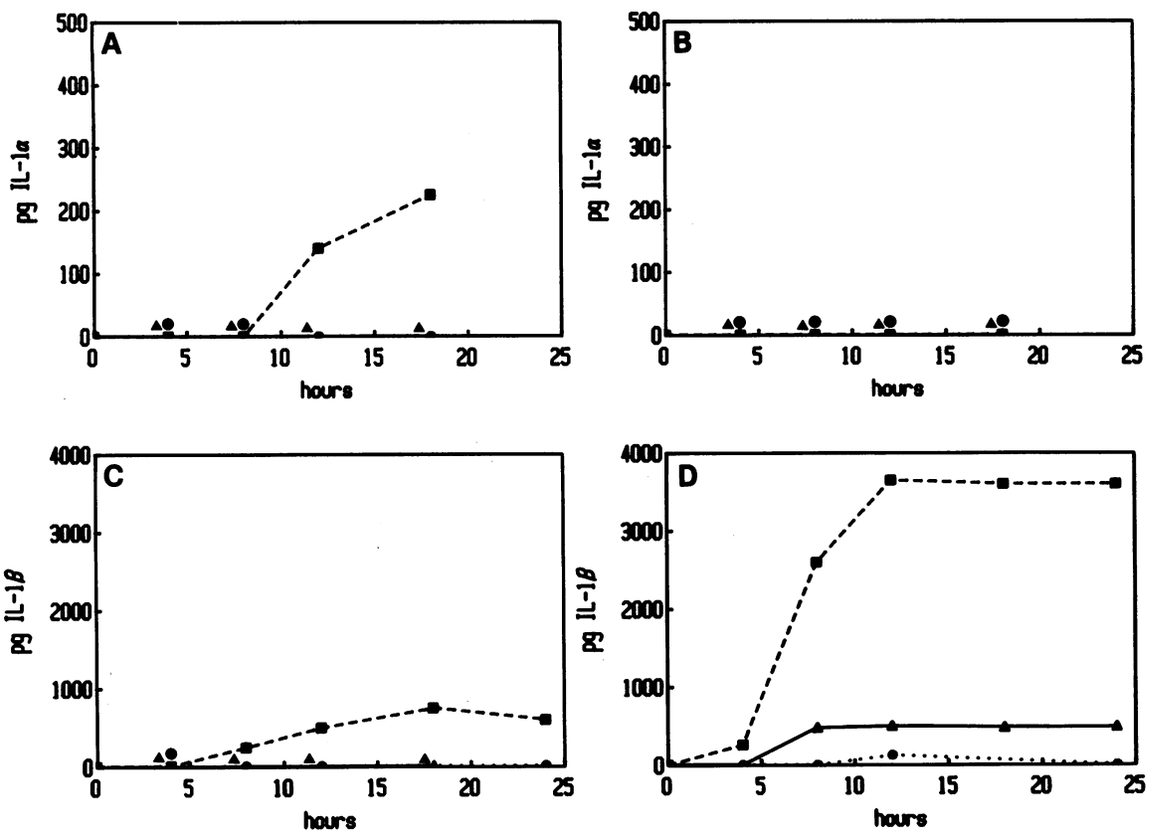

Figure 4. Released or cell-associated IL-1α or β immunoreactivity from PMNp or MNL stimulated with 10 μg/ml LPS as determined by ELISA. (*A*) IL-1α from cell lysates. (*B*) IL-1α released from cells. (*C*) IL-1β from cell lysates. (*D*) IL-1β released from cells. The values in pg/ml for PMNp (■) are from 9 × 10^6/cells/ml; the values from MNL (▲) are from 9 × 10^4 cells/ml (●) shows unstimulated PMNp. Note that for clarity the scales for IL-1α differ from IL-1β.

