Inflammatory cells accumulate within the lungs of cigarette smokers. Current concepts suggest that these cells can induce protease-antiprotease and/or oxidant-antioxidant imbalance(s), which may damage the normal lung alveolar and interstitial structures. Because type II pneumocytes line the alveolar space, and because the inflammatory cells migrate and reside at the alveolus, we postulated that the type II pneumocytes might release chemotactic activity for neutrophils and monocytes in response to smoke extract. To test this hypothesis, A549 cells were cultured and the supernatant fluids were evaluated for the neutrophil and monocyte chemotactic activity (NCA and MCA) by a blind-well chamber technique. A549 cells released NCA and MCA in response to smoke extract in a dose- and time-dependent manner (P < 0.05). Checkerboard analysis showed that the activity was chemotactic. Partial characterization of NCA and MCA revealed that the activity was partly heat labile, trypsin sensitive, and ethyl acetate extractable. Lipoxigenase inhibitors and cycloheximide inhibited the release of NCA and MCA. Molecular sieve column chromatography showed multiple peaks for both NCA and MCA. NCA was inhibited by anti-human interleukin (IL)-8 antibody, granulocyte colony-stimulating factor (G-CSF) antibody, or leukotriene (LT)B4 receptor antagonist. Monocyte chemotactant protein (MCP)-1 antibody or LTB4 receptor antagonist inhibited MCA. Immunoreactive IL-8, G-CSF, MCP-1, and LTB4 significantly increased in the supernatant fluids in response to smoke extract. These data suggest that the type II pneumocytes may release NCA and MCA and modulate the inflammatory cell recruitment into the lung. 

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1903
can activate complements.\textsuperscript{20} Robbins et al have shown that smoke activates the NCA of serum and inhibits the activity of chemotactic factor inactivator.\textsuperscript{21} However, the possibility that the alveolar type II epithelial cells could interact with cigarette smoke to release the chemotactic activity remains to be elucidated.

Because neutrophils and monocytes play important roles in the pathogenesis of pulmonary emphysema and because type II epithelial cells participate in lung inflammatory responses, we hypothesized that smoke extract might stimulate type II epithelial cells to release NCA and MCA. The results demonstrate that a human alveolar epithelial-like cell line, A549 cells, released NCA and MCA in response to smoke extract, including IL-8, granulocyte colony-stimulating factor (G-CSF), MCP-1, and leukotriene (LT)B\textsubscript{4}.

Materials and Methods

Preparation of A549 Type II Alveolar Epithelial Cells

Because of difficulty in obtaining primary human type II epithelial cells of sufficient purity, A549 cells (passage 75; American Type Culture Collection, Rockville, MD), a pulmonary type II epithelial cell line derived from an individual with alveolar cell carcinoma, was used.\textsuperscript{22} These cells retain many of the characteristics of the normal type II epithelial cells, such as surfactant production, cytoplasmic multilamellar inclusion bodies, and cuboidal appearance.\textsuperscript{16} A549 cells were grown as monolayers on 100-mm-diameter tissue culture dishes. A549 cells were incubated in 100% humidity and 5% CO\textsubscript{2} at 37°C with F-12 medium (GIBCO, Grand Island, NY) supplemented with penicillin (50 U/ml; GIBCO), streptomycin (50 μg/ml; GIBCO), fungizone (2 μg/ml; GIBCO), and 10% heat-inactivated fetal calf serum (FCS; GIBCO). The cells from monolayers were harvested with trypsin (0.25%) and EDTA (0.1%) in PBS (Sigma Chemical Co., St. Louis, MO), centrifuged at low speed (250 × g for 5 minutes), and resuspended in fresh medium at the concentration of 1.0 × 10\textsuperscript{6} cells/ml in 35-mm-diameter tissue culture dishes. The cells were grown to confluence during 5 to 7 days of incubation. After the cells reached confluence, the cells were used for the experiment.

