Normal Human Alveolar Macrophages Obtained by Bronchoalveolar Lavage Have a Limited Capacity to Release Interleukin-1

Mark D. Wewers, Stephen I. Rennard, Allan J. Hance, Peter B. Bitterman, and Ronald G. Crystal
Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205

Abstract. Interleukin-1 (IL-1) is a mediator released by stimulated mononuclear phagocytes that is thought to play an important role in modulating T and B lymphocyte activation as well as in contributing to the febrile response and other inflammatory processes. Circulating mononuclear phagocytes, blood monocytes, readily release IL-1 when stimulated. However, the ability of lung mononuclear phagocytes, alveolar macrophages, to dispose of the large daily burden of inhaled antigens without stimulating an inflammatory response suggests that the release of IL-1 by alveolar macrophages may differ significantly from that of blood monocytes. To evaluate this hypothesis, normal autologous alveolar macrophages, obtained by bronchoalveolar lavage, were compared with blood monocytes for their ability to release IL-1 in response to a standard stimulus, lipopolysaccharide (LPS). Alveolar macrophages were found to be at least 1,000 times less sensitive to LPS than blood monocytes. Furthermore, alveolar macrophages released significantly less IL-1 than blood monocytes (26 ± 11 vs. 128 ± 21 U/10^6 cells · 24 h, respectively, after stimulation with 10 μg/ml of LPS, $P < 0.001$). This difference was not due to the release of substances by macrophages, which inhibited lymphocyte proliferation in response to IL-1, or to degradation of IL-1 by macrophages. Culturing macrophages in the presence of indomethacin and dialysis of macrophage supernatants did not affect the difference, and culturing macrophages with monocytes did not decrease detectable IL-1 activity from the monocytes. The IL-1 produced by the two cell types was indistinguishable by anion-exchange chromatography, gel filtration, and isoelectric focusing. In addition, consistent with the findings for alveolar macrophages, macrophages generated by the in vitro maturation of blood monocytes were also deficient in their ability to release IL-1. These findings suggest that if the population of alveolar macrophages obtained by bronchoalveolar lavage represents the total in vivo population of alveolar macrophages, although normal human macrophages are capable of IL-1 release, they are relatively limited in this ability, and this limitation seems to be linked to the maturational state of the mononuclear phagocyte. These observations may explain, in part, the ability of alveolar macrophages to clear the airspaces of foreign antigens without extensive activation of other pulmonary inflammatory and immune effector cells.

Introduction

In response to immune or inflammatory stimuli, cells of the mononuclear phagocyte system release interleukin-1 (IL-1),[1] a 12–18,000-D protein that augments a variety of immune and inflammatory processes. For example, when blood monocytes are stimulated with a standard stimulus such as lipopolysaccharide (LPS), large amounts of IL-1 are released, and this IL-1 can magnify antigen- and mitogen-induced proliferation, enhance B lymphocyte immunoglobulin secretion, cause fever in laboratory animals, activate neutrophils, stimulate prostaglandin E_2 production by several target cells, stimulate the release of acute-phase reactants, and augment skin-fibroblast proliferation (1–18).

In this context, since the lower respiratory tract contains large numbers of mononuclear phagocytes (alveolar macrophages) that are constantly exposed to immune and inflammatory stimuli by virtue of their position in the airspaces, one might expect significant IL-1 release into the lower respiratory

Address correspondence to Dr. Wewers, National Institutes of Health, Bethesda, MD.

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1. *Abbreviations used in this paper:* FBS, fetal bovine serum; IL-1, interleukin-1; IL-2, interleukin-2; LPS, lipopolysaccharide.

tract with a concomitant activation of immune and inflammatory processes. However, this does not appear to be the case. The T and B lymphocytes recovered from the lower respiratory tract of normal individuals show little evidence of activation, and few neutrophils are present (19). Furthermore, while alveolar macrophages can present antigen to autologous T lymphocytes (a process thought to require IL-1), human alveolar macrophages function much less efficiently than human blood monocytes in antigen presentation (20–22). Thus, it appears that alveolar macrophages are relatively deficient in accessory-cell function when compared with blood monocytes. A possible explanation for this deficiency in alveolar-macrophage accessory cell function is that alveolar macrophages are relatively limited in their ability to release IL-1. Such a limitation would help explain the ability of alveolar macrophages to handle the usual burden of inhaled material without inappropriately triggering immune and inflammatory responses.

In this regard, the present study was designed to compare IL-1 release by human alveolar macrophages with that of human blood monocytes in response to a standard stimulus. Both cell types were evaluated for (*a*) their relative sensitivity to a standard stimulus, (*b*) the amount of IL-1 released in response to the same stimulus, and (*c*) the biochemical characteristics of the IL-1 released. In addition, since the alveolar macrophage represents a differentiated descendant of the blood monocyte, an in vitro analogue of monocyte maturation was examined to evaluate the effect of differentiation on the relative ability of the mononuclear phagocyte to release IL-1.

Methods

Isolation of alveolar macrophages and blood monocytes. Bronchoalveolar lavage cells were obtained from healthy normal subjects (n = 22) as previously described (19). Briefly, lavage of the right middle lobe, lingula, and left lower lobe was performed, instilling five 20-ml aliquots of 0.9% sterile saline into each lobe, respectively. The saline was gently aspirated, the recovered fluid was pooled, and the cells were separated by centrifugation at 500 g for 5 min. The cells were washed twice in RPMI 1640 (Microbiological Sciences, Inc., Elmsford, NY) containing penicillin (100 U/ml) and streptomycin (100 μg/ml) and resuspended at a concentration of 10^7 cells/ml. Viability, as determined by the ability to exclude 0.2% trypan blue, always exceeded 95%. Cell differentials were performed on Diff-Quick (American Hospital Supply Corp., McGaw Park, IL) stained cytocentrifuge preparations and in all cases showed alveolar macrophages >90%, lymphocytes ≤10%, and polymorphonuclear leukocytes <4%.

In cases where the alveolar macrophage populations represented <95% of the recovered cells, alveolar macrophages were further enriched by adherence to plastic. The recovered cells were plated at a density of 10^6 cells/ml RPMI 1640 with 50 μg/ml gentamicin (2 h, 37°C, 95% air-5% CO_2) in 24 well dishes (Costar, Cambridge, MA). Nonadherent cells were removed by repeated washings with RPMI 1640 and counted in a hemocytometer. The percentage of cells that remained adherent ranged between 70 and 90%. Fresh complete medium (RPMI 1640 containing 5% heat-inactivated fetal bovine serum [FBS; Associated Biomedics Systems Inc., Buffalo, NY] and 50 μg/ml gentamicin) was then added to the adherent cells. By morphological criteria these cells were always >95% alveolar macrophages.