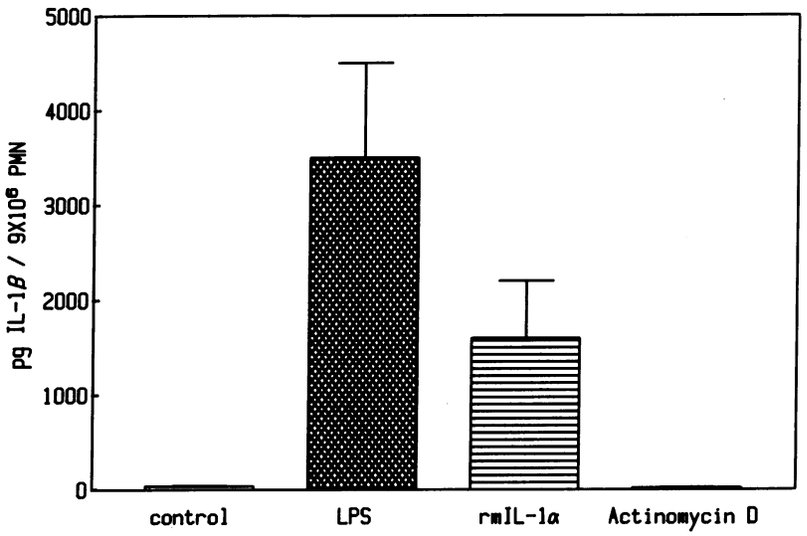

Figure 5. Synthesis and release of IL-1β immunoreactivity (ELISA) by PMNp stimulated with rmIL-1α (100 U/ml) for 18 h. LPS stimulation (10 μg/ml) is included for comparison. Also shown is the effect of 10 μg/ml of actinomycin D on synthesis of IL-1β by PMNp stimulated with 10 μg/ml LPS. Values are total synthesis (supernatant and lysate) and represent mean±SE of n = 4 for IL-1 and LPS and LPS plus actinomycin D.

was internalized by PMN after its synthesis and release by contaminating MNL. We have reported that human PMN contain receptors for IL-1 and that cell-associated IL-1α is rapidly internalized ($T_{1/2}$ = 30 min) (28). Internalization of IL-1β would be more likely than that of IL-1α, since little or no IL-1α was released by PMNp. To evaluate internalization of IL-1, we used the observation that a 1% contamination of MNL can release ~ 200 pg of IL-1β per 9 × 10^4 cells. PMNp were pretreated with cycloheximide (20 μg/ml) for 60 min to inhibit protein synthesis, and then the cells were incubated for another 60 min in the presence of 10^1–10^6 pg of recombinant human (rh) IL-1α (an insufficient quantity of rhIL-1β was available for these experiments). Although PMNp that were incubated with > 10^5 pg of rIL-1α stained positive for IL-1α, the preparations incubated with 10^1–10^4 pg of IL-1α were negative (not shown). This added support to the notion that the IL-1 observed in PMN was not internalized. Also, we incubated PMNp with a concentration of rmIL-1α (which does not cross-react with anti-human IL-1α antibody) which should effectively downregulate IL-1 receptors on PMN and thereby prevent exogenous IL-1β from being internalized induced. Such cells stained positive for IL-1β suggesting that IL-1β had been synthesized rather than internalized (not shown).

In vitro translation of IL-1 mRNA in PMNp and MNL. Although PMNp synthesized and released IL-1β and synthesized but did not clearly release IL-1α, they were considerably less efficient in protein synthesis than MNL. Thus, we compared translation of PMNp and MNL mRNAs in an in vitro assay. Total RNA was isolated from cells that had been stimulated for 9 h with LPS. An equal amount of PMNp or MNL RNA was then incubated in a rabbit reticulocyte in vitro translation system and protein products analyzed on polyacrylamide gels (Fig. 7). Lanes *1* and *2* show negative and positive controls, respectively. Lanes *3* and *4* demonstrate the total in vitro translated proteins from 5 μg of MNL or PMNp RNA. Lanes *5–8* and *9–12* are immunoprecipitations of IL-1 from 5 μg of MNL or PMNp RNA, respectively. The proteins translated by MNL included the 31–33-kD precursor IL-1α and β as shown by competition of the immunoprecipitation of either IL-1α (lane *6*) or IL-1β (lane *8*). The in vitro translated products of PMNp also included precursor IL-1α (lane *9*) and β (lane *11*), each of which was competed by the addition of unlabeled rIL-1α (lane *10*) or β (lane *12*). The IL-1β precursor was significantly elevated over that of IL-1α in both cell groups, confirming our Northern analysis and ELISA results that suggested that transcription of IL-1β mRNA exceeds that of IL-1α mRNA. Thus, there were no major differences between mRNA of PMNp or MNL in translating IL-1α or β mRNA in vitro.

