Alveolar Macrophage-derived Cytokines Induce Monocyte Chemoattractant Protein-1 Expression from Human Pulmonary Type II-like Epithelial Cells*

Theodore J. Standiford, Steven L. Kunkel†, Sem H. Phan‡, Barrett J. Rollins§, and Robert M. Strieter¶

From the †Departments of Internal Medicine and Pathology, Division of Pulmonary and Critical Care Medicine, The University of Michigan Medical School, Ann Arbor, Michigan 48109-0360 and the ¶Department of Medicine, Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts 02115

Many acute and chronic lung diseases are characterized by the presence of increased numbers of activated macrophages. These macrophages are derived predominantly from newly recruited peripheral blood monocytes and may play a role in the amplification and perpetuation of an initial lung insult. The process of inflammatory cell recruitment is poorly understood, although the expression of inflammatory cell-specific chemoattractants and subsequent generation of chemoattractant gradients is likely involved. Although immune cells such as macrophages and lymphocytes are known to generate several inflammatory cell chemoattractants, parenchymal cells can also synthesize and secrete a number of bioactive factors. We now demonstrate the generation of significant monocyte chemoattractant activity from tumor necrosis factor (TNF)-α and interleukin (IL)-1β-treated pulmonary type II-like epithelial cells (A549). The predominant inducible monocyte chemotaxin had an estimated molecular mass of approximately 14–15 kDa and was neutralized by specific antibody to human monocyte chemotactic protein-1 (MCP-1). Induction of activity was accompanied by increases in steady-state mRNA level for MCP-1. These data are consistent with the induction of MCP-1 expression from A549 cells by TNF and IL-1. MCP-1 production from A549 cells could be induced by lipopolysaccharide (LPS)-stimulated alveolar macrophage (AM)-conditioned media, but not by LPS alone. The inducing activity in AM-conditioned media was neutralized with specific antibodies to IL-1β, but not TNF-α. Our findings suggest that the alveolar epithelium can participate in inflammatory cell recruitment via the production of MCP-1 and that cytokine networking between contiguous alveolar macrophages and the pulmonary epithelium may be essential for parenchymal cell MCP-1 expression.

The recruitment of peripheral blood monocytes to the lung is essential for normal lung immune function, as well as in the generation and evolution of an inflammatory response to pulmonary injury. The alveolar macrophage, an important phagocyte of the pulmonary airspace and interstitium, is derived predominantly from differentiated peripheral blood monocytes and to a limited extent from local macrophage replication (1–3). Hence, ongoing monocyte recruitment is required to maintain the steady-state pool of alveolar and interstitial macrophages. During acute and chronic inflammation, the process of monocyte elicitation is accelerated resulting in transient or more prolonged increases in lung monocyte/macrophages (4, 5). Although elicited monocytes serve a vital role in the host defense against a number of bacterial, fungal, and protozoan organisms, the presence of increased numbers of activated monocyte/macrophages can lead to excessive tissue injury via the overzealous elaboration of inflammatory cytokines, proteolytic enzymes, and oxygen radicals (6, 7). This exaggerated monocyte/macrophage response is characteristic of several pulmonary disease states, including sarcoidosis (8, 9), idiopathic pulmonary fibrosis (10), and drug-induced lung injury (11, 12). The mediators involved and the cellular sources of the mediators that orchestrate pulmonary monocyte elicitation and activation in health and in disease remain poorly characterized.

The alveolar macrophage, in addition to its role in recognition and clearance of foreign substances, also functions as an important immune effector cell of the airspace via the generation of a vast array of bioactive mediators (13, 14). In addition to the production of inflammatory cell chemotaxins, alveolar macrophages secrete tumor necrosis factor (TNF)† (15) and interleukin-1 (IL-1) (16), cytokines which play a central role in mediating local pulmonary and systemic pathophysiology (17). Although numerous investigations have focused on the alveolar macrophage as the primary immune effector cell of the lung, it has only recently been recognized that non-immune parenchymal cells possess the ability to participate in immune responses. Cellular constituents of the alveolar capillary membrane, including endothelial cells and fibroblasts, have been shown to express several neutrophil and monocyte chemotaxins, including interleukin-8 (18, 19).

