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**DISCUSSION**

Dr. Henson: When we presented our somewhat similar findings at the ATS meetings, the first question asked by Dr. Ward related to the possible presence of endotoxin in our instillates. This is obviously something you're concerned about. It might be worth your making a comment about it since the question of initiating the cleavage of C5 is of some interest.

Dr. Kreutzer: Everything that we instill into the lungs of the hamsters we analyze by limus lysate routinely whether it's a C5 fragment or whole C5 we find 10-25 nanograms endotoxin equivalents/ml. We also find in our control substances, albumin or IgG, a similar content of endotoxin. So it implies that endotoxin per se, in mixtures of proteins, will not induce the reaction. We've also taken endotoxins both crude and in various degrees of purity, and instilled them in the lungs of the hamsters in the range of picogram through microgram quantities, and have not been able to induce any acute inflammatory reaction in the hamster lung.

Dr. Rylander: This is a most surprising result. We have been studying the effect of inhaled endotoxins in a variety of animal species, using the increase in the leukocyte number in the lung as the index. At the concentrations that you indicated we get a regular increase of leukocytes in the lungs of guinea pigs, hamsters, rats and rabbits; so there clearly must be some discrepancy.

Dr. Kreutzer: We're aware of papers that indicated aerosol endotoxin caused these reactions. We were instilling it transtracheally. All I can say is that in the hamster we were unable to induce significant poly influx at various doses of endotoxin using both crude and purified endotoxins which were prepared in different ways.

Dr. Ward: There might be a significant species difference among the animals with which you've been working.

Dr. Rylander: I don't think it's a species difference. We worked with hamsters as well as with rabbits and mice to make sure that the reaction we studied was not species-specific. It may be that intratracheal instillation is the difference.

Dr. Kreutzer: We have been extremely careful to control for effects of endotoxin in these reactions.

Dr. Merrill: Do you have any idea where the final end product resides once you instill it in the trachea? Have you labeled it and looked to see whether it's mainly deposited in alveoli or in airways? Also, what was the time course of the endotoxin experiments?

Dr. Kreutzer: To answer your first question, we find the polys only in the alveoli. We never see any polys in the upper airways. In regard to your second question, the data I presented here focuses on 1 and 4 hours. The endotoxin experiments included 1, 2, 4, 24 hour time points.

Dr. Merrill: Again, you don't really have any idea where most of the material winds up?

Dr. Kreutzer: No, I really can't say.

Dr. Merrill: Regarding the potential difference between tracheal instillation and aerosol, particle size may be quite different and the final residing place may be quite different.

Dr. Musson: Just a quick comment. I think Dr. Reynolds yesterday mentioned that C5 is one of the complement components found in lavage fluids. Is that correct? If C5 but not C5a is found in lavage fluid, then there is some sort of regulating mechanism to counteract the formation or activation of proteases in the lung which could convert C5 to C5a.

Dr. Kreutzer: The only comment I'd have on that is that in normal lung lavages there is an extremely low amount of C5. We're instilling 300 μg of C5 into the hamster lung and it may be that the amount of C5 that's delivered to the site is enough to cause a reaction. If there are small amounts of C5 in the normal lung, there isn't enough to cause a detectable reaction.

Dr. Reiss: I'd like to comment on the different methods of application of endotoxin. Endotoxin is a lipopolysaccharide and it's quite possible that this material forms different micelles depending on whether it's an aerosol or in solution. It's well known that enzymes can attack one micelle and not another.

Dr. Henson: I'd like to comment about the C5 cleavage again. I don't think you're going to have to have very much of your C5 cleaved to produce the type of inflammation that was seen. We get comparable effects with less than a microgram of C5a, which means that it would not require that very much C5 be instilled.

Dr. Kreutzer: We calculate that approximately 10 μg of inactive peptide would be generated from 300 μg of whole C5 if this was completely cleaved.

**Diffuse Pulmonary Interstitial Disease; An Immunohistologic Study**

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There has been increasing clinical, physiologic, histologic and immunologic evidence that diffuse pulmonary interstitial disease may develop in patients with autoimmune diseases associated with circulating antigen-antibody complexes (systemic lupus erythematosus and rheumatoid arthritis). 1-8 Several investigators believe this relationship is not coincidental. Others have postu

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lated a casual pathogenetic relationship between intravascular immunoglobulins and pulmonary interstitial disease. Tomasi and colleagues felt that precipitation of insoluble antigen-antibody complexes in the pulmonary capillary bed and interstitium might prove to be a pathogenetic mechanism for pulmonary disease. Bretjens et al demonstrated interstitial lung disease in animals following production of circulating immune complexes.

The purpose of this study was to determine if immune complexes are present in the lung tissue of patients with diffuse pulmonary interstitial disease, pulmonary fibrosis and interstitial disease associated with autoimmune diseases in order to determine whether there is a possibility that immune complexes may play a role in the genesis of pulmonary disease.

**Materials and Methods**

Lung tissue was obtained from 38 patients by open lung biopsy or from autopsy specimens. The lung tissue was quickly frozen in liquid nitrogen, cut into 6 micron sections, and then fixed by air drying for 30 minutes. These sections were then washed in phosphate-buffered saline solution to remove free proteins and serum and were then incubated for 30 minutes with Meloy Laboratory fluorescein conjugated antisera to human IgG, IgM, IgA, IgD, IgE, β2, C and albumin. The preparations were then viewed under a fluorescent microscope. Focal, diffuse, perivascular or peribronchial deposition was described and the intensity and extent arbitrarily graded from 0 to 4. Normal lung tissue was similarly prepared and evaluated.

