The Role of Proteolytic Enzymes in the Pathogenesis of Emphysema

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ABSTRACT

Alpha_{1}-antitrypsin (a_{1}AT) deficiency states, which predispose one to the development of pulmonary emphysema, have been described with increasing frequency. While the precise physiologic role of this protein is not known, it appears certain that it protects the lung from proteolytic digestion by a number of enzymes. Data are now available which mimic this situation in the experimental animal and demonstrate the protective effect of a_{1}AT. Mechanisms of this genetic model for autodigestion and a number of pathological and biochemical considerations are offered in this paper.

Laurell originally observed that the absence of the alpha-1 band on routine protein electrophoresis strips was associated with pulmonary disease. Subsequent studies by Eriksson, elucidating the clinical nature of pulmonary emphysema associated with deficiency of alpha-1-antitrypsin have led to increasing interest in the role of this acute phase protein, or lack of it, in the pathogenesis of chronic obstructive pulmonary disease. Furthermore, what appears to be a genetic model for “autodigestion of the lung” has led to studies focusing on this disease from various viewpoints.

The alpha-1-antitrypsin deficiency state represents less than five percent of the total emphysema population. It differs from classical emphysema only in that it occurs at a younger age and is evenly distributed between male and female. Afflicted individuals appear to have less of a bronchitic component than the emphysema population at large but, by all parameters of physiological testing no distinct abnormality of lung function separates a_{1}AT deficient patients from other emphysema patients. Alpha-1-antitrypsin deficient patients characteristically have radiological evidence of lower zone emphysema, loss of elastic recoil, and abnormal ventilation/perfusion ratios in the lower lobes in addition to evidence of obstructive lung disease by spirometry. Studies of frequency dependent compliance and other sophisticated procedures have been reported and have only confirmed the similarity between a_{1}AT deficient patients and the general population of emphysema patients.

Emphysematous destruction of the lower lobes with dilatation of the air spaces and
destruction of lung parenchyma, not unlike the general emphysematous lung of panacinar emphysema, is the most common finding. The loss of elastic tissue appears to be the common denominator for all the physiologic and pathologic abnormalities but, to date, the mechanism of such destruction is poorly understood.

Biochemical and Genetic Considerations

Alpha-l-antitrypsin (α1AT) is one of a number of protease inhibitors found in serum. Other protease inhibitors are chymotrypsin inhibitor (CI), inter-alpha-trypsin inhibitor (ia-AT) and alpha-2-macroglobulin (AαM). Alpha-l-antitrypsin is a low molecular weight glycoprotein (M.W. 54,000) that migrates with the alpha-1-globulin fraction on routine protein electrophoresis and is responsible for ninety percent of the anti-trypsin activity of serum. It is not specific for trypsin since it has activity against elastase,45 plasmin,14 leukocyte protease,31 kallikrein46 and thrombin.14 It has little activity against proteases of plant origin.

Alpha-l-antitrypsin is an acute reaction protein that is produced in liver parenchymal cells and can be identified in serum, lymph, semen, liver and lung tissue. Blood levels increase in infection, with estrogen therapy, during stress and in pregnancy. This rise is attributed to increased production.

Eight α1AT zones have been described by acid starch gel electrophoresis. Various combinations of these zones identify 17 variants in the so-called protease inhibitor (Pi) system. These α1AT variants are inherited as codominant alleles and are designated by the two zones in which the major protein concentration exists. Antigen-antibody crossed electrophoresis has given similar results.

To date, the α1AT type that is unequivocally associated with pulmonary emphysema is type PiZZ, whereas PiMM is the most common type in normals as well as the general emphysema population. There is still debate over whether some of the heterozygous variants, PiMZ, PiSZ and PiFZ, predispose one to the development of pulmonary emphysema. Current evidence supports the thesis that it does, but suggests that evolution of the disease takes longer and that it is generally less severe.

