Immune Complex Injury of the Lung

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Abundant evidence currently exists to suggest that immune complexes play an important role in inflammatory diseases of the lung. Clinically, idiopathic pulmonary fibrosis, eosinophilic granuloma of lung, and systemic lupus erythematosus have been shown to be associated with the presence of immune complexes both in lung and in the serum. Experimentally, there is compelling evidence that acute lung injury can be triggered by the deposition of complexes in vascular walls or by the presence of performed immune complexes instilled into the airways. The observed reactions are, as expected, complement- and neutrophil-dependent. The morphologic changes in lung caused by products of complement activation (C5a and related peptides) depend on whether complement activation occurs within the vasculature or within the airways. Airway activation is associated with intraalveolar accumulations of neutrophils, while intravascular activation leads to intracapillary sequestration of neutrophils. The chronic formation of immune complexes within the vasculature (in the model of "chronic serum sickness") leads to an interstitial fibrotic reaction and a thickening of basement membranes. Recent studies of intravascularly infused preformed immune complexes indicate a proclivity for certain types of complexes to localize within lung. These "lung-seeking" complexes differ from non-lung-seeking complexes only in the ratio of antigen to antibody. Complement does not seem to alter the tendancy for certain complexes to localize within lung. These studies emphasize the potential importance of immune complexes in lung injury and point out the variety of mechanisms involved in both the localization process and the injury process. (Am J Pathol 97:85-92, 1979)

THERE IS CONSIDERABLE CURRENT EVIDENCE that immune complexes play a significant role in the pathogenesis of interstitial inflammatory reactions in the lung. The clinical evidence is both direct and indirect (Table 1). In hypersensitivity pneumonitis, repeated exposure to inhaled antigen results in acute physiologic changes as well as progressive interstitial fibrosis.[1] Although patients with this disease have in their serums precipitating antibody to the offending antigen, the presence of antibody does not correlate with presence of disease.[2] The chief evidence of the role of immune complexes is entirely indirect, based on the appearance of pulmonary dysfunction 4–6 hours after aerosol challenge with antigen. A more convincing case for the presence (and presumed role) of immune complexes pertains to idiopathic pulmonary fibrosis. In individuals with this disease, immune complexes can be demonstrated in serum by the

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Table 1—Clinical Evidence for Immune Complexes in Inflammatory Diseases of the Lung

1.	Clinicopathologic features of hypersensitivity pneumonitis
2.	Idiopathic pulmonary fibrosis
	Immune complexes in serum
	Immune-complex–like material in bronchial lavage fluids
	Immunofluorescent evidence of complexes in lung tissue
3.	Eosinophilic granuloma
	Immune complexes in serum
4.	Systemic lupus erythematosus
	Complexes deposited in alveolar capillary walls and in pulmonary basement membranes

RAJI assay.[3] In addition, similar material, as defined by binding with C1q, has been found in bronchial lavage fluids.[4] Finally, immunofluorescence studies have revealed the presence in the lung interstitium of immunoglobulins and antigen in a pattern suggestive of immune complexes.[5] Immune-complex–like material has also been reported in the serums of patients with eosinophilic granuloma of the lung.[6] Finally, in systemic lupus erythematosus, there is, as expected, evidence for immune-complex deposits both in pulmonary vessels as well as in capillary and alveolar basement membranes.[7] Thus, there is substantial support for the presence and the presumed role of immune complexes in a variety of inflammatory interstitial diseases of the human lung.

There is abundant experimental evidence that immune complexes are pathogenic and produce inflammatory reactions in the lung (Table 2). Acute deposition with rat lung of IgG-containing immune complexes leads to an explosive hemorrhagic, interstitial, and intraalveolar inflammatory reaction.[8] This response is triggered by the airway instillation of antibody and the vascular injection of antigen. Being complement- and neutrophil-dependent, it resembles the reversed passive Arthus vasculitis reaction in dermis. Complement blockade or the lack of availability of neutrophils results in abolition of the lung parenchymal reaction, even though immune complexes can be demonstrated in the tissue. Presumably, the parenchymal damage can be related to protease release by leukocytes that have accumulated in response to the local generation of complement-dependent chemotactic factors. The intraperitoneal injection into guinea pigs of antibody-rich serum followed by aerosol challenge with antigen results in morphologic findings virtually identical to those described above.[9] In addition, the inflammatory reaction produced in this model is complement-dependent.

