Erythromycin Inhibits Neutrophil Chemotaxis in Bronchoalveoli of Diffuse Panbronchiolitis*

Hiroshige Oda, M.D.; Jun-ichi Kadota, M.D.; Shigeru Kohno, M.D., F.C.C.P.; and Kohei Hara, M.D., F.C.C.P.

The efficacy of low dose long-term erythromycin (EM) therapy in the treatment of chronic lower respiratory tract disease, including diffuse panbronchiolitis (DPB), has been reported, but its therapeutic mechanism is still unclear. In 13 patients receiving oral EM therapy the accumulation of neutrophils in bronchoalveolar lavage (BAL) fluid was significantly reduced (p<0.05), this reduction corresponds with an improvement in clinical symptoms. We sought to determine whether neutrophil chemotactic activity (NCA) in lavage fluid obtained from these 13 patients with DPB would respond to EM therapy. Pretreatment NCA in all patients was significantly elevated compared with levels in normal healthy nonsmoking volunteers (p<0.001), and the level was greatly reduced after EM therapy (p<0.001). In addition, this reduction correlated with increased percentages of neutrophils in the BAL fluid (r=0.737, p<0.01). Gel-filtration chromatography was also performed to characterize chemotactic factors. Pre-EM treatment BAL fluid revealed four NCA peaks (about molecular weight 15,000, 8,000, 1,500, and 300 daltons) in the elution pro-

file, and chemotactic activity was reduced in all areas after EM therapy. These findings indicate that NCA in lavage fluid from patients with DPB consists of various components. Although it was not clear which component is predominantly affected, these results indicate that EM may inhibit the migration of neutrophils to inflammatory sites by reducing the intrapulmonary chemotactic gradient, thus, ultimately reducing pulmonary inflammation. (Chest 1994; 106:1116-23)

Key words: bronchoalveolar lavage fluid; diffuse panbronchiolitis; erythromycin; neutrophil chemotactic factor.

Diffuse panbronchiolitis (DPB), an important pulmonary disease entity, was first reported in 1969 in Japan as an entity distinct from bronchial asthma, chronic bronchitis, chronic pulmonary emphysema, bronchiectasis, and alveolitis. This disease is characterized by chronic inflammation localized predominantly in the region of the respiratory bronchioles with inflammatory cell infiltration; its pathogenesis remains unknown. Although not uncommon in Japan, the disease is rare elsewhere. There have been recent reports, however, documented of two cases in white North American patients and of the first case in an Italian patient. The chief signs of this disease are chronic cough, pyomucous sputum, shortness of breath, wheezing, and hypoxemia. About three-quarters of these patients have associated chronic parasinusitis. Chest roentgenograms show bilateral fine nodular densities, together with hyperinflation of both lungs. Pulmonary function tests show a mixed ventilatory impairment consisting of slight restrictive and marked obstructive disturbance. The HLA-Bw54, found specifically in Japanese and Chinese people, is found more frequently in DPB cases than in healthy persons. Associated diseases include ulcerative colitis, allergic angitis and granulomatosis, and T cell leukemia.

Reports of chronic respiratory bronchiolitis in adults are not uncommon. Mecklem et al in 1971 reported seven cases of chronic obstructive pulmonary disease of the small airways and proposed a new definition of “small airway disease.” Homma et al reported that DPB patients had no evidence of a specific history of toxic gas inhalation, preceding pulmonary infection, or pathologic findings suggestive of chronic bronchitis, bronchiectasis, emphysema, or bronchial asthma. Eppler et al also suggested that an idiopathic form of bronchiolitis obliterans organizing pneumonia (BOOP) was a distinct clinical entity. The disease in patients with BOOP, however, was described as restrictive and not chronic. These lines of evidence suggest that DPB should be recognized as a disease entity, and should be differentiated from other more frequently ob-

*From the Second Department of Internal Medicine (Drs. Oda, Kadota, Kohno, and Hara), Nagasaki University School of Medicine; and the Department of Hygiene (Dr. Oda), Miyazaki Medical College, Japan. Manuscript received November 5, 1993; revision accepted January 26, 1994.

