Figure 2. Relation between antibody titer decline (expressed as mean percentage decline of the values obtained at two weeks after immunization ± SEM) of anti-HPH-IgG and anti-HPH-IgM titers, at 8 and 14 weeks after immunization in stage 1 patients (-----) compared with the COLD controls (------), and with "benign" thoracotomy controls (-------). For both antibody titers there was a significantly more rapid titer decline in patients compared with the control subjects (P<0.01 and P<0.001).


**DISCUSSION**

**Dr. Lawrence:** Did you measure the proliferative response in the patient's own serum?

**Dr. Jansen:** It was measured in 25 percent inactivated pooled human donor serum.

**Question:** Are the suppressor cells sensitive to indomethacin?

**Dr. Jansen:** Induction of lymphocyte response seems to be monocyte-dependent. In these patients, suppression of prostaglandin synthesis with indomethacin leads to increased lymphocyte response.

**Dr. Goodman:** Is there a defect in surveillance of killer cells here?

**Dr. Jansen:** We looked for NK cells and found no difference.

**Lung Lining Material As a Chemotactant for Alveolar Macrophages**

_Lester W. Schwartz, D.V.M., Ph.D., and Claudia A. Christman, B.A._

Critical functions performed by alveolar macrophages (AMΦ) as defenders of the pulmonary gas exchange surface include migration, phagocytosis, cytotoxicity, and generation of soluble mediators. Accumulation of AMΦ within the lung serves as an indicator of pulmonary insult. In the face of acute insult, recruitment and enhanced mobility aid in confinement of particulates and protection of the lung. Persistence of increased numbers of these inflammatory cells for prolonged periods upon the delicate alveolar membrane may proceed to be more detrimental than protective to the host.

Our study was designed to evaluate the influence of lung lining material on directional migration of AMΦ collected from rhesus monkeys. Motivation for the present study was derived from previous morphologic observations made on lungs of rodents and nonhuman primates exposed to low levels of oxidant air pollutants. Our observations indicated that the AMΦ was the principal inflammatory cell which accumulated within the lung during initial phases of insult and, in

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addition, persisted during prolonged insult. One or all of the following events could account for the increase in numbers of intraluminal AMϕ: (1) enhanced attraction of blood and/or interstitial monocytes into the alveolar lumen; (2) local intra-alveolar proliferation of existing AMϕ, and/or (3) reduced removal or inhibition of the usual intra-alveolar macrophage traffic. Kazmierowski et al\textsuperscript{1} have presented evidence that, following insult, the nonhuman primate lung contains at least two chemotactic substances for peripheral blood leukocytes.

We chose to focus on mechanisms which may function to initiate and regulate AMϕ migration. The limited use of AMϕ in cell migration studies stems from previous observations which indicated that AMϕ respond poorly in \textit{in vitro} chemotactic assay systems.\textsuperscript{3} This observation has recently been supported by Dauber and Daniele,\textsuperscript{4} but they stressed that the poor response to certain serum-derived chemotaxins was not sufficient evidence to suggest that the AMϕ lacked potential to respond chemotactically. They reported evidence that guinea pig AMϕ do respond to chemotactic formylmethionyl peptides. Without considering the identification of specific cell surface receptors for various chemotaxins, the variable response of the AMϕ may be attributed to metabolic requirements. Dauber and Daniele demonstrated that decreased PO\textsubscript{2} significantly decreased the \textit{in vitro} migratory response of alveolar but not peritoneal macrophages. An additional unique feature of the AMϕ is that \textit{in vitro} it functions within a complex, highly surface active milieu of lipids, proteins, and carbohydrates. La Force et al\textsuperscript{1} and Juers et al\textsuperscript{5} have suggested that components of lung lining material can alter AMϕ function, i.e., can enhance the bactericidal activity of the AMϕ.

### Methods

For this study, adult rhesus monkeys [\textit{Macaca mulatta}], weighing 8 to 10 kg and determined to be normal animals by physical examination and chest radiographs, were used. Lung lining material (LLM) was collected by bronchoalveolar lavage. Using light general anesthesia induced by ketamine hydrochloride and local anesthesia of the posterior pharynx with tetracaine hydrochloride, a double-lumen balloon bronchial catheter was positioned in a main stem bronchus. The balloon was inflated and 240 ml of phosphate-buffered saline solution was introduced in 80-ml aliquots and slowly withdrawn. Bacterial cultures of representative lavage samples failed to produce bacterial growth on blood agar within 38 hours. The lavage sample was centrifuged at 500 g for 15 minutes at room temperature and the cell-free supernatant collected. The supernatant lavage fluid was concentrated 20- to 25-fold by vacuum dialysis using a cellulose bag with a molecular weight cutoff of 12,000. A Lowry's protein determination was completed on the concentrated sample which was then refrigerated for no longer than three weeks prior to use.