Preparation of Cigarette Smoke Extract

Smoke extract was prepared by a modification of the method of Carp and Janoff.\textsuperscript{8} Briefly, two cigarettes without filters were combusted with a modified syringe-driven apparatus. The smoke was bubbled through 50 ml of Hanks’ balanced salt solution (HBSS; GIBCO). The resulting suspension was adjusted to pH 7.4 with concentrated NaOH and then filtered through a 0.20-μm pore filter (Lida Manufacturing Corp., Kenosha, WI) to remove bacteria and large particles. The resulting smoke extract was applied to A549 cell cultures within 30 minutes of preparation.

Exposure of A549 Cells to Smoke Extract

A549 cells were washed twice with serum-free F-12, and the cells were incubated in the presence and absence of smoke extract. To determine the dose- and time-dependent release of NCA and MCA, the cultures were incubated at various concentrations of smoke extract (0%, 0.5%, 1%, 5%, and 10%) for 12, 24, 48, 72, and 96 hours at 37°C in a humidified 5% CO\textsubscript{2} atmosphere. Smoke extract did not cause A549 cell injury (no deformity of cell shape and no detachment from tissue culture dish, and greater than 95% of cells were viable by trypan blue exclusion) after 96 hours of incubation at the maximal doses. The supernatant fluids were harvested and stored at −80°C until assayed. At least six separate A549 cell supernatant fluids were harvested from cultures for each experimental condition.

Measurement of NCA and MCA

Polymorphonuclear leukocytes were purified from heparinized normal human blood by the method of Boyum.\textsuperscript{23} Briefly, 15 ml of venous blood was obtained from healthy volunteers and then sedimented with 3% dextran in isotonic saline for 45 minutes to separate white blood cells from red blood cells. The leukocyte-rich upper layer was collected, and neutrophils were separated from mononuclear cells by Ficoll-Hypaque density centrifugation (Histopaque 1077, Sigma). The contaminating red blood cells were removed by lysing solution with 0.1% KHCO\textsubscript{3} and 0.83% NH\textsubscript{4}Cl. The suspension was then centrifuged at 400 × g for 5 minutes and washed three times in HBSS. The resulting cell pellet consisted of >96% neutrophils and >98% viable cells as determined by trypan blue and erythrocin exclusion. The cells were suspended in Gey’s balanced salt solution (GIBCO) containing 2% bovine serum albumin (BSA; Sigma) at pH 7.2 to give a final concentration of 3.0 × 10\textsuperscript{6} cells/ml. This suspension was used for the neutrophil chemotaxis assay.

Mononuclear cells for the chemotaxis assay were obtained from normal human volunteers by Ficoll-Hypaque density centrifugation to separate the red blood cells and neutrophils from the mononuclear cells. The mononuclear cells were harvested at the interface. The suspension was then centrifuged at 400 × g for 10 minutes and washed three times in HBSS. The preparation routinely consisted of 30% large monocytes and 70% small lymphocytes determined by morphology and α-naphthyl acetate esterase staining (Sigma) with >98% viability as assessed by trypan blue and erythrocin exclusion. The cells were suspended in Gey’s balanced salt solution (GIBCO) containing 2% bovine serum albumin (BSA; Sigma) at pH 7.2 to give a final concentration of 5.0 × 10\textsuperscript{6} cells/ml. This suspension was then used for the monocyte chemotaxis assay.

The chemotaxis assay was performed in a 48-well microchemotaxis chamber (NeuroProbe, Cabin John, MD) as previously described.\textsuperscript{24} The bottom wells of the chamber were filled with 25 μl of fluid containing the chemotactic stimulus or media in duplicate. A 10-μm-thick polyvinylpyrrolidone-free polycarbonate filter, with a
pore size of 3 μm for the neutrophil chemotaxis and 5 μm for the monocyte chemotaxis, was placed over the bottom wells. The silicon gasket and upper pieces of the chamber were applied, and 50 μl of the cell suspension was placed into the upper wells above the filter. The chambers were incubated in humidified air in 5% CO₂ at 37°C for 30 minutes for the neutrophil chemotaxis and 90 minutes for the monocyte chemotaxis. Nonmigrated cells were wiped away from the filter. The filter was then immersed in methanol for 5 minutes, stained with Diff-Quik, and mounted on a glass slide. The cells that completely migrated through the filter were counted using light microscopy in 10 random high-power fields (HPF, ×1000) per well.