Hematoxylin-eosin stained slides prepared from the fresh-frozen sections used for the immunologic portion of the study were read and interpreted by the UCLA Pathology Department.

**Results**

Lung tissue from 38 patients studied by direct immunofluorescent techniques fell into four groups. The first three groups included active interstitial pneumonia (seven), Pneumocystis carinii pneumonia (11), interstitial pneumonitis associated with autoimmune diseases (eight) and the fourth group, end-stage pulmonary fibrosis (12).

Our results revealed striking immunofluorescent deposition with conjugated anti-IgG, IgM and IgA antibody in the presence of β2C deposits in all seven patients with active interstitial pneumonia of unknown etiology. Albumin was present in three. IgD and IgE were absent. In a second group of 11 patients with Pneumocystis carinii pneumonia, seven had a combination of IgG or IgA associated with the deposition of complement. Albumin, IgD and IgE were not present. In a third group consisting of 12 patients with active interstitial disease associated with collagen-vascular disease, IgG, IgM, IgA and complement were present in every case. Albumin was found in one case and IgD and IgE in none.

Immunoglobulins G, M and A were present less frequently and found with complement only in one of 12 patients in the pulmonary fibrosis group.

The immunoglobulins and complement were found in diffuse or focal patterns on the thickened interstitium. There were no perivascular or peribronchial deposits.

**Discussion**

In active interstitial pulmonary disease, striking immunofluorescent deposition was found with conjugated anti-IgG, anti-IgM, anti-IgA and anti-complement in every patient with interstitial pneumonitis, in 82 percent (9/11) of the patients with Pneumocystis carinii pneumonias and in all eight cases with pulmonary interstitial disease associated with the collagen-vascular diseases (systemic lupus and rheumatoid arthritis). The pattern of deposition was coarsely granular throughout the interstitium.

The data suggest that immune complexes consisting of IgG, IgM or IgA have a propensity to be phagocytized by endothelial cells and macrophages or to be found on the phospholipid-containing cell or basement membranes. Because complement is present, it is possible that at the cell membrane adsorption results in complement fixation, which could result in inflammation and cell lysis. The presence of increased cellularity and antibody suggests an alternate pathogenic mechanism utilizing antibody-dependent cell mediated toxicity.

In the group of patients with pulmonary fibrosis, immunoglobulins were associated with complement in 8 percent of the patients. Immunoglobulins G, M or A were present in five of 12 patients with pulmonary fibrosis, whereas in active interstitial pneumonia, 25 patients had positive IF deposits and only one did not (Chi square = 14.7; P < .001). The immune complexes and complement were present during the active pre-fibrotic stage of pulmonary interstitial disease, but were infrequently present together in end-stage pulmonary fibrosis.

The literature suggests that immunoglobulins G and M are present with complement on lung tissue in patients with diffuse pulmonary interstitial disease associated with systemic lupus or rheumatoid arthritis. Our data revealed that immunoglobulins and complement were present in patients with other active pulmonary interstitial diseases, such as Pneumocystis carinii pneumonia, the usual interstitial pneumonias, as well as pulmonary interstitial disease associated with the collagen vascular diseases.

The presence of both immunoglobulins and complement in the pulmonary interstitium of patients with active pneumonitis supports the proposal that the pulmonary disorder is in part immunologic in origin.

**References**


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Pathogenetic Studies in Idiopathic Pulmonary Fibrosis*

Control of Neutrophil Migration by Immune Complexes

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Accumulation of neutrophils within the lung par-enchyma is characteristic of idiopathic pulmonary fibrosis (IPF) and likely plays a role in the interstitial abnormalities found in this disease. In order to assess factors which influence neutrophil traffic within the IPF lung, bronchoalveolar lavage was used to obtain effector cells and their products from the lower respiratory tract of seven IPF patients and six normal subjects. A 125I-C1q binding assay revealed a mean two-fold increase in C1q binding in IPF lavage fluid compared to normal subjects (P = 0.025), suggesting the presence of immune complexes in the lower respiratory tract of patients with IPF. The presence of local immune complexes in IPF was corroborated by the finding that alveolar macrophages obtained from these patients exhibited depressed antibody dependent cellular cytotoxicity. The pathogenetic significance of this immune complex-like material was suggested by the release of a potent neutrophil chemotactic factor (CF) from normal alveolar macrophages when incubated (3 hr, 37°C) with particulate (IgG-sheep RBC) or soluble (albumin-IgG antialbumin) immune complexes. Additionally, in a 3 hr culture, IPF alveolar macrophages "spontaneously" released CF in quantity comparable to that present in immune complex stimulated, normal AM supernatants. This finding was paralleled by the generation of CF by guinea pig alveolar macrophages incubated with IPF lavage fluid.

In summary 1) immune complex-like material is present in IPF lung and on the IPF alveolar macrophage surface; 2) both IPF lavage fluid and prepared immune complexes induce normal alveolar macrophages to secrete neutrophil CF, and 3) IPF alveolar macrophages "spontaneously" generate CF. These findings suggest immune complexes may produce the influx of neutrophils to IPF lung via interaction with the resident alveolar macrophage population.

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Discussion

Dr. Ward: Why do you exclude the role of the complement-fixing immune complexes and activation of complement?

Dr. Gadek: We have indirectly analyzed the potential role of soluble or serum-derived chemotactic factor, in particular complement-derived chemotactic factor, by attempting to look at evidence for conversion or turnover...