Blood mononuclear cells were obtained from heparinized venous blood taken from the normal subjects just before the bronchoalveolar lavage. The blood was diluted 1:1 with 0.9% saline (Biofluids, Inc., Rockville, MD), underlaid with lymphocyte separation medium (Litton Bionetics, Kensington, MD), and centrifuged at 200 g for 30 min. The interface cells were aspirated from the gradient, washed three times in Hanks' balanced salt solution (HBSS; Microbiological Sciences, Inc.), counted in a hemocytometer, and reconstituted to 10^7 cells/ml in RPMI 1640 with 50 μg/ml gentamicin. Cytocentrifuge preparations of this cell population consistently showed ~20% monocytes, 80% lymphocytes, and <1% neutrophils. To enrich the blood mononuclear cell populations for monocytes, the mononuclear cells were plated at a density of 2×10^6 cells/ml in RPMI 1640 with 50 μg/ml gentamicin for 2 h at 37°C with 5% CO_2 in 24 well dishes (Costar). Nonadherent cells were removed by repeated washings and adherent cells were quantitated as done for the bronchoalveolar lavage cells. Adherent cells represented 25–50% of the original population and consisted of 60–90% (average 75±4%) (all data are expressed as mean±SEM) monocytes with the remainder lymphocytes. Fresh complete medium was then added. These cells (referred to subsequently as monocytes) were then handled in an identical manner as the alveolar macrophages.

Assay for IL-1. Unless otherwise noted, IL-1 was quantified using the standard mouse thymocyte assay (23). Briefly, C3H/HeJ mice (The Jackson Laboratory, Bar Harbor, ME) 8–12 wk old were sacrificed by CO_2 inhalation. Using aseptic technique, the thymus glands were removed, teased into a single-cell suspension with a 15-scalpel blade, passed through one layer of surgical gauze to remove particulates, washed twice in HBSS, and resuspended in RPMI 1640 for counting. The cells (15×10^6/ml) were then placed into thymocyte medium [RPMI 1640 with 50 μg/ml gentamicin, 5% heat-inactivated FBS, and 2×10^{-5} M 2-mercaptoethanol (Sigma Chemical Co., St. Louis, MO)]. Just before plating, 2 μg/ml phytohaemagglutinin (The Wellcome Foundation, Ltd., Beckenham, England) was added to the thymocyte suspension, and 100 μl of the suspension (1.5×10^6 thymocytes) was plated into each well of a 96-well, round-bottomed microtiter plate (Linbro Chemical Co., Hamden, CT).

The samples to be evaluated for IL-1 activity were diluted in RPMI 1640 with 5% heat-inactivated FBS and added in 100-μl aliquots to the thymocytes. Each dilution was assayed in triplicate. The cultures were incubated (37°C, 95% air/5% CO_2) for 48 h, pulsed with 0.5 μCi of [^{3}H]thymidine (2 Ci/mM; Amersham Corp., Arlington Heights, IL) per well, incubated for an additional 24 h, harvested onto glass fiber filters using a cell harvester (MASH II, Microbiological Sciences, Inc.), and the incorporated radioactivity was quantified by scintillation counting.

Assay for interleukin-2. Supernatants were assayed for interleukin-2 (IL-2) activity by their ability to stimulate the incorporation of [^{3}H]thymidine by IL-2-dependent CT6 murine T lymphocytes, as previously described (24).

Quantitation of IL-1. IL-1 activity was quantified using probit analysis modified from the method of Gillis et al. (25). Briefly, each supernatant was assayed at fourfold dilutions (1:4–1:256) and the resulting thymocyte proliferation (as assessed by [^{3}H]thymidine incorporation) was compared with that obtained from dilutions of a partially purified IL-1 standard. (The standard was prepared from LPS-treated human blood monocytes by DEAE chromatography as described below.) The value resulting from each dilution of the test sample was

expressed as the percentage of thymocyte proliferation induced by the 1:20 dilution of the IL-1 standard; i.e., each value was expressed as: [(dpm of sample) − (dpm of medium control)] × 100/[(dpm of IL-1 standard) − (dpm of medium control)]. Each value was then plotted as a function of its reciprocal dilution on a $\log_2$ scale, thus generating a dilution curve for each sample. The reciprocal dilution of each sample that gave 50% of the maximum [^{3}H]thymidine incorporation was determined and compared (on a linear scale) with the reciprocal dilution of the standard that gave 50% of its maximum value. The standard was arbitrarily assigned the value of 100 U.

In the example shown (Fig. 1), the reciprocal dilution of the standard that produced 50% of the maximum [^{3}H]thymidine incorporation was 64; thus, by definition, the reciprocal dilution value of 64 corresponds to 100 U of IL-1 activity. For the monocyte sample, the reciprocal dilution that produced 50% of the maximum [^{3}H]thymidine incorporation was 125; thus, the monocyte sample was assigned 195 U (i.e., [125/64] × 100 U = 195 U). The alveolar macrophage sample produced 50% of the maximum [^{3}H]thymidine incorporation at a reciprocal dilution of 14 and thus was assigned 22 U (i.e., [14/64] × 100 = 22 U).

Comparison of alveolar macrophages and monocyte release of IL-1. Preliminary studies showed that incubation of monocytes in some batches of medium resulted in IL-1 release in the absence of the addition of exogenous stimulators of IL-1 release. Analysis of multiple lots of serum and medium components from several sources demonstrated that this spontaneous release of IL-1 correlated with the amount of endotoxin present as assayed by limulus amebocyte lysate gelation. These findings are consistent with previous observations of monocyte sensitivity to endotoxin (26, 27). However, when monocytes and alveolar macrophages were incubated in medium and serum that had been screened to eliminate endotoxin contamination, neither cell type spontaneously released IL-1. Furthermore, when a variety of stimuli including LPS, zymosan particles, *Staphylococcus epidermidis,* and ox erythrocyte-goat anti-ox erythrocyte immune complexes were tested, it was clear that LPS was the most potent stimulus for IL-1 release by both cell types (data not shown). For this reason, all subsequent experiments were performed in endotoxin-free medium using LPS as the standard stimulus.