To ensure that monocytes, but not lymphocytes, were the primary cells that migrated in the monocyte chemotaxis assay, some membranes were stained with the primary cells that migrated in the monocyte chemotaxis well.

Effects of Metabolic Inhibitors on the Release of NCA and MCA

The effects of nonspecific lipooxygenase inhibitors, nordihydroguaiaretic acid (NDGA, 100 μmol/L; Sigma), diethylcarbamazine (DEC, 1 mmol/L; Sigma), and 5-lipoxygenase inhibitor AA-861 (100 μmol/L; Takeda Pharmaceutical Co., Tokyo, Japan) on the release of NCA and MCA in response to 5% smoke extract for a 72-hour incubation were evaluated. To further examine the involvement of protein synthesis in the release of the chemotactic activity, cycloheximide (20 μg/ml; Sigma) was added to inhibit protein synthesis.26

Effects of LTB₄ and PAF Receptor Antagonists on NCA and MCA

Because the release of NCA and MCA was blocked by 5-lipoxygenase inhibitors, and because NCA and MCA were extracted into ethyl acetate, LTB₄ receptor antagonist (ONO 4057, ONO Pharmaceutical Co., Tokyo, Japan) and platelet-activating factor (PAF) receptor antagonist (TCV 309, Takeda Pharmaceutical Co.) at the concentration of 10⁻⁹ mol/L were used to evaluate the involvement of LTB₄ and PAF for NCA and MCA.27,28

Measurement of LTB₄ and PAF in the Supernatant Fluid

The concentration of LTB₄ in the supernatants was measured by radioimmunoassay (RIA) as previously described.29–31 Anti-LTB₄ serum, [5,6,8,9,11,12,14,15,3H(N)]-LTB₄, and synthetic LTB₄ were purchased from Amersham Co. (Arlington Heights, IL). Briefly, ethanol and supernatant mixtures were centrifuged at 5500 × g at 0°C. At a temperature of 37°C, the supernatants were evaporated under N₂ gas to remove ethanol. To each sample, 10 ml of distilled water was added. These samples were acidified to pH 4.0 with 0.1 mol/L hydrochloric acid and applied to Sep-Pak C₁₈ columns (Waters Associates, Milford, MA). The columns were washed with a 10-ml mixture of distilled water and 20 ml of petroleum ether and then eluted with 15 ml of methanol. These eluates were dried with N₂ gas at 37°C and then redissolved in 20 μl of methanol and 180 μl of RIA buffer (50 mmol/L Tris/HCl buffer containing 0.1% (w/v) gelatin, pH 8.6). [³H]LTB₄ was diluted in RIA buffer (100 μl, containing approximately 4000 dpm) and mixed with 100 μl of standards or samples in disposable siliconized tubes. Anti-LTB₄ serum, diluted by RIA buffer (100 μl), was added to siliconized tubes to give a total incubation volume of 400 μl. The mixture was incubated at 4°C for 18 hours. Free LTB₄ was absorbed onto dextran-coated charcoal. The supernatant, containing the antibody-bound LTB₄, was decanted into scintillation counter after centrifugation for 15 minutes at 2000 × g. Scintillation fluid (Aquazol 2, NEN Co., Boston, MA) was added, and radioactivity was counted by a scintillation counter (Tricarb-3255, Tackard Co., IL) for 4 minutes.