Alveolar macrophages were collected using a similar lavage technique. At least six rhesus monkeys were used for each determination, and cells from each monkey were used separately. After centrifugation, the cell pellet was resuspended in Medium 199 containing 10 percent fetal calf serum, penicillin (100 IU/ml), and streptomycin (100 µg/ml). Cell viability was determined using trypan blue exclusion, and differential cell counts were completed on smears stained with Wright-Giemsa stain.

Chemotaxis was measured using a modification of the agarose migration assay described by Nelson et al.\textsuperscript{6} A series of three wells (2.4 mm diameter) were cut in 1 percent agarose layered in a 35 × 10 mm plastic petri dish. The center well received cells (4.0 × 10\textsuperscript{6}), the outer well received chemotactant test material, and the inner well received Medium 199. Plates were incubated at 37°C in a 5 percent CO\textsubscript{2} humidified environment. After 24 hours of incubation the dishes were flooded with Karnovsky's fixative for 30 minutes, the agarose removed, and the dishes stained with 1 percent methylene blue. Quantification was completed by light microscopy using a 10 X objective and an ocular micrometer in a 10 X ocular. Cell numbers were also determined using a 4 mm\textsuperscript{2} ocular grid.

Rhesus monkey LLM was fractionated by gel filtration using Sephadex G-200 and a 1.5 × 90 cm column. Fractions were eluted at room temperature with 1.0 M NaCl in 0.1 M Tris chloride buffer, pH 7.4, containing 1 mM EDTA. Protein elution was monitored spectrophotometrically (280 nm) and the eluted fractions were evaluated for chemotactic activity with the agarose system.

### Results and Discussion

Generally, 84 percent of the lavage fluid was retrieved using this method of bronchoalveolar lavage. The mean protein concentration of the cell-free lavage fluid was 0.09 ± 0.02 mg/ml. Samples contaminated with red blood cells were discarded. The average number of cells

**Comparison of Chemotaxis of Alveolar Macrophages by Lung Lining Material and Rhesus Monkey Albumin**

**Figure 1.** The chemotactic effect of LLM and rhesus monkey albumin on alveolar macrophages collected from normal rhesus monkeys. Under agarose migration measured as described in the text. Medium 199 served as the control substance to determine spontaneous migration distances.
retrieved from a series of ten normal rhesus monkeys was 12.0 x 10^6 with a viability of 90.4 percent. The differential cell counts indicated 89.5 percent AM*, 8.9 percent lymphocytes, and 2.1 percent granulocytes. Cells were used as harvested and no attempt was made to purify the AM* population further.

Figure 1 summarizes migration data obtained from increasing the concentration of LLM based on protein content. Concentrations of LLM tested were 0.6, 1.2 and 2.4 mg protein/ml and the distances AM* migrated toward these concentrations were 340, 370 and 430 μm, respectively. These data indicate a linear relationship between the protein concentration of LLM and the distance AM* migrated. To provide evidence that a portion of this migratory response was in fact more than just enhanced random migration, an additional experiment was completed with results as shown in Table 1. AM* migration regardless of LLM influence is reported as random migration. The LLM-positive gradient was destroyed by addition of an equal concentration of LLM directly to the cell-containing well and the chemotactic well; this is reported as activated random migration. The distance migrated by AM* was significantly (P<0.01) greater than the random migration induced by Medium 199 alone. The influence on directional migration was demonstrated by addition of LLM to the chemotactic test well only, which produced a positive gradient, plus the evidence that in this positive gradient situation the migration distance consistently exceeded the no gradient situation. Directional migration in this later case was 396.2 μm which was significantly (P<0.02) greater than the activated random migration distance. The gradient of LLM was eliminated in another experiment by addition of LLM to the chemotactic test well three hours prior to addition of the cells. This increased diffusion time produced AM* migration distances of 298.3 ± 94.7 μm which were also significantly (P<0.001) less than the positive gradient situation. A chemotactic response to LLM is therefore indicated since destruction of the gradient in these two fashions significantly reduce the migration distances from that observed in positive gradient situations.