Reprint requests: Dr. Hara, Second Department of Internal Medicine, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852, Japan

1116 Erythromycin Inhibits Neutrophil Chemotaxis in Bronchoalveoli (Oda et al)
served obstructive pulmonary diseases.

Diffuse panbronchiolitis progresses insidiously and the prognosis, especially after *Pseudomonas aeruginosa* superinfection, is generally poor. Recently, low dose long-term erythromycin (EM) treatment has become the preferred therapy for chronic lower respiratory tract disease, including DPB.11 That erythromycin may act as an anti-inflammatory agent is indicated by recent reports that it raises natural killer cell activity,12 and has potent capacity to suppress the polymorphonuclear leukocyte (PMN) chemiluminescence induced by N-formyl-methionyl-leucyl-phenylalanine (FMLP), opsonized zymosan, and the calcium ionophore, A23187.13 The mechanism responsible for the therapeutic action of EM, however, is still unclear. Our recent finding indicated marked neutrophil accumulation in the lung of patients with DPB.14 Accordingly, in an attempt to elucidate this mechanism, we investigated the effect of EM on neutrophil migration, and we attempted to characterize the neutrophil chemotactic factors in the bronchoalveolar lavage (BAL) fluid of patients with DPB.

**Materials and Methods**

**Patient Population**

We evaluated neutrophil chemotactic activity (NCA) in 13 patients with DPB (10 men and 3 women; mean age 39.2 ± 4.9 years) who satisfied the diagnostic criteria for DPB set out by the Japanese Ministry of Health and Welfare. All the patients had the following clinical features: (1) symptoms of chronic cough with sputum production and exertional dyspnea, (2) physical signs of coarse crackles and rhonchi, (3) typical radiologic features on chest roentgenogram of diffuse nodular shadows and hyperinflation, (4) chronic parasal sinusitis, and (5) pulmonary function tests showing ventilatory defects that were obstructive, or rarely restrictive, and hypoxemia with or without hypercapnia. In 9 of the 13 patients the disease was histologically confirmed in open lung biopsies; in the remaining four patients, it was diagnosed clinically. All the patients were nonsmokers. When patients had signs or roentgenographic findings suggesting pneumonia or acute exacerbation of the disease before enrollment in the study, adequate antibiotics were administered. Thus, none of the patients had a pulmonary infection in the 1 month before enrollment in the study. For comparison, five healthy nonsmoking volunteers also were evaluated.

**Erythromycin Treatment**

All patients received 200 mg of erythromycin stearate orally 3 times per day for more than 6 months until BAL was repeated. The patients were instructed at each outpatient appointment to take one tablet every 8 hours, at home, and they were given a 4-week supply. They were instructed to bring back any remaining tablets at each visit to note that the numbers of remaining tablets could be checked. None of the patients received corticosteroids or antibiotics other than EM for the duration of this study.

**Bronchoalveolar Lavage**

The patients were premedicated intramuscularly with atropine (0.5 mg) and local anesthesia was produced with 2 percent lidocaine; airway examination was then carried out with a flexible fiberoptic bronchoscope (Olympus BF-P20 type; Olympus Corp. New Hyde Park, NY).

The bronchoscope was wedged securely into the subsegmental bronchus of the right middle lobe and 150 ml of sterile 0.9 percent NaCl at 37 °C was infused in three 50-ml aliquots, and gently aspirated immediately after each infusion. The recovered lavage fluid was pooled, passed through a double layer of gauze to remove gross mucus, then centrifuged. A differential cell count was performed on the pellet, and the supernatant was frozen at −80 °C until used.

**Isolation of Human Peripheral Blood Neutrophils**

Neutrophils for the chemotactic assay were isolated from a normal healthy volunteer by mono-poly resolving medium (M-PRM; Flow Laboratories, Irvine, Scotland) density gradient centrifugation.15 The neutrophils were suspended, at a density of 5X10⁶ cells/ml, in Hanks' solution (HBSS, Gibco), pH 7.2, containing 0.1 percent bovine serum albumin (BSA). More than 98 percent of the cells were neutrophils; cell viability was in excess of 95 percent, as determined by trypan blue exclusion.