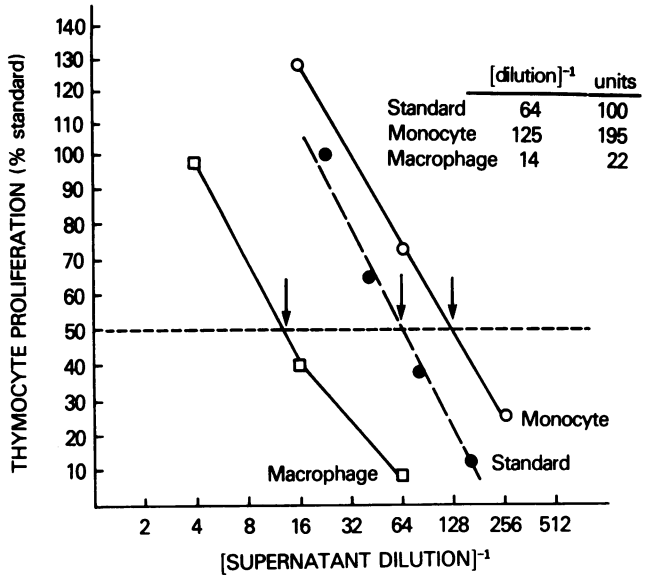

Figure 1. Quantitation of IL-1 using probit analysis. The amount of IL-1 in each supernatant sample was evaluated by determining thymocyte proliferation (assessed by [^{3}H]thymidine incorporation) in response to increasing dilutions of the supernatants. In the example given, the dilution curve for the partially purified IL-1 standard (●) is shown plotted with the test supernatants from alveolar macrophages (□) and blood monocytes (○) from the same individual. Quantitation of IL-1 was accomplished by comparing the sample with the standard curve at the dilution that produced 50% of the maximum proliferation induced by the standard. In this example, 50% proliferation occurred for the standard at a dilution of 64, which is arbitrarily defined as 100 U. The corresponding dilution at the 50% standard proliferation level was 125 for the monocyte sample (representing 195 U) and 14 for the macrophage sample (representing 22 U).

To determine the relative sensitivity of alveolar macrophages and blood monocytes to LPS, alveolar macrophages and monocytes from six normal individuals were cultured in 24 well dishes (Costar) in complete medium at 10^6 cells/ml. The cells were incubated for 24 h with *Escherichia coli* LPS, Westphal preparation (Difco Laboratories, Inc., Detroit, MI), in amounts ranging from 10^{-14} to 10^{-5} g/ml. The supernatants were harvested, filtered through 0.22-μm filters (Millipore Corp., Bedford, MA) to remove cells, and frozen at −20°C. All samples were assayed for IL-1 activity within 1 wk of harvesting.

To compare the relative amounts of IL-1 released by alveolar macrophages and monocytes maximally stimulated with LPS, alveolar macrophages and blood monocytes from 16 normal individuals were cultured in complete medium at 10^6 cells/ml in 24-well dishes (Costar) with or without 10^{-5} g/ml LPS for 24 h. Supernatants were handled as described above.

Since microgram quantities of LPS can induce thymidine incorporation by C3H/HeJ mouse thymocytes, each thymocyte assay included a medium control containing 10^{-5} g LPS/ml. In every instance, thymidine incorporation induced by medium containing LPS was approximately twice that induced by medium alone. When subjected to probit analysis, this nonspecific activity was insignificant compared with responses from stimulated mononuclear phagocyte supernatants (<1 U/ml for LPS medium control compared with an average 26 U/ml for alveolar macrophage supernatants and 128 U/ml for blood monocyte supernatants).

Evaluation of role of inhibitors modulating the measurement of IL-1 released by macrophages and monocytes. Since prostaglandin E_2 release may affect the proliferation of the thymocyte responder cells (28), alveolar macrophages and blood monocytes were incubated with indomethacin (10^{-6} M, Sigma) to determine if prostaglandin release by either of the two cell types influenced thymocyte proliferation. Indomethacin alone had no effect on the assay for IL-1 (data not shown). To test for the presence of dialyzable inhibitors in the cell supernatants that might suppress thymocyte proliferation, supernatants from monocytes and macrophage preparations stimulated with LPS were diluted 1:4 with complete medium and dialyzed against 1,000 vol of medium before assay. Finally, to determine if the alveolar macrophages might degrade released IL-1 or release nondialyzable inhibitors, monocytes plated at a density of 10^6 cells/ml were co-cultured with increasing numbers (1.25×10^5–10×10^5 cells/ml) of autologous alveolar macrophages. These co-cultures were stimulated and handled as previously described.

Human T cell assay for IL-1 activity. To validate that the difference observed between monocyte and alveolar macrophage IL-1 release was not related to the assay system used to detect IL-1, a similar study was carried out using human T lymphocytes as the detecting cell (10). Human peripheral blood mononuclear cells were isolated from buffy coat preparations obtained from normal donors using a lymphocyte separation medium gradient as described above. Monocytes were depleted by incubating the cells for 1 h at 37°C in 95% air/5% CO_2, and removing the nonadherent cells by gently washing the culture plates. The T lymphocytes in this preparation were then purified by rosetting with neuraminidase-treated sheep erythrocytes as previously described (29), and residual adherent cells were removed by culturing the cells overnight in a 100-mm tissue culture dish in complete medium. The nonadherent population was then removed, washed twice in RPMI 1640, resuspended at 15×10^6 cells/ml in heat-inactivated FBS containing 10% dimethyl sulfoxide, frozen in vials (Nunc, Kampstrup, DK) in an automated cell freezer, and stored at −70°C until use. Purity of the T cells as assessed by a T cell specific monoclonal antibody leu 4 (Becton Dickinson & Co., Oxnard, CA) (30) was >98% and cell viability always exceeded 90% as measured by trypan dye exclusion. On the day of assay, the T cells were rapidly thawed, washed three times in HBSS, and treated with anti-HLA-DR (Becton Dickinson) and rabbit complement (Cappel Laboratories, Cochranville, PA) to further deplete residual accessory cells. The remaining cells were washed twice in complete medium and were resuspended to 2×10^6 cells/ml in thymocyte medium as described for the thymocyte assay. The assays for IL-1 using the human T cells were performed as described for the mouse thymocyte assay, with the exceptions that the final concentration of phytohemoglutinin used was 0.1 μg/ml and the final cell concentration was 10^6 cells/ml.

