Pathogenetic Studies in Idiopathic
Pulmonary Fibrosis*

Control of Neutrophil Migration by Immune Complexes

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Accumulation of neutrophils within the lung parenchyma 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 pathogenic 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 anti-albumin) 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...
We've been unsuccessful in that attempt. In addition, of complement components obtained from lavage fluid. We've looked directly at the lavage fluid for evidence of intrinsic chemotactic activity present within the lavage fluid and presumably derived from serum or complement components and again have been unable to find it—possibly because of technical problems related to the sensitivity of those assays. I don't at all exclude the possible role of the more classic chemotactic factors.

Dr. Turner-Warwick: These again are beautiful studies. We can understand the accumulation of neutrophils in IPF. How do we explain the accumulation of the alveolar macrophage in these lungs?

Dr. Gadek: The increased number of alveolar macrophages obviously is a problem that has caused us some consternation as well. We think that in terms of the flux or traffic or inflammatory cells, the presence of relatively small numbers of neutrophils may be a more impressive phenomenon than the mononuclear phagocytes. However, one is still posed with the problem of explaining the predominance of the mononuclear phagocytes. Some preliminary studies demonstrated that once the neutrophils accumulate they elaborate a factor which is chemotactic both for mononuclear cells and/or polys. Whether those are products of cell death or specific cell-derived chemotactic factors isn't clear. Certainly once the neutrophils have intruded into the lung parenchyma then that opens the flood gates and at that point the serum or complement derived chemotactic factors play an important role.

Dr. Kohler: Did you characterize the immune complex-like material or the C1q binding material? Are they identical in the bronchial lavage vs the circulation, quantitatively?

Dr. Gadek: That's certainly a good point. We have yet to characterize them on a physicochemical basis.

Dr. Schwartz: I'd like to congratulate you on a very nice study. We've had the opportunity to study at least 15 cases of sarcoidosis with diffuse disease in which the alveolitis looks very similar to what is seen with the idiopathic variety and I have never seen the deposition of immunoglobulin or component within the alveolar walls. However, it's not unusual to see it around the granuloma. In other words, we see IgG, possibly IgM, and also complement on the periphery of the granuloma. I have one question to ask you. In the patients with IPF, what was the difference in the patients who were negative and positive for complexes and chemotactic factors?

Dr. Gadek: There was a correlation between the amount of immune complex activity, the amount of cellularity and the recovery of neutrophils in lavage, and the cellularity of the biopsies obtained. The immune complex activity seems to identify that subpopulation of IPF which is still active.

Dr. Kreutzer: Is the elaboration of chemotactic factor in sarcoidosis, in IPFs and in normal subjects blocked by inhibitors of protein synthesis? Is this really a de novo synthesis of chemotactic factor?

Dr. Gadek: Obviously we would like to think that this is de novo synthesis of a small peptide or a larger peptide in terms of the sarcoidosis-derived cells. We don't have information that directly relates to this particular experiment. In other experiments the release of chemotactic factor from these cells is blocked by inhibitors of protein synthesis. In addition, we've set up some long-term cultures where we have sampled supernatants at various time periods. The chemotactic factor has continued to be released despite the washing or other manipulations during culture.

Chronic Inflammation Involving Cellular Hypersensitivity*

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Chronic inflammation is a prominent feature of immunologic lung diseases. It involves a variety of interacting mechanisms. In our BCG model of chronic inflammation, we evaluated some of these mechanisms.

Persistence of Antigen. For inflammation to be chronic, the irritant must persist, but relatively little is known about persistent antigens or immune complexes as a chronic stimulus to inflammation. Histochemically, the peroxidase-antiperoxidase (PAP) immunocytochemical technique can detect persisting antigens. It was used to show that the waxes of tubercle bacilli persist in macrophages longer than their polysaccharides, which in turn persist longer than their protein components.

Production and Release of Hydrolytic Enzymes. Persistent irritants, directly and/or indirectly via immunoreactive lymphocytes and their lymphokines (cellular hypersensitivity), cause macrophages to secrete a variety of neutral-acting hydrolases, including collagenase, which perpetuate inflammation. Acid-acting hydrolases are also produced and released by autolysis and regeneration. Cathepsin D was found by histochemical techniques to adhere to necrotic tissue and remain active at least several days after cell death.

Turnover of Macrophages. Macrophages arriving from the blood stream may divide several times, but older macrophages do so infrequently. Most of the macrophages in BCG lesions are replaced in a week or ten days.

Differentiation of Macrophages. Some of the macrophages that enter the lesions differentiate into cells rich in acid-acting hydrolytic enzymes, and some differentiate into secretory macrophages. Preliminary work with histochemical stains for esterase, acid phosphatase and β-galactosidase suggests that secretory macrophages might be distinguished from ingesting and digesting macrophages in sections of chronic inflammatory lesions. When developed, the PAP technique for collagenase or elastase should also distinguish different.