**Neutrophil Chemotaxis Assay**

Neutrophil chemotactic activity was assessed using a 48-well microchamotaxis chamber (Neuroprobe Inc, Bethesda, MD) as described elsewhere.16 To each of the bottom wells, 25-μl aliquots of samples were added. A polycarbonate filter sheet (3-μm pores), not containing polyvinylpyrrolidone (NFB3-PVPF, Costar Corp, Cambridge, Mass), was placed on top of the wells in the bottom plate. The neutrophils (50-μl aliquots) were placed in the upper wells, at a concentration of 3X10⁶ cells/ml, in Hank's solution with 0.1 percent BSA. Chambers were incubated for 30 min at 37°C in a humidified atmosphere of 95 percent air: 5 percent CO₂; the filters were removed, fixed in absolute methanol, and stained with eosin-azure (Diff-Quik, Harleco; Gibbstown, NJ). The cells that migrated through the filter to the other side were counted. The NCA was measured as the mean number of cells per 10 high power fields (1,000X). The results were expressed as the percentage of the chemotactic response to FMLP 10⁻⁷ M. In each experiment, a negative control was assessed, using Hank's solution for the BAL fluid and the fraction fluid. The percent reduction of NCA was calculated according to the formula:14

\[
\text{NCA in Pre-EM tx BAL Fluid} - \\
\text{NCA in Post-EM tx BAL Fluid} \\
\times 100.
\]

The percent reduction of neutrophil percentage was calculated as:

\[
\text{Neutrophil Percentage in Pre-EM tx BAL Fluid} - \\
\text{Neutrophil Percentage in Post-EM tx BAL Fluid} \\
\times 100.
\]

**Gel-filtration**

Gel-filtration chromatography was performed on a 75 cm column (1.6-cm diameter, 60-cm long, Superdex, Pharmacia). One-milliliter aliquots of a ten-fold concentrated BAL fluid (freeze-dried 10-ml aliquots solubilized in 1 ml of distilled water) were applied to a column (Superdex) previously equilibrated with phosphate buffered saline (PBS, pH 7.2, Gibco). Chromatography was performed at 4°C, with a flow rate of 1.0 ml/min; 5 ml of each fraction was collected. The column was calibrated with molecular weight markers (blue dextran, cytochrome c, insulin, and phenol red). For fractions, 1.5 mg of protein was used.

**Statistical Analysis**

All data were expressed as the means ± standard error of the
Table 1—Clinical Characteristics of Ten Patients With Diffuse Panbronchiolitis

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>Chronic Sinusitis</th>
<th>Duration of EM (tx, mo)</th>
<th>Duration of Disease, yr</th>
<th>Sputum Culture</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>M</td>
<td>+</td>
<td>6</td>
<td>2</td>
<td>S aureus</td>
<td>OLB*</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
<td>F</td>
<td>+</td>
<td>12</td>
<td>10</td>
<td>H influenzae</td>
<td>OLB</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>M</td>
<td>+</td>
<td>6</td>
<td>5</td>
<td>Normal flora</td>
<td>OLB</td>
</tr>
<tr>
<td>4</td>
<td>52</td>
<td>M</td>
<td>+</td>
<td>12</td>
<td>10</td>
<td>P aeruginosa</td>
<td>OLB</td>
</tr>
<tr>
<td>5</td>
<td>63</td>
<td>F</td>
<td>+</td>
<td>8</td>
<td>20</td>
<td>Normal flora</td>
<td>Clinical</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>M</td>
<td>+</td>
<td>11</td>
<td>8</td>
<td>H influenzae</td>
<td>OLB</td>
</tr>
<tr>
<td>7</td>
<td>49</td>
<td>F</td>
<td>+</td>
<td>6</td>
<td>26</td>
<td>Normal flora</td>
<td>OLB</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>M</td>
<td>+</td>
<td>12</td>
<td>10</td>
<td>H influenzae</td>
<td>OLB</td>
</tr>
<tr>
<td>9</td>
<td>19</td>
<td>F</td>
<td>+</td>
<td>12</td>
<td>5</td>
<td>P aeruginosa</td>
<td>OLB</td>
</tr>
<tr>
<td>10</td>
<td>34</td>
<td>M</td>
<td>+</td>
<td>6</td>
<td>7</td>
<td>H influenzae</td>
<td>Clinical</td>
</tr>
<tr>
<td>11</td>
<td>49</td>
<td>M</td>
<td>+</td>
<td>12</td>
<td>5</td>
<td>Normal flora</td>
<td>OLB</td>
</tr>
<tr>
<td>12</td>
<td>14</td>
<td>M</td>
<td>+</td>
<td>6</td>
<td>4</td>
<td>Normal flora</td>
<td>OLB</td>
</tr>
<tr>
<td>13</td>
<td>41</td>
<td>M</td>
<td>+</td>
<td>12</td>
<td>5</td>
<td>P aeruginosa</td>
<td>OLB</td>
</tr>
</tbody>
</table>

Mean: 39.2±4.9

90.0±4.3 percent (p<0.01), FEV 1.0 percent from 68.6±4.2 percent to 75.2±4.0 percent (p<0.05), percent carbon monoxide diffusion capacity in the lungs (DLCO) from 55.8±3.8 percent to 69.6±3.9 percent (p<0.05) and V25/HT from 0.2±0.1 L/s/m to 0.5±0.1 L/s/m (p<0.05). There were no significant changes in the other parameters after EM therapy.