Partial purification of alveolar macrophage and blood monocyte IL-1. Blood mononuclear cells were obtained from the leukopheresis of a normal subject, cultured for 2 h in serum-free RPMI 1640, washed free of nonadherent cells, and then incubated for 24 h in complete medium in the presence of LPS (10 μg/ml). Alveolar macrophages were cultured for 24 h in the presence of LPS (10 μg/ml) in complete medium. Supernatant fluids obtained from the culture of alveolar macrophage from several individuals were pooled.

DEAE-52 chromatography. Supernatants from monocytes and macrophage cultures were dialyzed at 4°C against 10 mM NaCl, 20 mM Tris-HCl, pH 7.5, and applied to a 2.6×10 cm column of DEAE-52 cellulose (Whatman Ltd., Kent, England) that had been equilibrated in the same buffer. The column was eluted into 30 fractions using a 10–300 mM NaCl gradient. An aliquot of each fraction was diluted with RPMI 1640 containing 5% FBS, and assayed as described above.

Sephadex G-100 chromatography. Aliquots from the peak activity of IL-1 obtained from the DEAE-52 chromatography were lyophylized, reconstituted in distilled water to effect a 10-fold concentration, and applied to a 1.6×70-cm column of superfine Sephadex G-100 (Pharmacia Inc., Piscataway, NJ), and the column was eluted with 100 mM Tris HCl, 100 mM NaCl, pH 7.40, by gravity at a flow rate of 3 ml/h.

Isoelectric focusing. Supernatant fluid (100 ml) from blood monocyte or alveolar macrophage cultures was used for determining the isoelectric profile of IL-1 activity. The medium was dialyzed against 1% glycine, mixed with 5 g Ultradex (LKB Instruments, Inc., Rockville, MD) and 5 ml of Biolyte 3–10 (Bio-Rad Laboratories, Richmond, CA), and poured onto a focusing plate (LKB Instruments, Inc.). Focusing was done at 8 W constant power for 15 h at 4°C, and the resultant gradient was divided into 30 fractions. 1 ml of de-ionized water was added to each fraction, and the pH was determined. Each fraction was dialyzed against phosphate buffered saline (pH 7.4), diluted with complete medium, filtered through 0.22 μm filters, and assayed for IL-1 activity in the mouse thymocyte assay.

Monocyte maturation and IL-1 release. As an in vitro analogue of the maturation of monocytes into tissue macrophages, blood monocytes were allowed to mature in culture over an 11-d period. Maturation was confirmed by morphologic criteria. The cells were maintained at 10^6 cells/ml in endotoxin-free complete medium. Monocytes were tested for their ability to release IL-1 into the medium by stimulating the cells for 24 h with 10 μg/ml of LPS beginning at day 0, 1, 2, 3, 4, 7, and 11. Cell viability (>95% were viable at all time points) was assessed by trypan blue dye exclusion. Phagocytic function was evaluated by counting the percentage of cells able to phagocytose carbonyl iron particles (Sigma). Fibronectin released into the medium was measured by enzyme-linked immunosorbant assay (31). The molecular weight profile of newly synthesized secreted proteins was also determined by incubating cells for 24 h with 13.5 μCi [^{35}S]methionine/ml (1,485 Ci/ml; Amersham) at the time points indicated above. Supernatants were harvested, centrifuged, and frozen. The proteins present in the supernatants were precipitated with ethanol, resuspended in electrophoresis sample buffer containing 2% 2-mercaptoethanol, and electrophoresed into 10% sodium dodecyl sulfate-polyacrylamide gels (32). Autoradiograms were prepared by the method of Bonner et al. (33).

Preparation of supernatants from tissue macrophages other than alveolar macrophages. To compare the thymocyte mitogenic activity released from alveolar macrophage sources with that of other differentiated tissue mononuclear phagocytes, breast milk macrophages were isolated from the milk of five individuals by low-speed centrifugation, and peritoneal macrophages were obtained from the peritoneal fluid of one healthy female undergoing laparoscopy for an infertility evaluation.

These cells were cultured at 10^6 cells/ml as described for monocytes and alveolar macrophages. The supernatants were analyzed for IL-1 activity as described above.

Statistical analysis. Statistical significance was determined by the two-tailed t test.

Results

Relative sensitivity and extent of response of normal human blood monocytes and alveolar macrophages to LPS. Although neither monocytes nor alveolar macrophages released IL-1 when cultured in endotoxin-free medium, both cell types released IL-1 when stimulated with *E. coli* endotoxin (LPS). However, while both cell types could release IL-1, there was a remarkable difference in the response of the two cell types to LPS, both in relative sensitivity to LPS and in the quantity of the IL-1 produced after maximal stimulation (Fig. 2 *A*). In the six individuals studied quantitatively, blood monocytes released 124±26 U/10^6 cells·24 h of IL-1 at 10^{-9} g/ml of LPS and 211±24 U/10^6 cells·24 h at 10^{-5} g/ml of LPS. In marked contrast, alveolar macrophages released only 5±5 U/10^6 cells·24 h of IL-1 at 10^{-9} g/ml of LPS and 51±26 U/10^6 cells·24 h at 10^{-5} g/ml of LPS. The IL-1 release by the two cell types differed significantly at both the 10^{-9} and 10^{-5} g/ml level of LPS ($P < 0.005$ and $P < 0.02$, respectively).

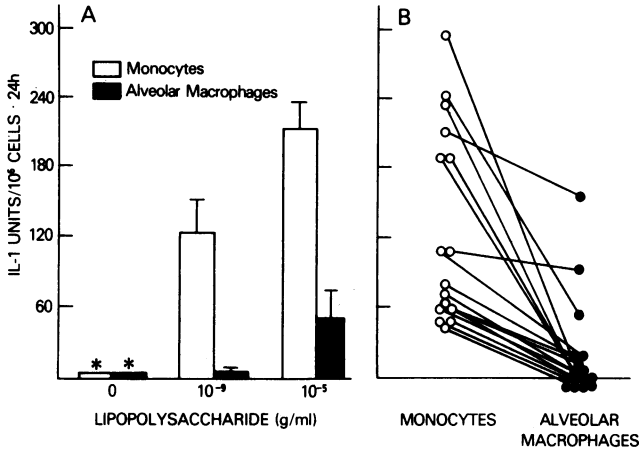

Figure 2. Comparison of the release of IL-1 by human blood monocytes and human alveolar macrophages in response to LPS. (*A*) Comparison of IL-1 release at different amounts of LPS. Blood monocytes (□) and alveolar macrophages (■) from three normal individuals were cultured for 24 h at 37°C with medium alone or with 10^{-9} or 10^{-5} g/ml of LPS. *, no detectable IL-1. (*B*) Comparison of the relative amount of IL-1 released by autologous human blood monocytes and alveolar macrophages in response to a standard stimulus. Blood monocytes (○) and alveolar macrophages (●) from 16 normal individuals were cultured for 24 h at 37°C in the presence of 10^{-5} μg/ml LPS, and the amount of IL-1 released into the supernatant was quantified. A line connects the data for each individual.