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† RJR Nabisco Research Scholar. To whom all correspondence and reprint requests should be addressed: Dept. of Internal Medicine, Division of Pulmonary and Critical Care, Box 0360, The University of Michigan Medical School, 3916 Taubman Ctr., Ann Arbor, MI 48109-0360; Tel: 313-763-6458; Fax: 313-764-2397.

¶ The abbreviations used are: TNF, tumor necrosis factor; IL-1, interleukin 1; IL-8, interleukin 8; MCP-1, monocyte chemotactic protein-1; LPS, lipopolysaccharide; TGF-β, transforming growth factor-β; LTB4, leukotriene B4; AM, alveolar macrophages; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC, high pressure liquid chromatography; FMLP, formylmethionyleucylphenylalanine; HBSS, Hanks' balanced salt solution; HPF, high powered field.
and the novel monocyte-specific cytokine monocyte chemoattractant protein-1 (MCP-1) (20-23).

MCP-1 is a 76-amino acid peptide initially isolated and cloned from the human THP-1 myelomonocytic and glioma cell lines (24-27). In addition to inducing monocyte chemotaxis, MCP-1 also activates monocytes, causing augmented cytosolic activity of monocytes against several tumor cell lines, stimulation of the respiratory burst, and inducing the release of lyosomal enzymes (27, 28). MCP-1 exists in two forms, MCP-1α, with a molecular mass of 13 kDa, and MCP-1β, with a molecular mass of 15 kDa (24, 28). These two forms of MCP-1 are derived from the same transcriptional product and are functionally identical, differing only by the extent of post-translational modifications. MCP-1 is produced by a variety of immune and non-immune cells, including blood mononuclear cells (24), endothelial cells (20, 21), fibroblasts (22, 23, 29), smooth muscle cells (30), epithelial cells, and several tumor cell lines (27, 30). Although endothelial cells synthesize MCP-1 in response to an exogenous stimulus such as lipopolysaccharide (20, 21), fibroblasts (20, 22) and epithelial cells require a host-generated signal (e.g. TNF or IL-1) before MCP-1 expression can occur. Immune cells (e.g. monocytes and tissue macrophages, do not express MCP-1 in response to LPS (32). These cells, however, may be capable of the paracrine induction of surrounding non-immune cell-derived MCP-1. Hence, cellular communication between immune and non-immune cells may be essential for the full expression of immune and non-immune cell-derived chemoattractants.

We now demonstrate the gene expression and synthesis of MCP-1 from type II-like pulmonary epithelial cells. MCP-1 production is induced by the macrophage-derived mediators TNF and IL-1, but not LPS. Monocyte/macrophages appear to be a likely in vivo source of these cytokines, as stimulated AM-conditioned media can induce the expression of MCP-1 from these cells. The effects of AM-conditioned media are mediated predominantly via the paracrine effects of TNF and IL-1, as this response is significantly abrogated by neutralization of these monokines. Our findings demonstrate that the alveolar epithelium can participate in inflammatory cell recruitment via the production of MCP-1 and that cytokine networking between alveolar macrophages and the pulmonary epithelium may be essential for induction of this response.

MATERIALS AND METHODS

Reagent Preparation—Human recombinant IL-1β, with a specific activity of 30 units/mg, and murine anti-human monoclonal IL-1β antibodies were the generous gift of the Upjohn Co. Human recombinant TNF-α with a specific activity of 22 units/mg was kindly provided by the Cetus Corp. Polyclonal anti-human recombinant TNF was produced by immunization of rabbits with recombinant TNF-α in multiple intradermal sites with complete Freund’s adjuvant. Polyclonal anti-human TNF utilized in this study was capable of neutralizing 10 ng of TNF, whereas the remainder were unstimulated. We used the A549 cell line in our studies because human type II cells are difficult to obtain in pure culture and are often contaminated with resident mononuclear phagocytic cells. Human A549 pulmonary epithelial cells were grown to confluence on 100-mm petri dishes (Corning Glass Works, Corning, NY) in complete RPMI 1640 plus 10% fetal calf serum. On the day of use, A549 monolayers were washed free of fetal calf serum in complete media and either cytokines or LPS was added for the specified times and doses. Cell-free supernatants were collected, and total cellular RNA was extracted as described below.