Partial Characterization of NCA and MCA

Partial characterization of NCA and MCA released from A549 cells was performed with the supernatant fluids harvested after a 72-hour incubation with 5% smoke extract. Sensitivity to proteases was tested by incubating the supernatant fluids with trypsin (100 μg/ml; Sigma) for 30 minutes at 37°C followed by the addition of a 1.5 mol/L excess of soybean trypsin inhibitor to terminate the proteolytic activity, and then the chemotactic activity was evaluated. The lipid solubility was evaluated by mixing the supernatant fluids twice with ethyl acetate, decanting the lipid phase after each extraction, evaporating the supernatant fluids twice with ethyl acetate, decanting the lipid phase after each extraction, evaporating the supernatant fluids twice with ethyl acetate, decanting the lipid phase after each extraction, evaporating the supernatant fluids twice with ethyl acetate, decanting the lipid phase after each extraction, evaporating the supernatant fluids twice with ethyl acetate, decanting the lipid phase after each extraction, evaporating the supernatant fluids twice with ethyl acetate, decanting the lipid phase after each extraction, evaporating the supernatant fluids twice with ethyl acetate, decanting the lipid phase after each extraction, evaporating the supernatant fluids twice with ethyl acetate, decanting the lipid phase after each extraction, evaporating the supernatant fluids twice with ethyl acetate, decanting the lipid phase after each extraction, evaporating the supernatant fluids twice with ethyl acetate, and evaporating the supernatant fluids twice with ethyl acetate.

Molecular Sieve Column Chromatographic Findings of NCA and MCA

To determine the approximate molecular weight of the released activity in the supernatant fluids harvested at 72 hours in response to 5% smoke extract, molecular sieve column chromatography was performed using Sephadex G-200 (Pharmacia, Piscataway, NJ). At a flow rate of 6 ml/hour, A549 cell culture supernatant fluid was eluted with PBS, and fractions were evaluated for NCA and MCA in duplicate.
PAF in the supernatant fluids was evaluated via the scintillation proximity assay system. Briefly, this assay system combined the use of a high-specific-activity tritiated PAF tracer with an antibody specific for PAF and a PAF standard similar to the methods of measurement of LTB₄.

Effects of Polyclonal Antibodies to IL-8, G-CSF, MCP-1, GM-CSF, RANTES, and TGF-β

The neutralizing antibodies to human IL-8, G-CSF, MCP-1, RANTES (regulated on activation, normal T cells, expressed and secreted), granulocyte-macrophage colony-stimulating factor (GM-CSF), and transforming growth factor (TGF)-β were purchased from Genzyme (Cambridge, MA). They were added to the A549 cell supernatant fluids that were harvested at 72 hours in response to 5% smoke extract at the suggested concentration to inhibit these cytokines and incubated for 30 minutes at 37°C. To evaluate the nonspecific effect of IgG, nonimmune IgG was added to the same supernatant fluids and incubated for 30 minutes at 37°C. These samples were then used for the chemotactic assay. These antibodies did not influence the chemotactic response to endotoxin-activated serum (data not shown).

Measurement of IL-8, G-CSF, MCP-1, GM-CSF, RANTES, and TGF-β in the Supernatant Fluids

The concentrations of IL-8, G-CSF, MCP-1, GM-CSF, RANTES, and TGF-β in A549 cell supernatant fluids cultured for 72 hours in response to 5% smoke extract were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s directions. GM-CSF and RANTES kits were purchased from Amersham (Little Chalfont, UK), and the minimal concentration detected by these methods was 2.00 pg/ml for GM-CSF and 15.6 pg/ml for RANTES. IL-8, MCP-1, and TGF-β kits were purchased from R&D Systems (Minneapolis, MN), and the minimal detectable concentration of IL-8, MCP-1, and TGF-β was 10.0, 31.3, and 310 pg/ml, respectively. The G-CSF kit was obtained from Chugai Pharmaceutical Co., Tokyo, Japan. The minimal concentration of G-CSF detected by this kit was 1.0 pg/ml.

Statistics

In experiments where multiple measurements were made, differences between groups were tested for significance using one-way analysis of variance with Duncan’s multiple range test applied to data at specific time and dose points. In experiments where a single measurement was made, the differences between groups were tested for significance using Student’s paired t-test. In all cases, a P value less than 0.05 was considered significant. The data in the figures and tables are expressed as means ± SEM.

Results

Dose- and Time-Dependent Release of NCA and MCA from A549 Cells

In response to smoke extract, A549 cells released NCA and MCA in a dose-dependent manner (P < 0.05; Figure 1, A and B). The lowest doses of smoke extract to stimulate A549 cells were 0.5% for neutrophils and 1% for monocytes. Increasing concentrations of smoke extract progressively increased the release of chemotactic activity up to 10%. A549 cells released NCA and MCA in
response to smoke extract in a time-dependent manner \( (P < 0.05; \text{Figure 2, A and B}) \). After the exposure to smoke extract, the release of NCA and MCA was significant after 48 hours \( (P < 0.05; \text{Figure 2, A and B}) \). Smoke extract itself was not chemotactic for neutrophils and monocytes (data not shown).