In another series of experiments, IL-1 release by both cell types was measured qualitatively (thymocyte [^{3}H]thymidine incorporation at a 1:4 dilution of supernatant from six individuals, using a much broader LPS dose range [10^{-14}–10^{-5} g/ml]). In this LPS range, monocytes invariably released detectable IL-1 activity at 10^{-12} g/ml of LPS and in some cases at 10^{-14} g/ml of LPS. In comparison, alveolar macrophages were much less sensitive. In no case was release of IL-1 by alveolar macrophages detectable at concentrations of LPS below 10^{-9} g/ml (data not shown). Thus the blood monocytes were at least 1,000 times more sensitive to LPS than were the alveolar macrophages.

The quantitative comparison of IL-1 release was performed on the paired blood monocytes and alveolar macrophages of 16 normal individuals at a standard LPS dose of 10^{-5} g/ml. For each individual, on a per cell basis, blood monocytes invariably released more IL-1 than did their paired alveolar macrophages (Fig. 2 *B*). On the average, blood monocytes released 128±21 U/10^6 cells·24 h and alveolar macrophages released 26±11 U/10^6 cells·24 h ($P < 0.001$). Two of the individuals studied were current smokers and their results did not differ from those of the group as a whole. Alveolar macrophages from eight of the individuals released no detectable IL-1 activity, but IL-1 was released in every instance from the blood monocytes.

To evaluate the possibility that inhibitors produced by the alveolar macrophages or blood monocytes interfered with the ability of the mouse thymocytes to respond to IL-1, alveolar macrophages and blood monocytes were stimulated with endotoxin and cultured in the presence of indomethacin (Table I). Culturing alveolar macrophages in the presence of indomethacin did not enhance measurable IL-1 activity. Culturing blood monocytes in the presence of indomethacin likewise had no significant effect on IL-1 activity. Furthermore, dialysis of the supernatants before assay was also ineffective in significantly altering the detectable IL-1 activity in either the alveolar macrophage or monocyte samples.

To exclude the possibility that alveolar macrophages may produce an inhibitor that is not dialyzable and whose synthesis is not blocked by indomethacin, or the possibility that alveolar macrophages may absorb or degrade IL-1 present in the culture medium, alveolar macrophages were co-cultured with blood monocytes during LPS stimulation. Culture of monocytes in the presence of alveolar macrophages at a ratio as high as 1:1 did not reduce the apparent IL-1 activity produced by stimulated monocytes (Table I).

To insure that the observed differences in IL-1 release by alveolar macrophages and blood monocytes were not peculiar to the mouse thymocyte assay, the supernatants of alveolar macrophages and blood monocytes were also evaluated for IL-1 activity using a second assay in which human T cells

Table I. Evaluation of the Possible Role of Inhibitors Modulating the Measurement of IL-1 Released by Macrophages and Monocytes

Condition	IL-1
	(U/10⁶ cells·24 h)*
Alveolar macrophage supernatants	6.4±0.8
Macrophages cultured with indomethacin‡	6.5±0.9
Supernatants dialyzed before assay§	7.4±0.9
Blood monocytes supernatants	53±3.0
Monocytes cultured with indomethacin‡	66±8.4
Supernatants dialyzed before assay§	63±3.2
Monocytes co-cultured with alveolar macrophages‖	
+1.25 × 10^5 macrophages	47±2.0
+2.5 × 10^5 macrophages	52±3.0
+5.0 × 10^5 macrophages	53±5.0
+10.0 × 10^5 macrophages	54±3.0

* Amount of IL-1 released into medium in response to LPS (10 μg/ml).

‡ Cells incubated with indomethacin (10^{-6} M) at time of stimulation with LPS.

§ Supernatants were dialyzed against 1,000 vol of RPMI 1640.

‖ Monocytes cultured at 10^6 cells/ml were incubated with increasing numbers of autologous alveolar macrophages.

served as the responding cell. IL-1 activity in supernatant fluid from cultures of LPS-stimulated blood monocytes and alveolar macrophages from three normal individuals were assayed using the human T lymphocyte assay. The results obtained using the human T lymphocyte assay closely paralleled results obtained using the mouse thymocyte assay. In each instance, the monocyte supernatant contained more activity than the macrophage supernatant ($P < 0.05$, data not shown).

Finally, to demonstrate that the observed IL-1 activity was not due to contaminating IL-2, samples were also tested for their ability to promote proliferation of the IL-2-dependent CT-6 T lymphocyte cell line. In no instance did alveolar macrophage or blood monocyte supernatants contain detectable IL-2 activity.

Comparison of the biochemical properties of alveolar macrophage and blood monocyte IL-1. By several biochemical criteria, human blood monocyte and alveolar macrophage IL-1 were similar. IL-1 activity from blood monocytes (10^9 cells) and alveolar macrophages (10^9 cells) eluted from DEAE-52 at the same ionic strength (Fig. 3). Furthermore, samples taken from the peak fractions of the DEAE column demonstrated that both monocyte and macrophage IL-1 activity eluted on Sephadex G-100, just preceding the cytochrome *c* marker corresponding to an estimated molecular weight of 13,000 (Fig. 4). In addition, the isoelectric points for monocyte and alveolar-macrophage IL-1 activity were very similar (Fig. 5), with both monocyte and macrophage IL-1 focusing consistently at an isoelectric point of ~6.8 and a somewhat more heterogeneous activity focusing at an isoelectric point between 5.2 and 5.4.