Discussion

The findings of this study clearly indicate that human blood PMN express IL-1α and β genes. PMNp exhibited increases in IL-1α and β mRNA in response to stimulation with LPS (Fig. 1). These increases could not be accounted for by the 1–2% MNL contamination, and the capacity of MNL and PMNp to increase IL-1β mRNA in response to LPS was only slightly greater in MNL (Fig. 2). IL-1α mRNA was not detected in unstimulated PMNp or PMNp stimulated with LPS for 2–3 h but was observed at low levels at 4 h and was most abundant after 9 h. In contrast, LPS-induced elevations in IL-1β mRNA were more abundant than IL-1-α mRNA at all time points, as reported for IL-1β mRNAs in monocytes (2, 5).

Our study of the release of IL-1 bioactivity did not completely eliminate the possibility that MNL and not PMN were synthesizing and releasing the bioactive IL-1 (Table II). However, the experiments using ELISA to quantitate released or cell-associated IL-1α or β in PMNp and MNL convincingly quantified differences between IL-1 synthesis and release by the different cell types (Fig. 4). By this analysis, it appeared that MNL were about seven times more efficient than PMNp in synthesis of IL-1β. Both cell-associated and released IL-1β were observed in PMNp, whereas IL-1α was predominantly if not exclusively cell associated. Similar differences were seen in MNL, as has been previously reported in human monocytes (29).

Final proof of IL-1 synthesis by PMN was provided by immunocytochemical analysis that was very sensitive in identifying IL-1α and β in individual PMN and monocytes. Virtually 100% of PMN were positive for IL-1α or β after stimulation with LPS for 1 h (Fig. 6). It was likely that PMN had synthe-

