IMMUNOLOGIC LUNG DISEASES (NON-ASTHMATIC)

Immunologic (Nonasthmatic) Disease of the Lung*

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The many experimental models of immunologically-induced pulmonary damage have proved that contact with inhaled antigen will induce acute and chronic inflammatory changes in lung, varying from acute, neutrophil-rich interstitial and intra-alveolar exudates, to granulomatous interstitial reactions and interstitial fibrosis. This knowledge is emphasized by the work presented in this Conference by Drs. Richerson and Moore and their colleagues. For most of the studies involving animal models, however, it has been difficult to be precise about the immunopathologic events involved, because of the likelihood that both cellular and humoral immunoreactivities are present. Based upon a great deal of laboratory work, it seems clear that both humoral as well as cell mediated immune reactions can induce pulmonary inflammatory reactions, either independently of one another, or synergistically. This would be analogous to immune complex-induced glomerulonephritis in which both anaphylactic as well as immune complex mechanisms are operative and result in the functional and morphologic features of glomerulonephritis.

From our own experimental studies in which the role of defined immunologic mechanisms in lung injury have been probed, and using reasonably unambiguous approaches, it has been demonstrated in a reverse passive Arthus type model that the in vitro deposition in lung airways of immune complexes results in an acute, neutrophil-rich interstitial and intra-alveolar reaction. The pulmonary damage seen in this model requires the participation of neutrophils, presumably through their delivery of hydrolytic enzymes that are inimical to substrates in structural elements (such as collagen, elastin, basement membrane and others) in the tissue. It has recently been possible to reproduce this lung inflammatory reaction in experimental animals using preformed immune complexes that are instilled intratracheally. Histologically, these resulting reactions are rather similar to those described above, in that there is a diffuse interstitial and intra-alveolar accumulation of neutrophils and considerable intra-alveolar edema and hemorrhage. Not surprisingly, these reactions also are complement and neutrophil-dependent. The phlogistic nature of the complexes is directly related to their size and, probably more importantly, to their complement fixing capacities. In antigen excess, as the complement fixing activity rapidly drops off (at antigen concentration > 3 times the point of equivalence), there is a concomitant fall-off in the ability of the complexes to cause vasopermeability changes in lung, an accumulation of neutrophils, and intra-alveolar hemorrhage. Whether the size of the complexes is related to their phlogistic potential is not currently known. While intratracheal instillation of preformed immune complexes does not reflect any naturally occurring disease process, the use of these preformed complexes does permit a probing of potential immunopathologic mechanisms in a manner that has not been successful with the kidney or any other organ. In the many immunopathologic studies of experimental glomerulonephritis, the infusion of preformed immune complexes has failed to induce glomerulonephritis. Thus, our own studies of lung-induced injury by preformed immune complexes has the potential of defining, for the first time, those immune complexes that are phlogistic for tissues, and they should permit a definition of the pathogenesis of the inflammatory reaction induced in lung.

The continued injection of antigen into rabbits sensitized to bovine serum albumin has been shown by others to result in a diffuse interstitial pulmonary fibrosis with thickening of vascular (capillary) basement membrane and intramembranous deposition of immune complexes. Both the manner in which these reactions have been induced, as well as the details of the immunopathology of these lesions are, in virtually every respect, identical with the earlier classic studies carried out in the kidney. These reactions are almost surely due to the deposition in tissues of large amounts of immune complexes, resulting in the triggering of a cellular inflammatory response that ultimately is associated with activation of interstitial fibroblasts and the deposition of large amounts of collagen. Thus, in studies using preformed immune complexes, the likelihood is high that, given the appropriate circumstances, pulmonary fibrosis should sooner or later ensue.

The role of immune complexes in the pathogenesis of human inflammatory lung diseases has been postulated in hypersensitivity pneumonitis where, initially, patients with farmer's lung or pigeon fancier's disease were found to have precipitating antibody in their sera. However, the subsequent lack of correlation between presence or absence of precipitating antibody in the serum and the presence or absence of pulmonary disease, and the demonstration of a better correlation of disease activity with lymphocyte reactivity (blastogenesis) to antigen have suggested that immune complexes may not be a proximate cause of the inflammatory changes in the lungs of patients with farmer's lung or pigeon fancier's disease.