Preparation of A549 Pulmonary Epithelial Cells—A549 pulmonary epithelial cells (American Type Culture Collection, Rockville, MD) are a cell line derived from a patient with alveolar cell carcinoma of the lung. These cells retain features of type II alveolar epithelial cells, including cytoplasmic multilamellar inclusion bodies and the synthetic property of surfactant (33). We used the A549 line in our studies because human type II cells are difficult to obtain in pure culture and are often contaminated with resident mononuclear phagocytic cells. Human A549 pulmonary epithelial cells were grown to confluency on 100-mm petri dishes (Corning Glass Works, Corning, NY) in complete RPMI 1640 plus 10% fetal calf serum. On the day of use, A549 monolayers were washed two additional times with phosphate-buffered saline (GIBCO) at a concentration of 3 × 10^5 AM/plate, adherence-purified, and then washed two additional times. LPS at a concentration of 10 μg/ml was added to some plates, whereas the remainder were unstimulated.

Conditioned media was collected after a 24-h incubation period at 37 °C. Twenty-four-hour AM-conditioned media (LPS-stimulated and unstimulated) was placed over A549 monolayers and RNA isolated at 4 h as described below.

Northern Blot Analysis—Total cellular RNA from A549 cells was isolated using a modification of Chirgwin et al. (34) and Jonas et al. (35). Briefly, A549 monolayers were scraped into a solution of 25 mM Tris, pH 8.0, containing 4.2 M guanidine isothiocyanate, 0.5% sarcosyl, and 0.1 M mercaptoethanol. After homogenization, the tissue supernatant was added per plate and cell pellets were washed twice with 10 mM EDTA and 1.0% sodium dodecyl sulfate. The mixture was then extracted with chloroform-phenol to chloroform-isooamyl alcohol. The RNA was alcohol-precipitated, and the pellet dissolved in 10 mM Tris EDTA to pH 8.0, containing 10 mM EDTA and 1.0% sodium dodecyl sulfate. The mixture was then extracted with chloroform-phenol and chloroform-isooamyl alcohol. The RNA was alcohol-precipitated and the pellet dissolved in 10 mM Tris EDTA to pH 8.0, containing 10 mM EDTA and 1.0% sodium dodecyl sulfate. The mixture was then extracted with chloroform-phenol and chloroform-

High Pressure Liquid Chromatography—Molecular mass estimates of monocyte chemotactic components of A549 conditioned media were made by high pressure liquid chromatography (HPLC) using a TSK 3000SW gel filtration column (1 × 45 cm) (Varian Instruments, Palo Alto, CA) fitted with a guard column. 15-ml aliquots of A549-conditioned media were dialyzed against 20 mM sodium phosphate buffer, pH 7.4, containing 0.1 M sodium chloride and analyzed by HPLC gel filtration performed, and isocratic elution undertaken with phosphate-buffered saline at a flow rate of 0.8 ml/min. Detection was at 220 nm; 1-min fractions were collected and assessed for chemotactic activity.

Monocyte Chemotactic Assay—Human mononuclear cells were prepared from peripheral blood by Ficoll-Hypaque density gradient centrifugation and then suspended in Hank’s balanced salt solution (HBSS) with calcium/magnesium (GIBCO) at a concentration of 3