Although the IL-1 from the two cell types was biochemically indistinguishable, a semiquantitative comparison of the net IL-1 activity, obtained by DEAE-52 anion exchange followed by Sephadex G-100 molecular sieving, demonstrated a significantly better yield from blood monocytes. For example, a 1:100 dilution of the blood monocyte DEAE-52 fraction produced activity similar to that of a 1:10 dilution of the alveolar macrophage DEAE-52 fractions. Similarly, after equal

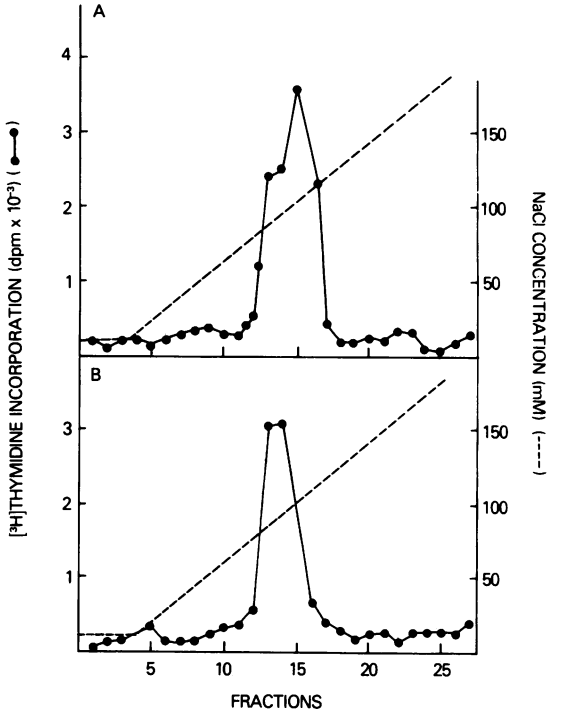

Figure 3. Comparison of DEAE-cellulose anion exchange chromatography of IL-1 released by human monocytes and alveolar macrophages. Supernatants from LPS-stimulated monocytes (10^9 cells) and LPS-stimulated alveolar macrophages (10^9 cells) were chromatographed on DEAE-cellulose using a 10–300 mM NaCl gradient. (*A*) The elution pattern for blood monocyte IL-1 (fractions diluted 1:100). (*B*) Elution pattern for alveolar macrophage IL-1 (fractions diluted 1:10). The dashed lines represent the NaCl gradient.

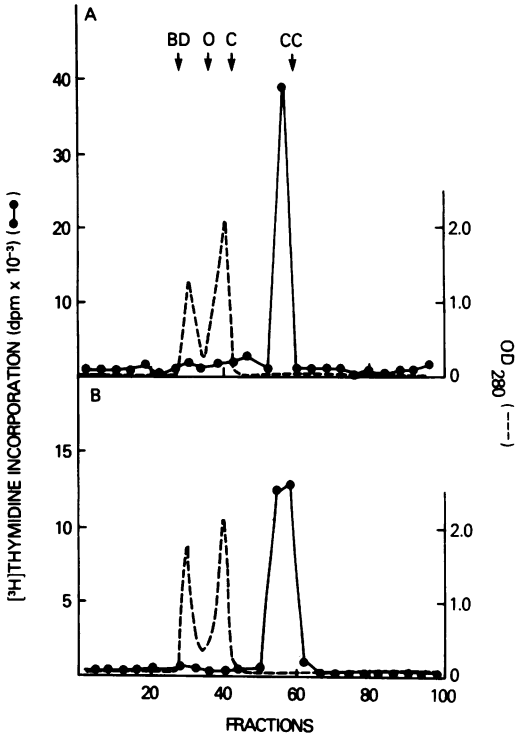

Figure 4. Comparison of Sephadex G-100 chromatography of DEAE-cellulose partially purified IL-1 released by blood monocytes and alveolar macrophages. Peak fractions (10 ml), each from the DEAE-cellulose column (Fig. 3), were concentrated to 1 ml and chromatographed by Sephadex G-100 gel filtration. (*A*) Blood monocytes (fractions diluted 1:10). (*B*) Alveolar macrophages (fractions diluted 1:10). Standards (arrows) include: blue dextran (BD, 2×10^6 D), and cytochrome *c* (CC, 12,000 D). The dashed lines represent the optical density of eluted fractions at 280 nm.

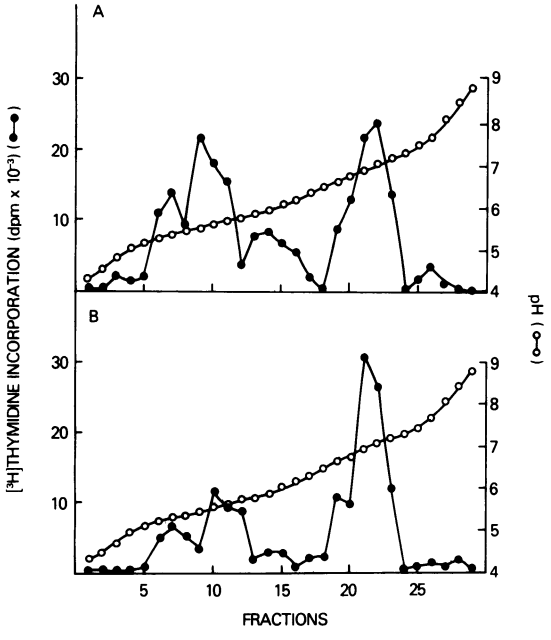

Figure 5. Comparison of the isoelectric focusing profile of IL-1 released by monocytes and alveolar macrophages. Supernatants from LPS-stimulated monocytes and alveolar macrophages were dialyzed into 1% glycine and focused for 15 h at 8 W constant power. IL-1 activity was measured after dialysis. (*A*) Blood monocytes. (*B*) Alveolar macrophages.

volumes from the DEAE-52 peaks were individually applied to Sephadex G-100 sieving, a 1:10 dilution of the fractions demonstrated a significantly better yield from the monocyte preparation.

Monocyte maturation and IL-1 release. Monocytes stimulated with LPS from time 0 until day 1 released large amounts of IL-1 activity (74 U/10^6 cells · 24 h; Fig. 6 *A*). However, monocytes maintained in culture for 1 d before being stimulated with LPS released <5 U/10^6 cells · 24 h, and those maintained for 48 h no longer could be stimulated to release detectable IL-1. In marked contrast, these same monocytes maintained the ability to phagocytize carbonyl iron particles (Fig. 6 *B*), and by day 11 in culture were secreting large amounts of fibronectin (Fig. 6 *C*). Furthermore, while the cultured monocytes could not produce detectable IL-1, the sodium dodecyl sulfate-polyacrylamide gel electrophoresis pattern of the [^{35}S]-methionine-labeled newly synthesized and secreted proteins suggested that these in vitro matured monocytes were capable of protein synthesis and secretion, and that some of the newly synthesized and secreted proteins at 11 d of maturation were different than those synthesized and secreted by day 1 (Fig. 6 *D*).