Table 2—Experimental Evidence for the Phlogistic Activity of Immune Complexes in the Lung

1.	Reverse passive Arthus type of reaction in lung
2.	Chronic model of "serum sickness"
3.	Reactions induced by airway instillation of preformed immune complexes

In a chronic model of immune-complex disease ("serum sickness") in rabbits, as described below, the intravascular production of immune complexes results in a series of irreversible interstitial and basement-membrane changes.[10]

Preformed immune complexes injected into the airways of rats are also intensely phlogistic for the lung parenchyma.[11] Diffuse, confluent, hemorrhagic reactions develop in the alveolar spaces, walls, and interstitium. The predominant cell type is the neutrophil. Like the reversed passive Arthus reaction in lung (see above), the reaction induced by preformed immune complexes is neutrophil- and complement-dependent. Presumably the same pathogenesis pertains: complement fixation with generation of complement mediators, influx of neutrophils, phagocytosis of complexes, release of lysosomal enzymes, and concomitant lung damage. The phlogistic activity of the preformed complexes is directly related to the ratio of antigen and antibody in the complexes. This ratio, in turn, is directly related to the complement fixing activity of the complexes. Thus, there is strong, direct evidence in support of the ability of immune complexes to inflict damage in the lung. To what extent the size of the immune complexes is relevant to the phlogistic activity of the complexes and what other factors are involved in the pathogenicity of immune complexes are now known. The ability to reproducibly trigger acute inflammatory reactions within the lung in a quantitatively reproducible manner provides the first opportunity in any organ to probe directly the phlogistic nature of immune complexes.

Based on a variety of clinical and experimental data, it seems that a general picture is emerging regarding the morphologic changes appearing in lung after exposure to immune complexes (Table 3). The acute changes are associated with the presence of neutrophils, fibrin, and hemorrhage, usually in an intraalveolar and interstitial location. Rarely are bronchiolar changes evident. Because of the compelling evidence that the immune-complex–induced reactions are complement-dependent, the presence of the fibrin deposits could well be related to the recent demonstration that C5a (a well-known product of complement activation and the chief chem-

Table 3—Morphologic Changes in Immune-Complex–Induced Lung Reactions

Acute changes (intraalveolar)
Neutrophils
Fibrin
Hemorrhage
Chronic changes (interstitial)
Chronic inflammatory cells
Fibrosis
Thickening and reduplication of alveolar and vascular basement membranes

otactic peptide derived from C5) induces the production of thromboplastic activity from leukocytes.[12] The chronic changes in immune-complex–inflicted damage to lung are primarily focused on the interstitial compartment. Here, chronic inflammatory cells accumulate, together with thick collagenous bands. A reduplication and thickening of both vascular (capillary) and epithelial (alveolar) basement membranes is also a prominent feature. No current explanation for these changes is available, although it is presumed that they are entirely due to the continued presence of immune complexes.

It has recently been possible to develop highly reproducible approaches to the quantitation of inflammatory reaction in the lungs.[13] In hamsters, homologous albumin has been labeled with ^{125}I, and homologous neutrophils have been labeled with ^{111}In, which has no influence on leukocytic functions, such as chemotaxis, a highly sensitively indicator of cell damage. These techniques have permitted a direct approach to the effects of preformed inflammatory mediators on the lung. Since it seemed highly likely that immune complexes trigger pulmonary inflammatory reactions by the generation of complement-derived chemotactic peptides (C5a), highly purified human C5a has been instilled into hamster lung (by intratracheal injection during inspiration). The instillation of 20–40 μg C5a into the airways results in a diffuse, confluent acute inflammatory response with large accumulations of neutrophils within alveolar spaces.[13] Less prominent, but present, is a peribronchial accumulation of neutrophils. The leukocytic accumulation can be measured as soon as 20 minutes after the instillation of C5a and shows a steady increase over a 4-hour period. Not surprisingly, in these same animals, little if any change in vascular permeability over the 4-hour period is seen, except for a slight rise (1.3 times that of controls) at 20 minutes. These data would suggest that when C5a is instilled into the airways, its effects are primarily on circulating leukocytes and are apparently minimal on endothelial cells and other structures relevant to vasopermeability changes. Using the same quantitative approaches, a doubling of the concentration of C5a considerably intensifies the accumulation of neutrophils and appears to speed up the process of leukocyte mobilization, which reaches a peak 60 minutes after the instillation of C5a. Thus, these data provide direct evidence for the phlogistic nature of C5a in the lung.