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**Notes:**

E. E. E., S. G., Strier, R. M., Elner, V. M., Rollins, B. J., Del Monte, M. A., and Kunkel, S. L. (1991) *Lab. Inves.*, in press.
ponents of A549-conditioned Media—To characterize the mo-
lecular mass of those components in monokine-treated A549-
conditioned media responsible for monocyte chemotactic ac-
tivity, we performed HPLC gel filtration on 24-h untreated,
TNF- and IL-1β-treated A549-conditioned media and as-
essed elution fractions for monocyte chemotactic activity. As
depicted in Fig. 2A, 24-h untreated conditioned media pro-
duced several small peaks of monocyte chemotactic activity
corresponding to molecular masses of 65, 28, and 13 kDa, and
less than 1,000 kDa. Similar peaks of chemotactic activity
were observed in IL-1β-treated conditioned media. In addi-
tion, however, a major peak of substantial monocyte chemo-
tactic activity was seen at fractions 37–39, corresponding to
an estimated molecular mass of 14,000–15,000. When consti-
tutive chemotactic activity (24-h control media) was sub-
tracted from IL-1β-stimulated A549 conditioned media, one
major inducible peak of chemotactic activity at 14–15 kDa,
whereas a smaller peak was observed at a molecular mass of
28 kDa. Elution fractions obtained by HPLC of TNF-treated
A549 conditioned media (Fig. 2B) revealed a major peak of
monocyte chemotactic activity from fractions 37–42, again
responding to an estimated molecular mass of 14–16 kDa.
To determine the dilution-effect of eluted fractions 33–41,
serial dilutions from 1/2 to 1/32 of IL-1β-treated conditioned
media eluted fractions 33–41 were made and then assessed
for chemotactic activity. Fraction 37 at a dilution of 1/2
possessed the greatest activity, with enhanced chemotactic
activity also seen in fractions 33, 38, and 39 at 1/16 and 1/32
dilutions (data not shown).

**RESULTS**

Generation of A549 Cell-derived Monocyte Chemotactic Ac-
tivity—Confluent A549 epithelial cell monolayers were stim-
ulated at time zero with either LPS, 10 µg/ml, TNF, 20 ng/
ml, or IL-1β, 20 ng/ml and assessed at specific time intervals
for the generation of supernatant monocyte chemotactic ac-
tivity. As shown in Fig. 1, treatment of these cells with TNF
resulted in the production of substantial chemotactic activity
that was seen as early as 1 h, plateaued by 8 h, with activity
further increased at 24-h post stimulation (100 ± 10% of
fMLP response). In a similar fashion, IL-1β-treated cells
generated an early rise in monocyte chemotactic activity that
plateaued by 8 h and was maximal 24-h post-stimulation (87
± 4.7% of fMLP response). In contrast, LPS-stimulated cells
generated only modest monocyte chemotactic activity at 24 h
(43 ± 4.1% of fMLP response) that was not different from
24-h unstimulated control (35 ± 2.5% of fMLP response, p =
0.7). Neither LPS, TNF, nor IL-1β were directly chemotactic
in these assays.

Determination of Monocyte Chemotactic Activity of the Com-
ponents of A549-conditioned Media—To characterize the mo-
lecular mass of those components in monokine-treated A549-
conditioned media responsible for monocyte chemotactic ac-
tivity, we performed HPLC gel filtration on 24-h untreated,
TNF- and IL-1β-treated A549-conditioned media and as-
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**Demonstration of the Time-dependent Expression of MCP-1 mRNA from A549 Epithelial Cells**—To establish whether the 14–15-kDa molecular mass monocyte chemotactic factor

![Fig. 1. Monocyte chemotactic activity produced by monokine-stimulated A549 epithelial cells. Time-dependent increase in monocyte chemotactic activity by TNF and IL-1β-stimulated A549 cell-conditioned media. Monocyte chemotaxis induced by unstimulated and LPS-stimulated A549-conditioned media at 24 h is also included. Chemotactic activity is expressed on the vertical axis as percent of fMLP control (10^-8 M). Values shown represent the mean ± S.E. of three individual experiments.](image-url)
MCP-1 Expression from Type II-like Human Pulmonary Epithelial Cells

might represent the recently isolated and cloned peptide MCP-1, we performed Northern blot analysis looking for evidence of inducible MCP-1 gene expression. A549 monolayers were treated at time zero with LPS 10 μg/ml, TNF 20 ng/ml, or IL-1β 20 ng/ml and at specific time intervals total RNA was extracted (n = 4). As depicted in Fig. 3, TNF induced the expression of MCP-1 mRNA by 2 h (41.9 ± 14.2% maximum), with peak expression at 8 h (84 ± 16% maximum), and continued detectable steady-state levels of MCP-1 mRNA 24-h post-stimulation (69 ± 16.8% maximum). Likewise, IL-1β-treated cells generated significant levels of MCP-1 mRNA by 2 h (43.1 ± 2.9% maximum), peak expression between 4 h (79.9 ± 0.87%) and 8 h (58.9 ± 13.1%), decreasing to 35.4 ± 6.3% by 24-h post-treatment. LPS, however, failed to induce MCP-1 mRNA from pulmonary epithelial cells at any of the time points assessed (data not shown).