Analysis of IL-1-like activity from tissue macrophages other than alveolar macrophages. To compare the observed alveolar macrophage release of IL-1 to other tissue macrophage sources, thymocyte-mitogenic activity generated by normal human breast milk macrophages from five individuals and normal human peritoneal macrophages from one individual were also studied. Of the crude supernatants evaluated from breast milk macrophages cultured in the presence of 10^{-5} g LPS/ml, three released no detectable thymocyte mitogenic activity at 24 h, while the remaining two released 11 and 13 U, respectively. The one source of peritoneal macrophages released 53 U/10^6 cells · 24 h. Although we do not have detailed biochemical confirmation that this activity resembles IL-1, nor have we evaluated these sources for the production of inhibitors to thymocyte mitogenesis, these preliminary results from other nonelicited sources of normal human tissue macrophages parallel the results with alveolar macrophages.

Discussion

IL-1 is a mononuclear phagocyte-derived protein that augments T-lymphocyte replication, enhances B-lymphocyte immunoglobulin secretion, initiates the febrile response, activates neutrophils, stimulates prostaglandin E_2 production by several cell types, triggers the release of acute phase reactants such as serum amyloid A, and augments skin fibroblast growth (1–18). The present study demonstrates that blood monocytes and alveolar macrophages can release IL-1 and that the molecules released are biochemically indistinguishable. However, there is a marked difference in the amounts of IL-1 these two cell types release. Importantly, in response to the same standard stimulus, on a per cell basis, alveolar macrophages release about fivefold less IL-1 than do autologous blood monocytes. Furthermore, blood monocytes matured in vitro rapidly lose the ability to release IL-1. This maturational change parallels the differences observed between blood monocytes and alveolar macrophages and suggests that relative to its precursor cell, the alveolar macrophage has a limited ability to release IL-1.

IL-1 release by human alveolar macrophages. When stimulated, normal human alveolar macrophages release IL-1 with properties very similar to the IL-1 released by human blood monocytes. The human alveolar macrophage and blood monocyte IL-1 demonstrated similar chromatographic behavior on ion-exchange chromatography, gel filtration, and isoelectric focusing. Furthermore, these properties are generally similar to the known properties of IL-1 released by human blood monocytes, human monocytic leukemia cells, human placental mononuclear phagocytes, transformed human lines, rabbit alveolar macrophages, and transformed murine macrophages (34–40). In addition to sharing biochemical properties with human blood monocyte IL-1, human alveolar macrophage IL-1 demonstrates similar biologic behavior in its ability to stimulate the proliferation of mouse thymocytes and highly purified human blood T cells. Thus, while differences may

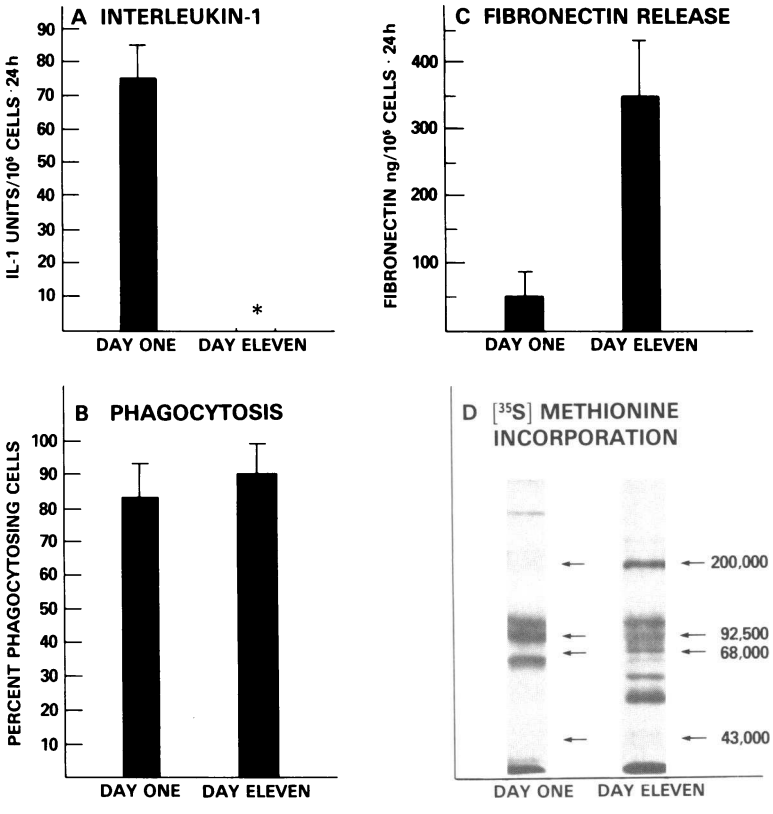

Figure 6. Effect of in vitro maturation on blood monocyte release of IL-1 compared with other monocyte functions. Blood monocytes were cultured from 1 to 11 d and stimulated with LPS (10 μg/ml) for 24 h before harvesting the supernatant. Shown are the results for day 1 and day 11 for (*A*) IL-1 release; *, no detectable IL-1; (*B*) phagocytosis of carbonyl iron particles; (*C*) fibronectin release; and (*D*) SDS-polyacrylamide gel electrophoresis of [^{35}S]methionine incorporated and released protein.

appear when the alveolar macrophage-derived IL-1 is purified to homogeneity and subsequently analyzed, the present data suggest that such differences, if any, will be minor.

Relative capacity of human alveolar macrophages to release IL-1. Strikingly, although the IL-1 of blood monocytes and alveolar macrophages is functionally and biochemically similar, the alveolar macrophage is clearly limited in its capacity to release this macromolecule. Several findings suggest this is an inherent property of the alveolar macrophage, and not a result of the in vitro conditions or the detection systems used. First, although individuals varied in the amount of IL-1 released by their monocytes and alveolar macrophages, in each case their monocytes produced more than did the alveolar macrophages for the same stimulus. Second, not only did the alveolar macrophages release less IL-1 than did the monocytes, but the alveolar macrophages were also less sensitive to a standard stimulus than the blood monocytes. Third, culturing alveolar macrophages in the presence of indomethacin, dialyzing macrophage supernatants, and subjecting macrophage supernatants to gel filtration did not unmask previously inhibited IL-1 activity, nor did co-culturing alveolar macrophages with blood monocytes inhibit blood-monocyte release of IL-1 activity. Fourth, the difference in the relative amount of IL-1 released by monocytes and alveolar macrophages was similar whether an assay system using mouse thymocytes or human T-cells as responder cells was used. Fifth, the measured IL-1 activity was not an artefact of IL-2 contamination, since the IL-2 assay failed to detect IL-2 activity. Sixth, the relative differences between the amount of IL-1 released by monocytes and alveolar macrophages were noted in response to stimuli other than LPS, including immune complexes, zymosan, and heat-killed bacteria, demonstrating that this difference is not stimulus specific.