The chemotactic responses to LTB4 at the concentration of \( 10^{-7} \) mol/L as positive control were 1020 ± 74 cells/10 HPF for neutrophils and 756 ± 34 cells/10 HPF for monocytes.

Checkerboard analysis revealed that the A549 cell supernatant fluids stimulated by smoke extract induced neutrophil and monocyte migration in the presence of a gradient across the membrane in a concentration-dependent manner. However, a smaller increase of the neutrophil and monocyte migration was observed in the absence of a gradient (Table 1). Thus, the migration of neutrophils and monocytes was predominantly consistent with chemotactic rather than chemokinetic activity.

Confirmation that the migrated cells were monocytes was provided by the following lines of evidence: 1) >90% of the migrated cells appeared to be monocytes morphologically by light microscopy; 2) >90% of the migrated cells were esterase positive; and 3) lymphocytes purified by allowing the monocytes to attach to plastic and tested in the chemotaxis assay yielded 0% to 20% of the chemotactic activity of the monocyte preparation.

**Partial Characterization of NCA and MCA**

The NCA and MCA were heterogeneous in character. Both NCA and MCA were partially but significantly sensitive to heat, extractable into ethyl acetate, and partially digested by trypsin (Figure 3, A and B).

**Molecular Sieve Column Chromatographic Findings of NCA and MCA**

The released chemotactic activity of the supernatant fluids was evaluated by molecular sieve column chromatography using Sephadex G-200. These experiments revealed that NCA obtained from the unstimulated cells was heterogeneous in size (Figure 4A). At least three peaks of activity were separated by column chromatography with two peaks near cytochrome c (molecular weight, 12,300) and an additional peak that eluted near quinacrine (molecular weight, 450). By stimulation of smoke extract, these peaks became prominent.

**Table 1. Checkerboard Analysis of the A549 Cell Culture Supernatant Fluid Harvested after 72 Hours in Response to 5% Smoke Extract**

<table>
<thead>
<tr>
<th>Lower well</th>
<th>F-12</th>
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<th>1:16</th>
<th>1:4</th>
<th>1:1</th>
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<tr>
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<td>6 ± 1</td>
<td>4 ± 1</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
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<tr>
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<td>6 ± 2</td>
<td>3 ± 1</td>
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<td>6 ± 1</td>
<td>5 ± 2</td>
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<tr>
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<td>11 ± 1</td>
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<td>20 ± 4</td>
<td>3 ± 1</td>
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<tr>
<td>Monocytes</td>
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</tr>
<tr>
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<td>9 ± 2</td>
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The vertical column represents the dilution of A549 cell supernatant fluids in the lower wells, and the horizontal row represents the dilutions of supernatant fluids in upper wells with cells.
The MCA from the unstimulated cells was also heterogeneous (Figure 4B). At least three peaks of activity were separated by column chromatography with two peaks between BSA and cytochrome c, and an additional peak eluted near quinacrine. When stimulated with smoke extract, each peak became prominent.

Effects of Metabolic Inhibitors on the Release of NCA and MCA

The 72-hour supernatant fluids incubated with 5% smoke extract in the presence of NDGA, DEC, and AA-861 showed a significant decrease in the release of NCA and MCA. Cycloheximide also inhibited the release of NCA and MCA ($P < 0.05$; Figure 5, A and B).

Effects of LTB$_4$ and PAF Receptor Antagonists on NCA and MCA

NCA and MCA in the supernatant fluids were significantly inhibited by the addition of LTB$_4$ receptor antagonist ONO4057, approximately 50% for NCA and 40% for MCA (Figure 6, A and B). ONO4057 also inhibited the chroma-
tography-separated lowest molecular weight peak more
than 80% for both NCA and MCA (data not shown). The
effects of PAF receptor antagonist TCV 309 on chemo-
tactic activity were not significant for NCA and MCA.
Each receptor antagonist at the concentration of 10^{-5}
mol/L completely inhibited the neutrophil migration in
response to 10^{-7} mol/L LTB_{4} and PAF, respectively, but
showed no inhibitory effects on activated-serum-induced
neutrophil and monocyte chemotaxis (data not shown).