Dose-dependent Expression of MCP-1 mRNA by A549 Epithelial Cells—To determine if monokine-induced MCP-1 gene expression was dose-dependent, confluent A549 monolayers were stimulated with graded doses of LPS (1 ng to 10 μg/ml), TNF (20 pg to 20 ng/ml), or IL-1β (20 pg to 20 ng/ml) and total RNA extracted 4-h post-stimulation (Fig. 4). In each of three experiments, the threshold concentration of TNF or IL-1β to induce significant levels of MCP-1 mRNA was 2 and 0.2 ng/ml, respectively. Maximal steady-state mRNA levels were observed at 20 ng/ml of TNF and 2 ng/ml of IL-1β, whereas concentrations above these did not lead to any further increases in MCP-1 mRNA. In contrast, LPS in doses from 1 pg to 10 μg/ml failed to induce MCP-1 mRNA from epithelial cell monolayers (data not shown).

Neutralization of Inducible Monocyte Chemotactic Activity from A549-conditioned Media Fractions—Having demonstrated inducible monocyte chemotactic activity from IL-1β- and TNF-stimulated A549 cell-conditioned media corresponding to a molecular mass similar to that of MCP-1, we combined the fractions making up the major peak of activity and reassessed bioactivity in the presence and absence of neutralizing rabbit anti-human MCP-1 antiserum. As shown in Table I, anti-MCP-1 antiserum did not alter monocyte chemotaxis of IL-1β-treated eluted fractions 33-35. In contrast, when fractions 36-39 were combined with anti-MCP-1 antiserum and assessed for monocyte chemotactic activity in an undiluted state and after 1:8 dilution, there was a highly significant reduction in monocyte chemotaxis by 69 and 84%, respectively. The anti-human MCP-1 antiserum used in this study reduced monocyte chemotaxis by 88 and 58%, respectively, and by 1 and 10 ng/ml of MCP-1β by 82 and 92%, respectively. As the minor peak of inducible chemotactic activity (fractions 33-35) was observed at a molecular mass of approximately 28 kDa, which corresponded to the molecular mass of another known monocyte chemotaxin, TGF-β (36), we performed neutralization studies with those fractions utilizing rabbit anti-

![Fig. 3. Time-dependent gene-expression of MCP-1 from monokine-stimulated A549 epithelial cells. Northern blot analysis depicting the time-dependent increase in MCP-1 mRNA from A549 condition media. A is a Northern blot representing steady-state levels of MCP-1 mRNA. B is the laser densitometry of each Northern blot and expressed on the vertical axis as the percent of maximum response. 28 and 18 S rRNA bands are shown in C. The data depicted are representative of four individual experiments.](image-url)
human TGF-β antiserum. The antiserum significantly reduced monocyte chemotactic activity induced by fractions 33–35 by 86% (p < 0.01), whereas anti-human TGF-β antiserum had no effect on the chemotactic activity induced by fractions 36–39 (data not shown). Treatment of fractions 37–40 and fraction 42 from TNF-treated A549 conditioned media with MCP-1 antiserum (1:100 dilution) resulted in an 89 and 84% reduction in chemotactic activity, respectively (data not shown).