Recently, sera from patients with idiopathic pulmonary fibrosis have been shown to contain a complement fixing factor that binds to the surfaces of RAJI lymphocytes. This has been interpreted as evidence for the presence of immune complexes in the sera of these patients. In this Conference, evidence is presented that bronchial lavage fluids from the same patients yield a similar substance (putative immune complexes) as de-
tected by Clq-binding assays. These data suggest that patients with idiopathic pulmonary fibrosis have immune complexes both in their lungs, as well as in their blood stream. The relevance of these observations to the underlying disease process is entirely unknown, but this appears to be the first clear-cut association between the presence of detectable immune complexes and lung disease in humans.

To what extent humoral and/or cellular mechanisms are responsible for the inflammatory diseases seen in humans is still largely unknown. The development of animal models to study fundamental reactions of lung to inflammatory mediators, together with the newly developing techniques for retrieval from the lung of soluble factors and cells, should provide the basis for an understanding of the pathogenesis of a large number of inflammatory lung diseases.

REFERENCES


DISCUSSION

Dr. Musson: At what time did you examine the injury in neutrophil-depleted and complement-depleted animals? Dr. Ward: Most of these studies were done either at 6, 12 and 24 hours. The Table presented had data from animals at 6 hours.

Dr. Kohler: Do you ever see any capillary or vascular injury, particularly when you do a reverse passive Arthus?

Dr. Ward: We never see a vasculitis per se. There is clear evidence that there is vascular injury as indicated by the leakage of the markers which persists for about a 2-day period; the evidence of hemorrhage also indicates that vascular damage has occurred. We’ve not yet done ultrastructural studies; so I can’t tell you what the capillaries look like or in fact what some of the venules and arterioles look like in the lungs.

Induction of Acute Inflammatory Reactions in Lung following Intrapulmonary Instillation of Preformed Chemotactic Peptides and Purified Complement Components

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Inflammatory reactions induced by infections, pollutant or immunologic mechanisms may result in extensive pulmonary injury. As in the case with many other organs, this tissue damage, at least in part, is considered to be due to the effects of various hydrolytic enzymes released from leukocytes. Pulmonary inflammatory reactions induced by the formation in lung of immune complexes are complement-dependent. Similar reactions occurring in walls of dermal blood vessels have been shown to be mediated by the generation of chemotactic factors derived from the complement system. Thus, in a variety of reactions, leukotactic mediators appear to play a central role in the outcome of the reaction. In this study we present results that directly demonstrate the ability of preformed chemotactic mediators to induce acute inflammatory reactions in lung.

MATERIALS AND METHODS

Chemotactic and Complement Factors

Chemotactic factors were characterized with modified Boyden chambers, using rabbit and hamster leukocytes as indicator cells. Functional chemotactic activity was expressed in ED₅₀ of chemotaxis (i.e one ED₅₀ of chemotaxis equals the amount of chemotactic required to give 5% maximal chemotactic response). The chemotactic factor from the fifth component of complement (C₅ fr) was isolated from zymosan-EACA activated human serum by gel filtration in Sephadex G100 and G75. The synthetic peptide formylmethionyl-leucyl-phenylalanine (F-Met-Leu-Phe) was obtained from Dr. Elmer Becker and was dissolved in dimethyl sulfoxide (DMSO) and buffered containing 5 mg/ml bovine serum albumin. DMSO alone and DMSO-BSA were used as negative controls in all experiments in which F-Met-Leu-Phe was employed. Whole C₅ was isolated from human serum by the method of Nilsson et al. The purified protein gave a single band when studied analytically in either basic or SDS polyacrylamide gel electrophoresis.

Intrapulmonary Instillation

The various factors were instilled intratracheally by direct tracheal cannulation under direct laryngoscopic visualization. Adult male hamsters weighing 75-100 gm were used for these studies. After appropriate intervals, animals were sacrificed and lungs processed for routine light microscopy.

*From the Department of Pathology, University of Connecticut Health Center, Farmington. Supported in part by National Institutes of Health Grants HL 22437, HL 23122, AI 09851, HL 07202. Reprint requests: Dr. Kreutzer, University of Connecticut Health Center, Farmington 06032

CHEST, 75: 2, FEBRUARY, 1979 SUPPLEMENT

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