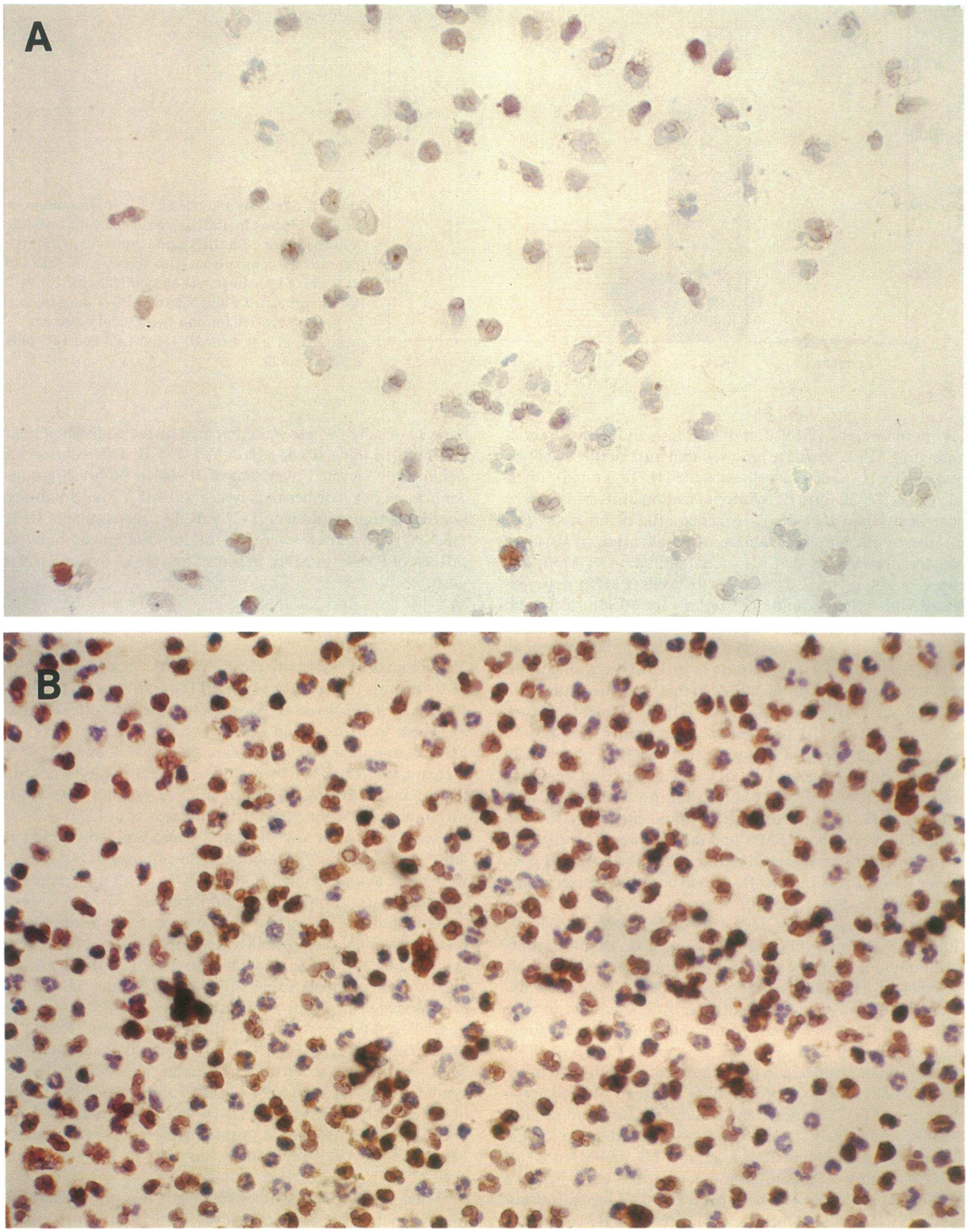

Figure 6. Immunocytochemical staining of IL-1α or β in PMN. PMNp were stimulated for 60 min with 10 μg/ml LPS and prepared as described in Methods. *A* represents a zero time control PMNp using preimmune serum. *B* shows IL-1α staining, and *C* IL-1β staining in PMN.