Inhibition of Monocyte Chemotactic Activity of A549 Cell-conditioned Media by Anti-human MCP-1 Antiserum—Having demonstrated the gene expression of MCP-1 from A549 pulmonary epithelial cells, as well as the generation of a monocyte chemotaxin with a molecular mass identical to MCP-1, we then determined the proportion of A549-derived monocyte chemotactic activity that was attributable to MCP-1. 24-h unstimulated LPS, TNF, or IL-1β-treated A549 conditioned media was preincubated with control (rabbit preimmune) or rabbit anti-human MCP-1 antiserum and then assessed for chemotactic activity. MCP-1 antiserum had no effect on monocyte chemotaxis induced by fMLP 10^{-8} M, unstimulated or LPS-treated A549 cell-conditioned media (Table II). In contrast, MCP-1 antiserum caused significant abrogation of the monocyte chemotactic activity induced by TNF and IL-1β-treated A549-conditioned media (46 and 44% suppression, respectively). Maximal inhibition of monocyte chemotactic activity occurred at an antibody concentration of 1:10,000, with decreasing suppression at increasing dilutions of antibody. MCP-1 antiserum failed to inhibit chemotactic activity of cytokine-treated A549-conditioned media at dilutions of 1:10,000 and greater (data not shown).

**AM-induced MCP-1 Gene Expression from A549 Epithelial Cells**—Our previous experiments have demonstrated the gene expression and synthesis of MCP-1 from A549 pulmonary epithelial cells in response to host-generated cytokines (i.e. TNF and IL-1), but no exogenous stimuli (i.e. LPS). Because of the close proximity of the alveolar macrophage to the pulmonary epithelium within the alveolar space, we hypothesized that the alveolar macrophage may produce soluble mediators that can induce the expression of MCP-1 by pulmonary epithelial cells. Cell-free human AM-conditioned media (24-h LPS-stimulated or -unstimulated) was incubated with A549 monolayers and total RNA extracted at 4 h (n = 5). As shown in Fig. 5, LPS-stimulated AM-conditioned media induced significant expression of MCP-1 steady-state mRNA. Unstimulated AM-conditioned media also induced MCP-1 mRNA from these cells, although steady-state MCP-1 mRNA levels were only 13.4 ± 4.6% of those seen in the presence of LPS-stimulated AM-conditioned media. Control media (unconditioned) failed to induce MCP-1 mRNA. To better define the relative contributions of the specific AM-derived monokines TNF and IL-1, neutralization studies were performed (Fig. 6). LPS-stimulated AM-conditioned media was preincubated for 30 min with appropriate control antiserum and/or IgG (n = 3). In addition, AM-conditioned media was preincubated with either murine anti-human IL-1β monoclonal antibody (75 μg/ml), rabbit anti-human TNF monoclonal antiserum (1:100) or both antibody and antiserum in combination and then overlayed on A549 monolayers. Total RNA was again extracted at 4 h. As shown in Fig. 6, LPS-stimulated AM-conditioned media induced maximal MCP-1 mRNA levels from A549 cells that was reduced to 57.3 ± 15.7% maximum in the presence of IL-1β-neutralizing antibodies. Although rabbit anti-human TNF antiserum did not significantly reduce AM-conditioned media-induced MCP-1 mRNA (77.6 ± 12.2% maximum, p = 0.28), the combination of TNF and IL-1β neutralizing antibodies displayed synergistic effects in reducing A549 cell-derived MCP-1 mRNA to 27 ± 7.6% maximum (p = 0.01). Control rabbit serum or murine IgG, alone or in combination, did not alter AM-conditioned media-induced MCP-1 mRNA levels from pulmonary epithelial cells.

**TABLE II**

Neutralization of monocyte chemotactic activity with rabbit antihuman MCP-1 antiserum

Human A549 epithelial cells were treated as described, supernatants collected, and incubated with either control or immune serum for 30 min at 37°C prior to assessment of chemotactic activity. Each value represents the mean number of cells/HPF of five individual fields performed in duplicate (n = 10) minus HBSS control.