Since albumin contributes a major portion to the protein component of LLM, and albumin has been shown to have chemotactic properties, the migratory influence of rhesus monkey albumin (RMA) was evaluated and is also shown in Figure 1. Concentrations of RMA tested were 0.52, 1.0 and 2.1 mg/ml and the distances AM* migrated towards these concentrations were 320, 330 and 310 μm, respectively. Clearly, albumin also enhanced AM* migration, but a linear response was not observed with increasing concentrations of RMA and differences between concentrations were not detected.

Partial characterization of the chemotactic activity of LLM was attempted by gel filtration with Sephadex G-200. The elution pattern is shown in Figure 2. Four fractions were detected spectrophotometrically. Calibration of the Sephadex column with proteins of known molecular weight suggested that fraction I was 800,000 daltons (above the fractionation range of Sephadex G-200), fraction II was 173,000 daltons, fraction III was 75,000 daltons, and fraction IV was eluted from the column below the molecular weight.

![Figure 1](http://journal.publications.chestnet.org/)

![Figure 2](http://journal.publications.chestnet.org/)

**Table 1—Evidence that Lung Lining Material (LLM) Produces Random and Directional Migration of Alveolar Macrophages**

<table>
<thead>
<tr>
<th>Type of Migration</th>
<th>Well Contents</th>
<th>Distance (μm) Cells Migrated under Agarose towards Outer Well</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random</td>
<td>Medium 199</td>
<td>Medium 199 and cells</td>
</tr>
<tr>
<td>Activated random</td>
<td>Medium 199</td>
<td>Medium 199, cells, and LLM</td>
</tr>
<tr>
<td>Directional</td>
<td>Medium 199</td>
<td>Medium 199 and cells</td>
</tr>
</tbody>
</table>

*Data obtained from 6 rhesus monkeys, each evaluated in triplicate.

**P<0.01 for activated random migration compared with Medium 199.

†P<0.02 for directional migration compared with activated random migration.
fractionation range of Sephadex C-200, i.e., less than 5,000 daltons.

Since the migration distance was a linear response to the protein content of LLM, the chemotactic activity of the four fractions was assessed and reported as distance migrated in μm per μg protein. Figure 2 illustrates the influence of each fraction on AMφ migration, and evidence suggests that the major activity is present within the small molecular weight fraction IV. Additional characterization is presently being completed, particularly of fraction IV.

This study has presented evidence that cell-free lining material collected from the normal rhesus monkey lung stimulates random migration of AMφ and will produce a directional migration of these cells. This observation supports the concept that the AMφ does respond to chemotactic stimuli and that this function may occur in vivo as a mechanism to regulate intraluminal AMφ numbers. Additional studies are in progress to identify features of the chemotxin (s) within LLM.

REFERENCES
3 Mellick PW, Dungworth DL, Schwartz LW, et al: Short term morphologic effects of high ambient levels of ozone on lungs of rhesus monkeys. Lab Invest 36:82-90, 1977

DISCUSSION
Dr. Sanderson: Do you think that the surface interaction between the cell and plate effects migration?
Dr. Schwartz: We have not specifically evaluated the influence of altered surface tension and cell migration in the agarose system. Test situations in which positive gradients of LLM were eliminated by the addition of LLM to both the cell-containing well and chemotaxin test well would appear to provide the maximum change in surface tension for this system, and with these conditions, cells migrated less than when a positive gradient was produced. This evidence suggests that altered surface tension alone would not produce maximum cell migration.

Dr. Murphy: Were any of these monkey lungs inflamed?
Dr. Schwartz: In this study, cells and lung lining material were collected only from lungs of essentially normal monkeys.

Dr. Murphy: Have you looked at the influence of LLM from lungs that are inflamed?
Dr. Schwartz: No, this has not been rigorously evaluated. Our approach has been to collect LLM and serum from normal rhesus monkeys which were then ozonized in vitro and tested for migration effects. We have not observed significant changes in alveolar macrophage migration induced by ozonized LLM or serum. In general, our impression is that chemotaxis as a mechanism responsible for macrophage accumulation is probably of limited significance. Perhaps more important is suppression of migration of the usual macrophage traffic and/or in situ proliferation. We have observed that the migration of alveolar macrophages collected from ozone-exposed monkeys is suppressed. In addition, an enhanced proliferative capacity is reflected by an increased number of colony-forming units in methylcellulose culture produced by cells retrieved from ozone-damaged rat lung.