Alpha-l-antitrypsin protease activity is commonly measured in the laboratory by assay of its inhibitory effect on trypsin, hence the name. Benzoyl-D, L-arginine-p-nitroanalide (BAPA) is a suitable substrate which gives the yellow color of p-nitroanaline when reacted with trypsin. Protein concentration of α1AT is determined immunologically by either the radial immunodiffusion technique of Mancini or by the "rocket" technique of Laurell. There is general correlation between the amount of immunologically identifiable protein and trypsin inhibitory capacity (T.I.C.) of serum.

Affected families and population surveys show a trimodal distribution of values in which very low values represent homozygous PiZZ states and intermediate values correlate with heterozygosity, PiSZ, PiFZ and PiMZ. Misidentification of the patient with intermediate deficiency as normal is an occasional problem because these patients respond to infection and the other factors by increased production of α1AT and thus may have normal values under such conditions. To be sure of heterozygous deficiency either genetic studies of the family or identification of the Pi variant must be performed. Homozygously deficient patients of the PiZZ type cannot similarly respond to reach levels which would be confused with intermediate values.

In addition to differential migration in an electrophoretic field, there is evidence to suggest that abnormal variants of α1AT are structurally different from normal.
Lieberman has observed large membrane bound globules crowding the rough endoplasmic reticulum of hepatic cells in patients with α1AT deficiency. Similar changes are seen in juvenile cirrhosis associated with α1AT deficiency. These globules are PAS positive, diastase resistant and contain α1AT by immunofluorescent staining. Changes have not been observed in normal livers. The reason for these changes is not known. Lieberman postulated that the α1AT molecule could not be released into the circulation owing to either the absence of a releasing factor or structural alteration which would not permit the molecule to enter the extracellular space. Our data on one patient confirm Lieberman’s observation. Although it was possible to identify the globules as α1AT by immunofluorescent staining, little protease inhibitor activity was detected in sucrose, buffered saline, tween-80 or in 0.001 M HCl extracts. Residual α1AT immunofluorescent activity remained in the unsolubilized pellet. It is suggested that α1AT in the liver of such patients is membrane bound and structurally different from that circulating in the blood.

Patients with α1AT deficiency of the Pi type do not necessarily develop hepatic scarring nor have patients with juvenile cirrhosis been observed to have emphysema. Furthermore, there are a number of instances of α1AT deficient patients of the Pi type who have neither pulmonary emphysema nor hepatic cirrhosis. This evidence suggests that there may be variants with the Pi type itself and that the Pi variety may simply be a marker for a yet unidentified genetic system.

Lieberman suggested that abnormal protein variants of α1AT deteriorate in vivo. He observed a “double ring” around all immunodiffusion wells of heterozygously deficient patients’ serum. Lieberman postulated that a portion of what appears to be a homogenous protein variant forms dimers which migrate differently in immunoochemical gel. Confirmation of this thesis awaits turnover studies of specific Pi varieties and verification that the plates used contained monospecific antibody.

Present evidence indicates that α1AT binds reversibly to the lysine residue of trypsin. However, there is no free trypsin in the extracellular or vascular compartments of the body. More consideration must be given to the known actions of α1AT against other proteases, especially elastase. To date, the mechanism of action of α1AT in vivo and its role in homeostasis is mere speculation with evidence to support various theories.

Pathophysiology

Since Gross first described the destructive properties of papain in the lungs of experimental animals and Goldring observed elastic fiber destruction in the lungs of these animals, research has centered on the search for an endogenous enzyme with elastoproteinase activity. Janoff recently characterized an esteroprotease in granules of polymorphonuclear leukocytes and macrophages. He observed that this enzyme was active at physiologic pH and was more destructive to the lung of experimental animals than collagenase. This enzyme, leukocyte elastase, when purified was found to be one-tenth as active as pancreatic elastase and more active than papain, trypsin and chymotrypsin against synthetic substrates in vitro.