Another interesting piece of information has come from these studies: airway instillation of 200 μg intact human C5 (but not human C4, or hamster IgG or albumin) results in an intense, confluent intrapulmonary inflammatory reaction characterized by large numbers of neutrophils in alveolar spaces. The C5-induced reaction is delayed, with few changes

occurring during the first hour, but with an intense reaction developing by the fourth hour.[13] We have recently shown that this reaction is correlated with the intrapulmonary cleavage of C5, presumably due to the presence of alveolar enzymes such as those derived from alveolar macrophages. Details regarding the pathogenesis of this reaction are not yet known.

The intravascular infusion of C5a results in a somewhat different series of changes in the lung. Airway and interstitial changes are minimal and mainly focused on the vasculature. C5a (as well as other chemotactic factors), when incubated *in vitro* with neutrophils, induces at least two changes that can be measured by physical alterations of cells: a slight swelling and the formation of cell–cell aggregates (tricellular collections representing the average size of the aggregates).[14] It has been known for some time that the intravenous infusion of C5a or other chemotactic factors results in profound neutropenia, and it has been postulated that this is due to the trapping of neutrophils within the pulmonary capillaries.[15,16] This theory has been supported by morphologic observations at the levels of light and electron microscopy. Although there is no compelling evidence that this entrapment causes significant lung damage, the lung sequestration of neutrophils following intravascular contact with chemotactic factors deserves careful investigation.

Although, as stressed above, complement depletion interferes with immune-complex damage of lung, the complement-fixing activity of the complexes relates to their phlogistic activity in the lung; and while airway instillation of C5a mimics the lung inflammatory reaction, perhaps the most compelling evidence for the role of C5-derived chemotactic factors has come from the demonstration that the C5 chemotactic-factor inactivator (purified from human serum) is a potent suppressor of the acute lung inflammatory reaction induced by airway instillation of antibody and intravenous injection of antigen.[17] The admixture of minute amounts (10 μg) of the purified chemotactic-factor inactivator with the antibody completely abolishes the ability of the forming complexes to induce lung injury.

Finally, the recent experimental studies described above have emphasized the ability of immune complexes found on the air side of the lung parenchyma to trigger violent, lung-damaging inflammatory reactions. There is experimental support for the ability of immune complexes formed within the vasculature over a long period of time to induce irreversible pulmonary vascular and interstitial changes.[10] The vascular changes include a membrane proliferative reaction with immune complexes embedded within the membranes. The interstitium contains a dif-

fuse confluent chronic inflammatory reaction with advanced fibrotic changes. While it seems likely that all of these changes can be attributed to the presence of the complexes, this conclusion is inferential; and it is not possible to exclude the participation of other immune reactions, such as cell-mediated systems, in the pathogenesis of the lung-related inflammatory changes. There is a recent report suggesting that products (lymphokines) of activated lymphocytes have the ability to activate fibroblasts and increase collagen synthesis,[18] thus adding to the potential mechanisms in the immune system that may result in a fibrogenic response.

Recently we noted that the intravascular infusion of heterologous immune complexes into mice results in a significant localization of these complexes in the lung, as compared with the localization of the same complexes in the spleen, the liver, and the kidney.[19] The localization occurs rapidly (peaking at 1 hour) after infusion of the complexes, the localization is enhanced by histamine but not serotonin, and complement does not seem to affect the amounts of complexes localized within lung. No information is available about where precisely within the vascular network the complexes are localized. By immunofluorescence it appears that these lung-seeking immune complexes follow the capillary outlines; but whether the complexes are on, within, or beyond the endothelial cell boundary is not known. The preliminary studies suggest that there may be something unique about the lung for the localization of immune complexes. At this point, nothing is known about the more long-range effects of the presence of these complexes on the pulmonary vascular bed.

Obviously, little is really known about lung injury induced by immune complexes. However, as pointed out above, there is gathering evidence that complexes play an increasingly common role in inflammatory diseases of the lung. A detailed understanding of these mechanisms awaits extensive experimental studies.

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