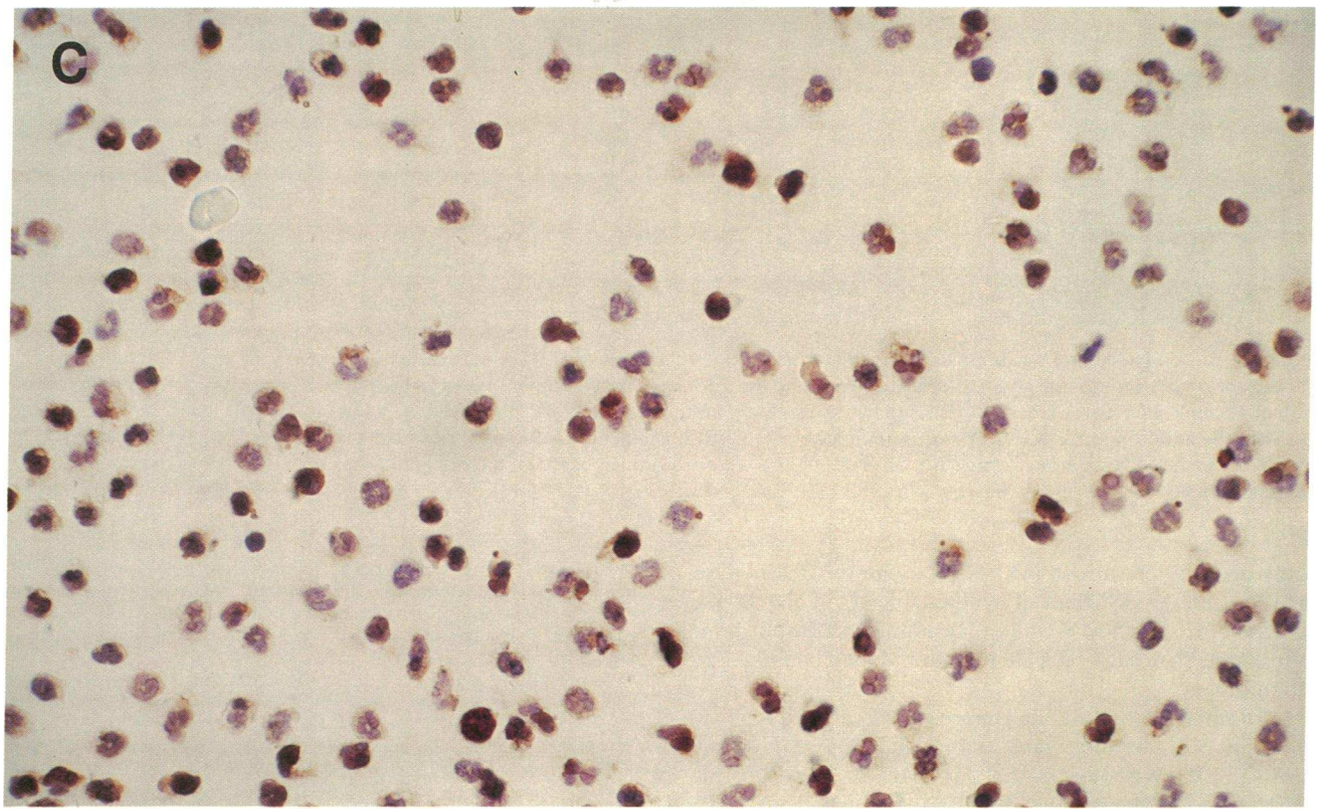

Figure 6 (Continued)

sized rather than internalized the cell-associated, immunoreactive IL-1 since little or no IL-1α was released by PMNp or MNL and PMN stained strongly positive for IL-1α. Also, putative downregulation of the IL-1 receptor by rmIL-1α did not significantly alter the staining pattern of PMN for IL-1β, suggesting that IL-1β had not been internalized and that receptor-mediated responses to rmIL-1α induced IL-1β synthesis. The ELISA results of stimulation of IL-1β synthesis by rmIL-1α supported this notion. The early appearance of IL-1 by cytochemical analysis differed from our other analyses, a finding that we cannot explain. It might simply reflect sensitivity of the assay. Another possibility is that adherence of cells and stimulation by LPS are synergistic for IL-1 synthesis.

The observation that IL-1 can stimulate production of IL-1 in PMN is interesting. Such has been reported in human monocytes (30). While the manuscript was being reviewed Marucha et al. (31) reported their findings of IL-1β synthesis in human PMN induced by IL-1α.

This induction of IL-1β by IL-1 in PMN is the first functional response after receptor-binding of IL-1 in PMN that we have detected. For example, we (28) and others (32) have not found that IL-1 receptor-linked stimulation of PMN by rhIL-1α or rmIL-1α induces chemotaxis, a respiratory burst, or degranulation. However, there are reports that IL-1 is a weak stimulus for the respiratory burst and degranulation of human PMN (33, 34). We have also found that PMN stimulated with rhIL-1α do not produce PGE_2, or elevate intracellular calcium (unpublished observations). An attractive concept is that regulation of gene expression in PMN can occur without inducing the production and release of potential autotoxins such as proteases and O_2-derived moieties; such could be advantageous in inflammation. In support of this notion are our observations that radiolabeled IL-1α internalizes and may translocate to the nucleus in PMN following receptor-mediated binding (28).

A surprising and novel observation was that while intact PMN were efficient at transcribing mRNA for IL-1α and β they were inefficient in translating these messages when compared with intact MNL. In contrast, PMN and MNL IL-1 (and other) mRNAs were translated with equal efficiency in reticulocyte lysates (Fig. 7). The reason for the relative deficiency in translation of IL-1α and β mRNA by PMN is not known. One possibility is that PMN might require more than one stimulus or transductional signal to effectively translate IL-1 mRNAs. Such has been reported in macrophages (35) where thioglycollate-elicited peritoneal macrophages contained a pool of TNFα mRNA that was not efficiently translated until the cells were stimulated with LPS. Posttranscriptional control of IL-1β synthesis also occurs in fibroblasts (35). However, we have not found that PMN stimulated with LPS plus other agonists such as phorbol myristate acetate, the chemotactic peptide *N*-formyl-methionyl-leucine, or the calcium ionophore A23187 significantly increase synthesis of IL-1α or β (unpublished observations). Another possibility is that PMN contain a specific inhibitor of translation of IL-1 mRNAs, or that PMN have a generalized deficiency in translating their mRNA. The latter notion is supported by observations of Jack and Fearon who found that PMN were less efficient than monocytes in synthesizing complement receptor CR-1 protein (13). PMN have relatively few ribosomes and endoplasmic reticulum (6), and they contain less total RNA than monocytes or MNL (our observations and 14). The low number of ribosomes in PMN might limit the rate of total synthesis of protein by PMN, depending