Alpha-1-antitrypsin has been shown to inhibit the fibrinolytic activity of leukocytes. However, Gans was unable to demonstrate lack of leukocyte fibrinolytic inhibition in α1AT deficiency states. Lieberman has recently clarified this area by characterizing four neutral proteases in extracts of purulent sputum, only one of which is fibrinolysin. One is similar to leukoclastase, as described by Janoff, and the others are referred to as stable protease
Figure 1. Photomicrographs of rat lung after instillation of 1 mg. of papain transtracheally showing patchy alveolar destruction similar to centrilobular emphysema. a. Control at 24 h. b. Control at 48 h. c. Control at 6 days. d. Test animal at 24 h. e. Test animal at 48 h. f. Test animal at 6 days.
and liable protease. These proteases were not present in non-purulent mucoid sputum and were unrelated to the infecting organism.

Using hemoglobin substrates, it can be demonstrated in vitro that the proteolytic activity of the stable and liable protease is inhibited by normal serum, less so by heterozygous $\alpha_1$AT serum and only minimally by homozygous $\alpha_1$AT serum when equivalent dilutions are used. These leukoproteases in vivo cause disintegration of alveolar structure in hamster lung.

Normal serum can be shown to inhibit the rate of spontaneous proteolysis of homogenized human lungs. Furthermore, spontaneous autolysis of $\alpha_1$AT deficient lungs is higher than normal lungs and can be accentuated by the addition of leukocyte protease, an effect which is readily preventable by the addition of normal serum.

Intense macrophage activity has been observed when the lungs are exposed to cigarette smoke. McLaughlin and Tueller have observed a pigmented macrophage, thought to be a component of cigarette smoke, in lungs of patients with early emphysema. This pigmented macrophage is shed in the sputum of smokers and is absent in non-smokers. It can be recovered continually from the lungs of patients as long as five years after cessation of smoking. Harris postulated release of lysosomal enzymes from these macrophages as a factor which leads to alveolar damage in smokers.

Loss of elasticity is characteristic of all forms of pulmonary emphysema. Debate exists as to whether or not there is less elastic tissue per se, but it is clear that the elastic tissue of emphysematous lung is abnormal. Keller has shown that, in contrast to normal lung elastic tissue, neutral amino acid residues are decreased and polar amino acid residues are increased in emphysematous lung. This results in decreased cross linking and loss of overall elasticity. Similar changes have been observed in experimental emphysema.

Bacteria have been shown to produce inactivation of alpha-1-antitrypsin. Mashowitz has shown that species of Proteus and Pseudomonas produce large amounts of this so-called antitrypsin inactivator (AI) and suggests that lung destruction could result from AI overwhelming the protective protease inhibitor proteins of the $\alpha_1$AT deficient lung.

There appears to be no free elastolytic activity in the blood, as previously reported. With careful preparations, all elastase properties of blood are found in polymorphonuclear leukocytes and monocytes. Loeven has observed higher pancreatic levels of elastoproteinase in senile emphysema and a number of other degenerative conditions. This elastoproteinase attacks lung elastic tissue vigorously and increases destruction of lung in the experimental animal when superimposed pneumonia is present.

Werle has suggested that kallikrein, a potent substance active in the lung, may be responsible for some of the changes in pulmonary emphysema. It is only slowly inhibited by $\alpha_1$AT, even when $\alpha_1$AT is in excess, but may be active in the pulmonary capillaries when $\alpha_1$AT is overwhelmed or in $\alpha_1$AT deficiency states. Mustafa has suggested that the primary induction of protease release from macrophages and leukocytes may be caused by membrane associated biochemical reactions of lipase and has experimental evidence to confirm this reaction. Dukor has demonstrated that lysosomal proteases are under the control of cyclic AMP and the release of these enzymes is inhibited by colchicine.

**Experimental Emphysema**

One of the main thrusts of emphysema research has been to develop an experimental animal model in which to study the pathogenesis of the disease. Gross instilled papain into the tracheas of rats and dem-
onstrated anatomical changes not unlike centrilobular emphysema. Previous exposure to silica dust had a protective effect on these lungs. Goldring produced similar changes by aerosol and further demonstrated disruption of the elastic fibers.