Dr. Brody: How does lung lining material effect phagocytes and where do you think it is coming from?
Dr. Schwartz: Lung lining material is a very general term referring to all cellular material lining both alveoli and airways; we do not know the source of the components influencing migration. We are, of course, interested in possible sources. A suggested source and function would be that cells of small or large airways release products that influence the central migration of macrophages, thus aiding in lung clearance mechanisms.

Dr. Schuyler: Have you compared the response of pulmonary alveolar macrophages in this system with known potent chemotactic agents?

Dr. Schwartz: Very few known potent chemotaxins for alveolar macrophages have been identified. We presently are comparing LLM to formulated peptides.

Dr. Repine: Dr. Schwartz, you said that the stimulus gradient on macrophage chemotaxis caused by lung lining material would have differed and been gone by 4 hours. If the cells are still moving at 24 hours in the absence of a gradient, as your work indicates, could it be because the cells have been given an orientation as a result of their initial exposure to the gradient?

Dr. Schwartz: I do not know the answer to your question; however, it is generally accepted that for directional migration to occur a gradient must be present.

Dr. Repine: I believe careful kinetic studies would help answer the question. If the kinetic studies showed that the macrophage continues to exhibit simulated locomotion after the gradient was gone, this would be very
A Possible Link Between the Pulmonary and Urinary Tract IgA Response*

Inger Mattsby, Sc.; L. A. Hansson, M.D.; and B. Keil, M.D.

Using as a model ascending pyelonephritis in rats caused by E coli O6, we investigated the antibody response in serum as well as in bronchial lavage.

Nine rats immunized orally with live E coli O6 two weeks before injection of the same bacteria into the urinary bladder gave rise to anti-O6 antibodies of the IgG, IgM and IgA classes in both serum and bronchial washings one week after the injection. Only two of eight animals not pre-immunized orally gave rise to IgM and IgA antibodies in serum and bronchial washings after bladder injection. The preimmunized rats showed a higher anti-O6 IgA titer in the bronchial washings than the not pre-immunized ones.

The anti-O6 antibody levels in serum and bronchial lavage were determined in infected rats killed two to four months after the introduction of bacteria into their bladders. A significant correlation was found for IgG and IgM antibodies (P < 0.02 and < 0.001) but not for the IgA antibodies (P < 0.1) comparing serum and bronchial washings. The IgG anti-O6/IgA anti-O6 ratios were higher in serum than in bronchial lavage. This indicates the possibility that some of the IgA antibodies appearing in the lung may be produced locally or that serum IgA antibodies more readily diffuse into the air spaces of the lung than IgG or IgM antibodies.

These observations suggest that IgA antibodies in the lung may be part of a local antibody response transferred via a "homing" mechanism for IgA producing lymphocytes. A transport of E coli antigens via the blood to the pulmonary lymphoid tissue seems less likely. Further experiments are required to substantiate this extension of the homing mechanism hypothesis which would agree with the findings of Rudzik et al and Bienenstock et al in that lymphoid cells from various sources as BALT, GALT and mesenteric lymph nodes are "homing" to various mucosal sites.

REFERENCES


DISCUSSION

Dr. Richerson: How did you compare serum antibody levels to those in bronchoalveolar lavage fluids?

Dr. Mattsby: We compared the relation between antibody levels of IgA, IgG and IgM classes in serum to the respective antibody levels in bronchoalveolar fluid. We also compared the ratios of the antibody titres of IgG class to the antibody titres of IgA or IgM in serum with the same ratios in bronchoalveolar fluid.

Dr. Richerson: Dr. Mattsby, where are the urinary antibodies being produced and how do they get into the bronchial fluid without being detected in the blood?

Dr. Mattsby: The urinary antibodies are probably mainly of local origin, and we have found antibodies of all three Ig classes in the urine of rats. The local formation may perhaps partly be attributed to the lymphoid cells and cell aggregates observed in the submucosa of the rat urinary bladder. The submucosal lymphoid cells may perhaps function in a similar mode as has been reported for lymphoid cells from Peyer's patches or BAL in that the cells may migrate to other submucosal sites of the body giving rise to preferentially IgA producing lymphocytes. Intestinal colonization of pregnant women with a bacterial strain give rise to antibodies in their milk but not in serum, maybe also indicating migration of antigen-stimulated lymphoid cells. IgA antibodies could not be detected in serum at the dilution used as compared to our reference sera.