| Control serum | Immune serum | Suppression | p value |
|---------------|--------------|-------------|---------|
| (1:100)       | (1:100)      | (1:100)     |         |
| **mean**      |              |             |         |
| cells/HPF     |              |             |         |
| **(×1000)**   |              |             |         |
| Control       | 3.5 ± 0.3    | 3.1 ± 0.5   | 11      | 0.56   |
| fMLP (10^{-8} M) | 12.9 ± 0.6  | 13.5 ± 1.5  | 19      | 0.46   |
| 24°C, control | 15.1 ± 1.6   | 12.3 ± 1.9  | 9       | 0.81   |
| 24°C, LPS (1 μg/ml) | 12.1 ± 1.9  | 12.7 ± 1.1  | 46      | 0.02   |
| 24°C, TNF (20 ng/ml) | 27.5 ± 2.2  | 14.9 ± 2.5  | 44      | <0.01   |
| 24°C, IL-1 (20 ng/ml) | 30.3 ± 3.4  | 16.9 ± 2.1  |         |        |

*Data are statistically significant.

**DISCUSSION**

The migration of mononuclear phagocytes from the peripheral blood to the lung interstitium and airspace is an important event both to maintain the pool of lung phagocytes (1–3) as well as to respond to a variety of lung insults (3–12). Although the process of inflammatory cell recruitment is complex and poorly understood, it is clear that the local generation of specific immune cell chemotaxis is required. The nature and cellular sources of these chemotaxins conti-
The data depicted are representative of three individual experiments. Suppression of LPS-stimulated AM-conditioned media-induced neutralizing antibodies. Northern blot analysis depicting the epithelial cells in the presence and absence MCP-1 gene expression from pulmonary fibrosis (10). Activated lymphocytes isolated from induced by lung macrophages in response to bleomycin (11) and chemotaxin with a molecular mass of approximately 10-15 kDa (9).

Monocyte chemotactic factors have been shown to be produced by lung macrophages in response to bleomycin (11) and cyclophosphamide (12), as well as in patients with idiopathic pulmonary fibrosis (10). Activated lymphocytes isolated from the lungs of patients with sarcoidosis produce a monocyte chemotaxin with a molecular mass of approximately 10-15 kDa (9).

Whereas pulmonary immune cells produce a number of bioactive substances, the lung parenchymal and epithelial cells have generally been considered targets of inflammatory cell products rather than active participants in the immune response. More recently, however, these cells have been recognized as important effector cells in many organ-specific inflammatory responses. Bovine bronchial epithelial cells (39) and bleomycin-treated rat type II cells (40) can express inflammatory cell chemotractants. Pulmonary type II-like epithelial cells (41), as well as fibroblasts (19) and endothelial cells (18), produce the neutrophil and lymphocyte-specific chemotactic factor, IL-8. Furthermore, endothelial cells, fibroblasts, and smooth muscle cells express the monocyte-specific chemotaxin MCP-1 (20-23, 30).

We now demonstrate the gene expression of MCP-1 from monokine-stimulated type II-like pulmonary epithelial cells. MCP-1 gene expression is associated with the generation of significant inducible monocyte chemotactic activity which is due predominantly to the expression of a 14-15-kDa factor that is neutralized with anti-human MCP-1 antiserum. This data strongly supports our conclusion that A549 pulmonary epithelial cells synthesize and secrete MCP-1 and that MCP-1 is one of the major monocyte chemoattractants produced by these cells.

Although MCP-1 is one of the monocyte chemoattractants secreted from A549 epithelial cells, it is certainly not responsible for all of the chemotactic activity observed. Several lines of evidence would suggest the presence of other bioactive substances. Significant monocyte chemotactic activity was produced by unstimulated and LPS-stimulated A549 cells in the absence of MCP-1 gene expression. In addition, significant monocyte chemotactic activity persisted in the supernatants of TNF and IL-1-treated cells after incubation with neutralizing MCP-1 antiserum. Furthermore, several additional peaks of monocyte chemotactic activity were seen in the HPLC elutions corresponding to molecular masses of approximately 6, 28, and <1,000 kDa. A549 cells are known to produce a latent form of TGF-β (42), which can be activated by a number of proteolytic enzymes. The molecular mass and neutralization of this peak's chemotactic activity with anti-human TGF-β antiserum would indicate that this peak likely represents TGF-β and that this factor is inducible upon stimulation and present in its active state. The substances responsible for the chemotactic activity seen at 65 kDa and <1,000 kDa remain unknown, although the larger molecular mass fractions may represent fibronectin fragments or other matrix degradation products, whereas the smaller molecular mass fractions may represent bioactive lipid mediators. The low molecular mass substance does not appear to be LTβ or other 5-lipoxygenase products, as epithelial cells have little 5-lipoxygenase activity (43), and no detectable LTβ was detected by radioimmunoassay (data not shown). Finally, type II cells are known to synthesize and secrete complement, proteins that are chemotactic for both neutrophils and monocytes (44). Although a number of bioactive substances likely contribute to the significant constitutive production of chemotactic activity by A549 epithelial cells, MCP-1 appears to be the predominant inducible monocyte chemoattractant produced by these cells.