**Effects of Smoke Extract on the Release of LTB_{4} and PAF**

The measurement of LTB_{4} by RIA revealed that A549
cells released LTB_{4} in the baseline culture condition.
The addition of smoke extract at the concentration of
5% for 72 hours induced a significant increase in LTB_{4}
release from A549 cells ($P < 0.05$; Figure 7). In con-

![Figure 5](image_url)

**Figure 5.** Effects of nordihydroguaiaretic acid (NDGA), diethylcarbamazine (DEC), AA-861, and cycloheximide (CYCLO) on the release of the neutrophil (A) and monocyte (B) chemotactic activity in response to 5% smoke extract harvested after a 72-hour incubation ($n = 8$). Percentage of chemotactic activity is on the ordinate, and the experimental groups are on the abscissa. *$P < 0.05$ compared with the smoke-extract-exposed supernatant fluids.

![Figure 6](image_url)

**Figure 6.** Effects of LTB_{4} (ONO 4057) and PAF receptor (TCV 309) antagonists on the released neutrophil (A) and monocyte (B) chemotactic activity obtained from A549 cell monolayers incubated with 5% smoke extract for 72 hours ($n = 8$). Percentage of the chemotactic activity is on the ordinate, and the experimental groups are on the abscissa. *$P < 0.05$ compared with the smoke-extract-exposed supernatant fluids.
contrast, PAF was not detected in the baseline and smoke-extract-stimulated supernatant fluids.

Effects of Polyclonal Antibodies to IL-8, G-CSF, MCP-1, GM-CSF, RANTES, and TGF-β

We evaluated the capacity of polyclonal blocking antibodies to IL-8, G-CSF, MCP-1, GM-CSF, RANTES, or TGF-β to reduce NCA and MCA. Anti-IL-8 and G-CSF antibodies significantly blocked NCA. Anti-MCP-1 antibody significantly reduced MCA (Figure 8). Nonimmune IgG did not have any effects on NCA and MCA. We evaluated the effects of IL-8, G-CSF, and MCP-1 antibodies on the column-chromatography-separated high-molecular-weight peaks. These antibodies inhibited the chemotactic activity at the corresponding molecular weight peak (data not shown).

Effects of Smoke Extract on the Release of IL-8, G-CSF, MCP-1, GM-CSF, RANTES, and TGF-β

The measurement of chemokines by ELISA revealed that A549 cells released IL-8, G-CSF, MCP-1, and TGF-β constitutively. Smoke extract stimulated the release of IL-8 G-CSF, and MCP-1 significantly (Figures 9, A and B, and 10A), but smoke extract did not stimulate the release

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**Figure 7.** The release of LTB4 from A549 cell monolayer in response to 5% smoke extract harvested after a 72-hour incubation (n = 6). The concentration of LTB4 is on the ordinate, and the experimental groups are on the abscissa. *P* < 0.05 compared with the supernatant fluids without smoke extract.

**Figure 8.** Effects of anti-IL-8, G-CSF, GM-CSF, TGF-β, RANTES, and MCP-1 polyclonal antibodies on the released neutrophil (A) and monocyte (B) chemotactic activity obtained from A549 cell monolayers incubated with 5% smoke extract for 72 hours (n = 6). Percentage of the chemotactic activity is on the ordinate, and the experimental groups are on the abscissa. *P* < 0.05 compared with the smoke-extract-exposed supernatant fluid.

**Figure 9.** The release of IL-8 (A) and G-CSF (B) from A549 cell monolayers in response to 5% smoke extract harvested after a 72-hour incubation (n = 6). The concentration is on the ordinate, and the experimental groups are on the abscissa. *P* < 0.05 compared with the supernatant fluids without smoke extract.