Additional support for the concept that lung-mononuclear phagocytes differ from blood monocytes in their ability to release IL-1 can be inferred from the work by Atkins et al., demonstrating that mononuclear cells were sources of endogenous pyrogen (i.e., likely IL-1) (41). When equivalent numbers of mononuclear cells were compared, rabbit blood mononuclear cells released significantly more endogenous pyrogen than rabbit lung mononuclear cells, suggesting that (*a*) rabbit alveolar macrophages release less IL-1 than rabbit blood monocytes in response to a standard stimulus, and (*b*) a pyrogen assay system also detects the difference in the relative amount of IL-1 released by lung- and blood-mononuclear phagocytes.

One hypothesis to explain this difference between the capacity of blood monocytes and alveolar macrophages to release IL-1 is that monocytes lose the ability to release significant amounts of IL-1 as they mature into alveolar macrophages. In this context, since alveolar macrophages are

a part of the mononuclear phagocyte system, and since the blood monocyte is the precursor of the alveolar macrophage (42), one model of mononuclear phagocyte differentiation is to maintain the monocytes in culture for several days. Under these conditions, the monocytes spread out and take on many of the morphologic and functional characteristics of alveolar macrophages (43–46). If this model is accepted as a reasonable parallel of the in vivo situation, then further support for the concept that alveolar macrophages have a limited capacity to release IL-1 comes from the data presented here, as well as in previous reports (11, 46) that blood monocytes maintained in culture rapidly lose their ability to release IL-1 in response to a standard stimulus. This observation is even more striking in the context of the findings that these cells remain viable, can phagocytose, continue to synthesize protein, and secrete more fibronectin than freshly cultured blood monocytes.

That this limited release of IL-1 by alveolar macrophages and in vitro matured macrophages may also be characteristic of other mature tissue macrophages is implied by the results obtained from peritoneal macrophages and breast milk macrophages. However, given the limited number of subjects evaluated, we are reluctant to generalize the results with alveolar macrophages to all tissue macrophages.

Why alveolar macrophages are relatively limited in their ability to release IL-1. One explanation, implied by the in vitro monocyte maturational studies, is that since the ability to release IL-1 decreases significantly with mononuclear phagocyte maturation, the IL-1 detected in the alveolar macrophage cultures is actually being produced primarily by the young, newly recruited cells that have not yet differentiated fully into mature alveolar macrophages. In this context, the limited release of IL-1 by resident alveolar macrophages may simply reflect a limited number of recently recruited mononuclear phagocytes. Indirect evidence in support of this concept is provided by studies demonstrating that BCG administration will cause a rapid influx of circulating monocytes into the lungs (42) and that BCG administration can induce an alveolar macrophage population that is enhanced in its ability to release IL-1 (38). Therefore, if indeed young mononuclear phagocytes are the prime source of IL-1, it may be that IL-1 release in the lung is regulated, at least in part, by factors that control the influx of young monocytes.

Against this concept, however, are studies in a murine system demonstrating no correlation between the percentage of peroxidase-positive peritoneal mononuclear phagocytes (i.e., younger mononuclear phagocytes) and the ability of these cells to release IL-1 (47). In addition, Whisler et al. (12) have shown that human monocyte subsets, as defined by the presence or absence of complement receptors, differ 4–12-fold in their ability to release IL-1. Thus, a second explanation for the alveolar-macrophage limitation in IL-1 release (albeit not entirely separate from the maturational concept) is that IL-1 is released only by a subpopulation of mononuclear phagocytes that are normally underrepresented in the lung.

Another explanation for the observed limitation in alveolar macrophage IL-1 release is that the lung cells obtained by bronchoalveolar lavage may not represent the total alveolar macrophage population. That is, maybe lavageable alveolar macrophages are a select group of mononuclear phagocytes that have either already been stimulated to release IL-1 and hence have repressed their IL-1 release, or they are simply senescent cells with depressed overall metabolic function. This is a methodological problem inherent to all bronchoalveolar lavage studies.

A final explanation of why alveolar macrophages are limited in their ability to release IL-1 may be found in the marked differences in sensitivity to LPS between alveolar macrophages and blood monocytes. Whereas blood monocytes invariably responded to 10^{-12} g LPS/ml, alveolar macrophages did not respond to amounts $<10^{-9}$ g LPS/ml. Therefore, alveolar macrophages may not sense potentially immunogenic material in the same way as do blood monocytes, and are hence better suited for an environment of continual antigenic loading.

Interestingly, the use of LPS to demonstrate the relative inability of alveolar macrophages to produce IL-1, compared with blood monocytes, pointed out the high sensitivity of blood monocytes to endotoxin. Detecting 10^{-12} g LPS/ml far exceeds the sensitivity of the standard limulus amebocyte-lysate gelation assay. Duff and Atkins (26) compared the sensitivity to LPS of blood monocyte-endogenous pyrogen production and limulus amebocyte-lysate gelation, and found monocyte-endogenous pyrogen production to be 10-fold more sensitive to LPS. Since endogenous pyrogen and IL-1 are indistinguishable, and since the mouse thymocyte is 10^3–10^4 times more sensitive to IL-1-endogenous pyrogen than is the rabbit pyrogen assay (48), it is reasonable that the mouse thymocyte assay can detect 10^5-fold lower amounts of endotoxin than standard limulus amebocyte lysate gelatin (using fresh-blood monocytes as endotoxin detectors and thymocyte-mitogenic activity as the response).

Consequences of the relative impotence of alveolar macrophages as producers of IL-1. Although the release of IL-1 by mononuclear phagocytes is thought to play a central role in a number of immune and inflammatory processes (1–18), in the context of the lung, the most important local effects are probably those related to mononuclear phagocyte-mediated responses to antigens. In this regard, the fact that alveolar macrophages have a limited capacity to release IL-1 may be responsible, at least in part, for the relatively subdued response of the pulmonary immune system to antigen stimulation compared with blood, i.e., IL-1 may play a rate-limiting role in the ability of the antigens that reach the lung to induce T lymphocyte proliferation and immunoglobulin production. Since the lung is one of the sites in the body where there is high antigen load, this may be one mechanism that limits the immune response. However, when the population of alveolar macrophages changes, as occurs in many chronic inflammatory

lung disorders, it is possible that the immune response within the lower respiratory tract may be significantly altered.

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