A549 cells produce MCP-1 in response to TNF and IL-1 but do not express this peptide in response to an exogenous stimulus such as LPS. The signal-specific synthesis of MCP is also displayed by other non-immune cells, including fibroblasts (20, 22) and other epithelial cells. Hence, the host must first recognize an exogenous stimulus (i.e. LPS) and respond by elaborating the appropriate signals to allow for nonimmune cells to participate in the inflammatory response. In contrast, endothelial cells, cells that are likely to come in direct contact with a foreign substance, can produce MCP-1 in response to either endogenous or exogenous signals (20, 21). Fibroblasts (19) and epithelial cells (41) display similar signal specificity in their expression of IL-8, as they respond to TNF and IL-1, but not LPS.

Cellular communication between immune and non-immune cells is likely an essential process in the initiation, maintenance, and resolution of an inflammatory response. Although type II cells cannot directly synthesize LTβ, these cells can release free arachidonic acid which can be synthesized by alveolar macrophages to 5-lipoxygenase products including LTβ (43). In addition, networking between neighboring cells is operative in the generation of several cytokines. Platelet-derived growth factor, a product of a number of different cell types including monocyte/macrophages (45), serves as a stimulus for the secretion of MCP-1 by human fibroblasts (23). As discussed previously, the macrophage products TNF and IL-1 are important signals for the expression of MCP and IL-8 by non-immune cells. Because of the close proximity of the alveolar macrophage and the pulmonary epithelium within
the alveolar space, the alveolar macrophage would appear to be a likely source of the endogenous signals necessary to induce the expression of MCP-1 from pulmonary epithelial cells. In this study, we demonstrate that soluble mediators produced by LPS-stimulated AMs can induce the gene expression of MCP-1 from type II-like epithelial cells, and that TNF and especially IL-1α are the major cytokines involved. Although alveolar macrophages produce platelet-derived growth factor, this factor does not appear to be an important macrophage-derived inducing agent of MCP-1 from A549 pulmonary epithelial cells. The aforementioned observations are of special importance in that we have not previously detected inducible MCP-1 gene expression from peripheral blood monocytes or alveolar macrophages. Monocyte/macrophages can, however, indirectly elicit monocytes to an area of injury by inducing MCP-1 production from surrounding cells.

A number of previous studies have utilized the human A549 epithelial cell line to assess type II pneumocyte effector cell function (36, 46–50). Whereas these investigations have generated some controversy. For example, the use of this cell line to study surfactant synthesis may not accurately reflect the production of this substance by normal pneumocytes (46–48). Thus, the use of a cell line to represent primary cell cultures must be weighed when interpreting the scientific data. As previously stated, however, these cells have proven to be useful models in studying the production and secretion of a number of bioactive factors, including complement components (49), TGF-β (36), and urokinase plasminogen activator (31). In addition, A549 cells make an attractive model of human pulmonary epithelial cell immune responses, because they are readily assessable and can be grown in pure culture without contaminating tissue macrophages, a frequent problem of techniques used to isolate primary pulmonary epithelial cells.

Our findings suggest that the pulmonary epithelium may participate in the dynamic process of monocyte recruitment via the generation of MCP-1, and the expression of this peptide appears to be dependent upon cytokine networking between macrophage/monocytes and neighboring non-immune cells. Future investigations may further define the role of MCP-1 in the process of monocyte migration and activation in health and in disease states such as pulmonary fibrosis, sarcoidosis, and other granulomatous lung diseases.

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