**Figure 10.** The release of MCP-1 (A) and RANTES (B) from A549 cell monolayers in response to 5% smoke extract harvested after a 72-hour incubation (n = 6). The concentration is on the ordinate, and the experimental groups are on the abscissa. *P* < 0.05 compared with the supernatant fluids without smoke extract.
reported that A549 cells released chemoattractant activity for monocytes spontaneously. The present study demonstrated that A549 cells can also release NCA and MCA in response to smoke extract and suggested the possibility that the type II alveolar epithelial cells play a role in defining the lung inflammatory environment.

The present study demonstrates that several chemotactic factors were released by A549 cells that may contribute to the inflammatory cell recruitment. Partial characterization revealed that the released NCA and MCA were partly ethyl acetate extractable. Pretreatment with AA-861, NDGA, and DEC inhibited the release of NCA and MCA. Molecular sieve column chromatography showed that there was a large chemotactic peak in the lowest molecular range. The chemotactic activity in the lowest molecular peak was inhibited by LTB4 receptor antagonist. Furthermore, the concentration of LTB4 assessed by RIA is high enough to produce neutrophil and monocyte chemotactic activity. Smoke extract increased the release of LTB4 into A549 cell culture supernatant fluids. In this context, LTB4 may be the predominant chemotactic activity.

In contrast, the trypsin sensitivity of the chemotactic activity along with the inhibition of the release by cycloheximide suggests that the activity was at least partly dependent on protein synthesis. Molecular sieve column chromatography revealed increases in the high molecular weight peaks of chemotactic activity in response to smoke extract. The antibodies to IL-8, G-CSF, and MCP-1 inhibited the NCA and MCA. IL-8, G-CSF, and MCP-1 were significantly increased in the supernatant fluid in response to smoke extract. These concentrations of IL-8, G-CSF and MCP-1 were chemotactic for neutrophils and monocytes, respectively. These data suggest that these cytokines may play important roles in the recruitment of inflammatory cells into the lungs of smokers.

Early descriptions of cytokines focused on their production by immune and inflammatory effector cells. However, it is apparent that structural cells are also capable of releasing many cytokines. A549 cells are known to produce a variety of cytokines, including IL-8, G-CSF, TGF-β, and MCP-1, in response to a variety of stimuli. However, the relation between smoking and the release of these cytokines has not been established. The present study demonstrated that A549 cells released these cytokines as chemotactic factors in response to smoke extract and suggest that these cytokines may play a role in smoking-induced lung disease by recruiting inflammatory cells.

Although TGF-β was detected in the supernatant fluid, TGF-β antibody did not attenuate monocyte chemotactic activity. TGF-β induces monocyte chemotaxis at concentrations from 0.1 to 10 pg/ml. At higher concentration, the chemotactic response of monocytes declines. It was reported that the biologically inactive form of TGF-β, which constitutes more than 98% of autocrine TGF-β, is secreted by 12 different cell types. TGF-β5 was unable to bind to the receptor without previous proteolytic activation. The release of inactive TGF-β may account for the lack of inhibition of MCA in the A549 cell supernatant fluids by anti-TGF-β.
G-CSF could be an important factor determining the number and functional activity of neutrophils. G-CSF has been reported to induce neutrophil migration at concentrations of more than 10 to 100 U/ml (7 to 10 ng/ml). The concentration of G-CSF in the supernatant fluids released from A549 cells was relatively low in the present study. However, the blocking antibody of G-CSF inhibited chemotactic response of neutrophils up to 46%. Recently, we have found that doses of 10 to 100 pg/ml G-CSF will induce significant NCA. Although G-CSF may be facilitating the chemotactic response of other cytokines, the concentration of G-CSF in the culture supernatant fluids exceeded the lower chemotactic threshold observed in our laboratory.

In conclusion, A549 cells released chemotactic activity toward neutrophils and monocytes in response to cigarette smoke extract. The released activity was lipid and peptide in its nature and involved LTB4, IL-8, G-CSF, and MCP-1. These data suggest the possibility that the type II alveolar epithelial cells may play an important role in the recruitment of the inflammatory cells in the lung in response to cigarette smoke.

References