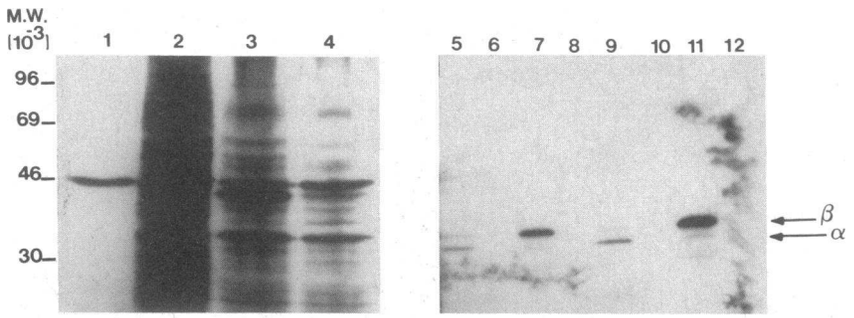

Figure 7. In vitro translation of RNA from PMNp or MNL. 5 μg of total PMNp or MNL RNA from LPS-stimulated PMNp or MNL were incubated in a rabbit reticulocyte in vitro translation system and subsequently electrophoresed on 12.5% SDS-polyacrylamide gel or immunoprecipitated with either IL-1α or β antisera, with and without the addition of unlabeled IL-1α or β protein, respectively. Lane *1*, no RNA added to in vitro translation system; lane *2*, yeast mRNA as a positive control. Lane *3*, 5 μg of MNL RNA; lane *4*, 5 μg of PMNp RNA. Lanes *5–8*, immunoprecipitation of a sample identical to lane 3 divided into two samples. (*A*) without the addition of unlabeled IL-1α protein (lane *5*) or with the addition of unlabeled IL-1α protein (lane *6*); (*B*) without the addition of unlabeled IL-β (lane *7*) or with the addition of IL-1β protein (lane *8*). Lanes *9–12*, immunoprecipitation of a sample identical to lane 4 divided into two samples and immunoprecipitated (*A*) without the addition of unlabeled IL-1α protein (lane *9*) or with the addition of unlabeled IL-1α protein (lane *10*), or (*B*) without the addition of unlabeled IL-1β protein (lane *11*) or with the addition of unlabeled IL-1β protein (lane *12*). Sizes of proteins were determined based on their migration relative to protein standards of known molecular weights (*M.W.*).

on the quantity of various mRNAs. Despite the observation that PMN are less efficient at synthesizing IL-1 than MNL, synthesis of IL-1 by PMN could play an important role in inflammation when such cells are present in blood and tissue in high numbers.

In summary, a multifaceted approach was used to evaluate expression of IL-1α and β genes in human blood PMN stimulated with LPS. We conclude that: (*a*) PMN can transcribe and translate IL-1α and β genes; (*b*) PMN stimulated with LPS increase their levels of IL-1 mRNA with an efficiency similar to that of MNL; (*c*) IL-1β mRNA of PMN reaches a higher level than IL-1α mRNA; (*d*) more IL-1β protein than IL-1α protein is synthesized by PMN; (*e*) IL-1β but little or no IL-α protein is released by PMN; (*f*) translation of IL-1α or β mRNA is less efficient in intact PMNp than in MNL, but both IL-1α and β mRNAs from PMNp and MNL are similarly translated to IL-1 α and β in an in vitro translational system; and (*g*) IL-1 can induce its own synthesis. Finally, we stress the importance of considering the contribution of even small numbers of contaminating MNL when studying gene expression in preparations of PMN.

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