Results

Clinical Characteristics of Patients

Neutrophil chemotactic activity was measured in the pre- and post-EM treatment BAL fluid of the 13 DPB patients. As shown in Table 1, their mean duration of disease was 9.0±1.9 years; the onset was insidious. Sputum cultures at the time of admission yielded Hemophilus influenzae in four patients (cases 6, 8, 10, and 11), Pseudomonas aeruginosa in three (cases 4, 9, and 13), Staphylococcus aureus in one (case 1), and normal flora in 5 (cases 2, 3, 5, 7, and 12). Erythromycin was administered to the patients for 9.3±0.8 months. After the treatment, H influenzae was eradicated in three patients but replaced by P aeruginosa in one case. In all 3 patients originally exhibiting P aeruginosa in cultures, the organism was eradicated. In the one patient (case 1) with S aureus, this organism was eradicated, but replaced by H influenzae. In one of the patients with normal flora, these were replaced by H influenzae; in the other such patient, these flora were replaced by P aeruginosa. The bacteria eradication rate was 75.0 percent (6/8).

Changes in Respiratory Function Test Results of Patients

The mean values for respiratory function parameters and arterial blood gas analysis before and after EM treatment are shown in Table 2. After EM treatment, the mean values for four parameters of respiratory function were markedly improved, ie, percent VC increased from 72.7±4.5 percent to 90.0±4.3 percent (p<0.01), FEV 1.0 percent from 68.6±4.2 percent to 75.2±4.0 percent (p<0.05), percent carbon monoxide diffusion capacity in the lungs (DLCO) from 55.8±3.8 percent to 69.6±3.9 percent (p<0.05) and V25/HT from 0.2±0.1 L/s/m to 0.5±0.1 L/s/m (p<0.05). There were no significant changes in the other parameters after EM therapy.

Changes in BAL Findings

The mean values for BAL parameters before and after EM therapy are shown in Table 3. The mean values for total cell number and neutrophil percentage before EM treatment were significantly higher than values in healthy volunteers; the values were significantly reduced after EM therapy (p<0.05).
corresponding with an improvement in clinical symptoms and findings. Changes in the recovery rate pre- and post-EM treatment were not significant (48.3 ± 4.3 vs 46.5 ± 5.9 percent).

**Changes in Neutrophil Chemotactic Activity in BAL Fluid**

Figure 1 shows the changes in NCA pre- and post-EM treatment in the BAL fluid of patients with DBP. The mean NCA value before EM treatment was 52.9 ± 2.9 percent; and there was a marked reduction in this value after the treatment (30.8 ± 2.5 percent, p<0.001). There were no significant differences between values for albumin concentration in initial BAL fluid samples and in repeated samples (41.8 ± 10.8 vs 39.3 ± 6.8 mg/dl, p=0.85). Additionally, there were no significant correlations between the recovery rate and NCA either in initial BAL fluid samples (r=0.017, p>0.6) or in repeated samples (r=0.316, p>0.6). Albumin concentration and NCA did not correlate in either the initial or the repeated samples of BAL fluid (initial samples: r=0.102, p>0.6; repeated samples: r=0.254, p>0.6). Thus, we regarded NCA as being unaffected by recovery rate or by albumin concentration, and we, therefore, expressed without correction. Although BAL fluid samples from DBP patients may contain more bacterial endotoxin than samples from healthy subjects, we believe it to be that the endotoxin would not have affected the chemotaxis findings, both in the light of report by Issekutz and Bhimji\textsuperscript{17} that endotoxin did not induce neutrophil chemotaxis in the in vitro assay and in the light of our observation here that neither of the two endotoxin preparations (\textit{P. aeruginosa} serotype 10 and \textit{Salmonella typhosa}; Sigma) tested induced neutrophil chemotaxis in the in vitro assay at concentrations 10 to 100 times higher than those inducing leukocyte infiltration in vivo (data not shown).