Papain and other plant enzymes cause direct injury to the alveolus followed by inflammation, frank pneumonia and finally fibrosis and alveolar destruction (figure 1). When papain is directly instilled, centrilobular emphysema is produced; when it is aerosolized, panacinar emphysema is the usual lesion. Hamsters, which do not have antitrypsin in their serum, show an intense reaction to proteases which are instilled into the lung. Persistence of alveolar macrophages is observed after the inflammatory reaction. The detrimental effect on lung of other plant proteases in enzyme detergents has been confirmed in the experimental animal. Alcalase and maxatase, proteases produced by *B. Subtilis*, produce focal emphysema in hamster lung but the lesions are less profound than those of papain.

Kimbel produced emphysema in dogs by aerosolization of leukocyte protease harvested from human polymorphonuclear leukocytes, dog polymorphonuclear leukocytes and dog alveolar macrophages. Rabbit PMN extracts had little activity and did not produce convincing lesions. Janoff has subsequently shown that rabbit leukocytes do not have demonstrable elastolytic protease. Progesterone has been shown to protect experimental animals from papain emphysema without a permanent rise in the T.I.C. or increase in serum a1AT. Although the mechanism is not known, Lieb-erman surmised it is due to the anti-inflammatory properties of progesterone rather than its ability to increase circulating a1AT.

Turpentine by injection and nitrogen dioxide by inhalation, agents which are known to increase serum trypsic activity, have been given in conjunction with papain to experimental animals. Although there are marked increases in serum trypsic activity which are seen transiently, these substances did not prevent the eventual development of emphysema.

Mandl has produced emphysema in rats by injecting thermolysin and pancreatic elastase directly into the systemic circulation. No damage was seen in other organs. The loss of elasticity in the lungs of such animals was attributed to enzymatic attack of the valine and isolucine in the outer shell of elastin. In addition, a decreased ratio of polar to nonpolar amino acids was observed in these lungs. When papain is injected systematically rather than administered locally to the lungs, the supporting capillary network of elastic tissue in the lung is destroyed. Harley suggested that this is the primary event in papain emphysema and that the alveolar damage and loss of capillary bed is a secondary phenomenon.

Nitrogen dioxide (NO₂), a known pollutant found in cigarette smoke, increases proteolytic activity in lungs of exposed rats. Lunan demonstrated increased protein synthesis and increased protein soluble filtrate in such lungs when they were inflated with BAPA substrate. Since NO₂ oxidizes α-tocopherol and other fatty acid components of cell membranes, Lunan suggested that protease release in such animals is secondary to initial destruction of lipid membranes of cells which are undergoing rapid turnover.

The mechanics of papain lung in experimental animals have been studied by Park and Caldwell. Increased lung compliance and decreased elastic recoil have been demonstrated by plethysmography without change in the airway resistance. Park maintains that emphysema in such animals can develop without predisposing airway disease. Caldwell concluded that the abnormal mechanical properties of
such lungs is due to the loss of tethering properties of the airway rather than to changes in the viscoelastic properties of the airway wall.

**Summary**

The etiology and pathogenesis of pulmonary emphysema is still unknown. However, there is good evidence to suggest that autodigestion of lung parenchyma by proteolytic enzymes with elastase properties occurs in response to noxious inhalants and pulmonary infection. The source of these enzymes is most likely from endogenous polymorphonuclear leukocytes and macrophages which migrate from the capillary bed to alveolar space and subsequently lose their cellular integrity. Alpha-1-antitrypsin, a potent protease inhibitor with elastase inhibitory properties, protects the lung from proteolytic damage, in most instances. However, it is postulated that emphysema can occur when this enzyme inhibitor is abnormally low as in the cases of $a_1$AT deficiency.

**References**


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