of complement components obtained from lavage fluid. We've been unsuccessful in that attempt. In addition, 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 Clq 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. Schwaarz: 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**

Arthur M. Dannenberg, Jr., M.D., and Sadanobu Higuchi, M.D.

*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

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populations of macrophages.

Increased Blood Supply. The number of capillaries supplying the BCG lesions was increased two to three times over the number in normal skin and remained at this level until the lesion healed.

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DISCUSSION
Dr. Henson: I'd like to ask a couple of questions about the quantitative release of enzymes into the chamber. Could you not interpret the data as showing that as cells die all the enzymes are released, including lysozyme? And can you determine that the enzymes come from macrophages?

Dr. Dannenberg: When you study something in vivo, it is very complex. The cells are entering, dying (or leaving). In the chamber bed most of the cells are macrophages. Macrophage collagenase (which is secreted and not stored) would come from live (not dead) cells. However, lysozyme is both secreted and stored and much of the PMN collagenase is stored and released by regurgitation or cell death. The number of PMN decreased after 4 days, but the number of activated macrophages peaked at 11 and 18 days. At this time, the amount of collagenase in the chamber fluid also peaked. We therefore feel we were measuring macrophage (and not PMN) collagenase.

Dr. Brooks: In histologic studies reported, there are usually only a few organisms in the solid caseum. Do you have any information on the make-up of caseum and how it affects multiplication of the tubercle bacilli?

Dr. Dannenberg: Caseum is known to be inhibitory to the growth of tubercle bacilli, probably from some of the fatty acids and the anoxic condition that is present in it. However, bacilli grow well in caseous material after it liquefies. During liquefaction, enzymes partly hydrolyze the lipids, proteins and nucleic acids in caseous tissue, so that its osmotic pressure increases. As soon as that occurs, fluids rush in and probably oxygen, especially if a bronchus or bronchiolo is eroded. Since tubercle bacilli multiply readily in liquefied caseous material, the whole disease exacerbates.

Dr. Davis: Your very elegant histochemical stains raise the possibility of looking at different functional populations of macrophages, as well as different morphologic ones, and I wonder if time-course studies or any other studies allow you to predict whether these are sequences in the lifespan of the macrophage. In other words, are the same cells going through these functional states? Or do you view the secretory cell as being an end-stage macrophage of one type and the lysozyme-rich macrophage as being an end-stage cell of a different type?

Dr. Dannenberg: Macrophages probably differentiate for different functions. Macrophage secreting factors which effect lymphocytes or secreting colony stimulating factors which effect bone marrow cells are probably not rich in all the digestive enzymes, but this remains to be shown. There may be markers by which you could identify different macrophage populations and it is possible that local factors make macrophages differentiate in different directions. In addition, some macrophages may both secrete and digest.

Comparison of the Alveolitis of Sarcoidosis and Idiopathic Pulmonary Fibrosis*


Sarcoidosis and idiopathic pulmonary fibrosis (IPF) are associated with a parenchymal accumulation of inflammatory and immune effector cells (EC). Sarcoid, in contrast to IPF, is associated with parenchymal granulomata, suggesting that the EC present in the lung in sarcoid may differ from those in IPF. To evaluate this hypothesis, EC were isolated from the lung by bronchoalveolar lavage (BAL) (five sarcoid, five IPF, six normal subjects) and compared to EC in blood. To demonstrate that the EC present in BAL were representative of those present in the lung parenchyma, we also isolated from open lung biopsies (three sarcoid, three IPF). In IPF, the BAL was characterized by an increase in the percentage of neutrophils compared to normals (23 ± 10 vs 1 ± 1). In sarcoid, BAL was characterized by increased percentage of lymphocytes compared to normal subjects (7 ± 7 vs 7 ± 1, P < 0.001). In controls and IPF, the percentage of T-lymphocytes were similar in BAL and blood (P > 0.2 all comparisons). In marked contrast, sarcoid patients had a higher percentage T-lymphocytes in BAL compared to normals.

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