**Correlation Between Reduction in Neutrophil Percentage and Neutrophil Chemotactic Activity in Pre- and Post-EM Treatment BAL Fluid**

Neutrophil chemotactic activity and neutrophil percentage in the BAL fluid of the 13 patients with DBP were compared before and after EM therapy. There was a significant correlation between the reduction in neutrophil percentage and NCA (r=0.737, p<0.01; Fig 2).

**FIGURE 1.** Total neutrophil chemotactic activity in pre- and post-EM treatment BAL fluid obtained from 13 patients with diffuse panbronchiolitis and from 5 healthy nonsmoking volunteers (HV). NCA, assessed by the blindwell chamber technique is expressed as a percentage of the chemotactic response to 10\textsuperscript{−7} mol/L \textit{N}-formyl-methionyl-leucyl-phenylalanine. Each value represents mean ± standard error. Shaded area shows background activity of this assay using Hank’s solution. The chemotactic activity in pre-EM treatment BAL fluid obtained from the DBP patients is significantly elevated compared with that of the HV. The chemotactic activity of BAL fluid from the DBP patients was significantly reduced after EM treatment (p<0.001).

**FIGURE 2.** Correlation between reduction in neutrophil chemotactic activity and neutrophil percentage in BAL fluid. The vertical and horizontal axes express the percent reduction of NCA and neutrophil percentage, respectively. There was a significant correlation between the reduction in neutrophil percentage and chemotactic activity (p<0.01).
Characterization of NCF in Pre- and Post-EM Treatment BAL Fluid of a Patient With DPB

Analysis of pre-EM treatment BAL fluid obtained from one patient with DPB (case 4) resulted in four peaks of activity; these were estimated to be 15,000, 8,000, 1,500, and 300 daltons (Fig 3). All four peaks of chemotactic activity in the BAL fluid from this patient were reduced after EM treatment (Fig 4).

DISCUSSION

Diffuse panbronchiolitis is a chronic inflammatory disease that is manifested in a diffuse fashion in both lungs in the region of the respiratory bronchioles. Typical features of the lesions are thickening of the walls of the bronchioles, with infiltration of lymphocytes, plasma cells, and histiocytes; proliferation of lymphofollicles; accumulation of foamy cells within the wall and neighboring area; and extension of these inflammatory changes toward the peribronchiolar tissues.1,2

The treatment for this disease used to involve steroid administration and oxygen inhalation in the first stage, and antibiotics, expectorants, and bronchodilators in the advanced stage. Prognosis was poor, however, especially when there was superinfection with P aeruginosa. In 1984, Kudoh et al11 reported that low dose long-term EM therapy was effective in chronic lower respiratory tract disease, including DPB, and that great improvements were shown in clinical symptoms. Since that time, this has been the preferred therapy for DPB. Its mechanism of action, however, remains obscure.

Antibiotics generally act directly on infecting micro-organisms. For example, the tetracyclines inhibit bacterial protein synthesis by binding to the 30S subunits of bacterial ribosomes.18 Chloramphenicol acts on the 50S ribosomal subunit and suppresses peptidyl transferase.19 Erythromycin, a broad-spectrum macrolide antibiotic commonly used in patients with lower respiratory infections, also interferes with bacterial protein synthesis by binding to the ribosomal subunit.20 On the other hand, many investigators have found that some commonly used antibiotics exert their effects by influencing host defense mechanisms, eg, by acting on neutrophil chemotaxis, lymphocyte transformation, delayed hypersensitivity, and so on. In 1950, Munoz and Geister21 showed that chlortetracycline inhibited normal human leukocyte phagocytosis, and they addressed the question of antibiotic modulation of the immune system.
Other antibiotics, including doxycycline, lymecycline, and rifampin, have also been found to inhibit chemotaxis of human neutrophils and to suppress phytohemagglutinin-induced lymphocyte transformation in vitro. Recently, FK506, a macrolide antibiotic, has been shown to inhibit T-cell activation by a mechanism that appears similar to that of cyclosporin A. Various studies have reported the influence of EM on the host defense mechanisms of patients with DPB, in terms of the changes in lymphocyte subsets, increased natural killer cell activity, suppression of oxygen radical products, and suppression of elastase activity. We have previously reported that the BAL fluid supernatant obtained from patients with DPB enhanced superoxide production by neutrophils isolated from healthy volunteers, and that this enhancing effect was reduced to near normal after EM treatment. Nagai et al reported a pharmacokinetic study of EM in 11 DPB patients (8 responders and 3 nonresponders) after long-term low-dose administration. They showed that the maximal serum and sputum levels of EM were below the MIC of several clinically pathogenic bacteria often isolated from the sputum of such patients. They also observed no difference between responders and nonresponders in EM absorption. Thus, these studies have led to the conclusion that the therapeutic action of EM in DPB patients is anti-inflammatory rather than antibacterial.

Hopkins et al suggested that the accumulation of neutrophils in the alveoli was a characteristic pathologic finding in bacterial pneumonia, while Martin et al showed that chronic bronchitis patients with chronic cough or with phlegm production had elevated percentages of neutrophils in BAL fluid. In our study, markedly increased numbers of neutrophils were observed in the pretreatment BAL fluid of patients with DPB, consistent with previous reports. Neutrophils serve as the first line of host defense by controlling the expansion and dissemination of microbes by killing and removing pathogenic microorganisms. The mobilization of neutrophils from the intravascular compartment into the respiratory tract is crucial for effective host defense. In other clinical settings, however, such as adult respiratory distress syndrome and chronic lower respiratory tract infection, neutrophils are injurious to the host in terms of generating oxygen radicals and in terms of the action of elastase. Thus, excessive inflammation contributes to tissue damage. Several researchers...
have described the inhibitory effect of EM on chemotaxis in vitro\(^\text{35,36}\) and on neutrophil migration into the murine lung in response to Proteus mirabilis in vivo.\(^\text{37}\) In our present study, we found that treatment with EM significantly reduced the number of intrapulmonary neutrophils, indicating that, in DPB, EM might act first by reducing neutrophil migration and then by inhibiting inappropriate and excessive inflammation in the lower respiratory tract.

The accumulation of neutrophils is induced by a number of chemotactic mediators, including C5a, IL-1, TNFα, IL-8, granulocyte-CSF, and granulocyte-macrophage-CSF.\(^\text{38-45}\) We evaluated NCA in pre- and post-EM treatment lavage fluid from DPB patients and found that in post-EM BAL fluid, NCA was significantly reduced, and, interestingly, that this correlated with improvements in neutrophil percentages. In a recent study, Hirata et al.\(^\text{46}\) found that EM had potently suppressed neutrophil chemiluminescence (CL) induced by FMLP stimulation. In contrast, phorbol myristate acetate-induced CL was much less affected. Phorbol myristate acetate activates neutrophils, probably through the activation of protein kinase C.\(^\text{47}\) Although the concentration of EM in serum was below the MIC for several pathogenic bacteria, EM was 10- to 25-fold concentrated in neutrophils and alveolar macrophages.\(^\text{47}\) All these results indicate that EM acts on the intracellular signal pathway, reduces chemotactic activity directly or indirectly, and plays a crucial role in modulating inflammation in the lungs of patients with DPB, all of the effects from EM are from its action in suppressing the oxidative and proteolytic products of neutrophils.

Our chromatographic data showed that the weight after molecular sieving of three of the fractions closely corresponded to the molecular weights of C5a,\(^\text{39}\) interleukin-8,\(^\text{41}\) and a lipid-containing substance,\(^\text{42,48}\) this being a lipoxygenase pathway metabolite of arachidonic acid. All chemotactic factors were reduced after EM treatment, suggesting that these factors are important in neutrophil recruitment. Which of these factors, however, is quantitatively the most important in this regard was not determined from our findings in this study. Nevertheless, it is likely that EM prevents airway damage in this disorder by suppressing neutrophil accumulation, as a consequence of reducing NCA in the inflammatory sites in the lung.

**REFERENCES**

15. Ferrante A, Thong YH. A rapid one-step procedure for purification of mononucleocal and polynonuclear leukocytes from human blood using a modification of the hypaque-ficol tech-

**Erythromycin Inhibits Neutrophil Chemotaxis in Bronchoalveolar (Oda et al)**


40 Ming WJ, Berson L, Mantovani A. Tumor necrosis factor is chemotactic for monocytes and polymorphonuclear leukocytes. J Immunol 1987; 138:1469-74


45 Cybulsky MI, McComb DJ, Movat HZ. Neutrophil leukocyte emigration induced by endotoxin. J Immunol 1988; 140:3144-49

