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α_1-Antitrypsin Deficiency and Susceptibility to Lung Disease

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α_1-Antitrypsin (AAT), the major component of α_1-globulin, is capable of inhibiting proteolytic enzymes in polymorphonuclear leukocytes (PMN) and pulmonary alveolar macrophages (PAM). AAT is ubiquitously distributed in body fluids and tissues. It is a glycoprotein synthesized in the liver with a molecular weight of 50,000 daltons, a half-life of 3-6 days, and a sedimentation constant of 3.3 S. Enzymes inhibited include elastase, collagnenase, thrombin, kallikrein, and chymotrypsin. Serum AAT concentration is determined by multiple codominant alleles. The normal phenotype is M, the most severe deficiency state, Z; intermediate levels are associated with the MZ phenotype. Ethnicity is a major factor in determining phenotypic prevalence. The homozygous Z state occurs in 1/4000-1/8000 Caucasians.

Numerous disease processes, mainly involving lungs and liver, have been associated with AAT deficiency. Establishing an unequivocal etiologic relationship, however, between AAT deficiency and lung disease has been difficult. Studies in this area have varied widely in the number of individuals included, whether phenotypic analysis, or only quantitative determinations, were carried out, in the range of clinical and pulmonary function studies performed and whether the population was community or hospital based. Other confounding variables include the degree of industrial exposure, cigarette smoking, a history of childhood lung disease, and levels of proteolytic enzymes in PAMs and PMNs.

It has been estimated that about 80% of those with homozygous Z phenotype will develop obstructive pulmonary disease. The prevalence of lung disease in other non-M phenotypes, especially among heterozygotes is uncertain. Those investigations which support, and others which refute, an association will be reviewed.

Further investigations, especially those emphasizing levels of proteolytic enzymes, are needed to clarify the interrelationship of these variant phenotypes, environmental risks and the predisposition to chronic obstructive lung disease.

Background

α_1-Antitrypsin (AAT) is the major component of α_1-globulin. It is capable of inhibiting proteolytic enzymes in polymorphonuclear leukocytes (PMNs) and pulmonary alveolar macrophages. Its concentration is determined by about 30 codominant alleles. There are five other serum proteins such as α_2-macroglobulin, which also inhibit proteolytic enzymes, but these are of lesser importance, unless AAT is deficient. The capacity to inhibit enzymes is ubiquitously distributed in all body fluids and tissues and is found widely at all levels of the phylogenetic scale. Even leguminous plants such as cabbage, lettuce, and soya bean have inhibitory capacity. ATT is found in polymorphonuclear leukocytes, platelets, and bronchial lining. Enzymes inhibited by ATT include chymotrypsin, thrombin, collagenase, kallikrein, and elastase. AAT is a glycoprotein synthesized in the liver with a molecular weight of 50,000 daltons. It has a brief half-life, 3-6 days, and a sedimentation constant of 3.3 S. The normal phenotype is designated as M. The severest deficiences (10% of normal serum concentration) are associated with the ZZ phenotype, and intermediate levels are associated with the MZ phenotype. There are strong ethnic determinants of allele prevalence. The S allele is particularly common among individuals of Hispanic origin. Non-M phenotypes are rarely observed among blacks. The frequency of the ZZ phenotype in Caucasians is 1/4000 to 1/8000. There is a rare homozygous null state in which there is no detectable AAT.

AAT serum concentration can be estimated by using the Mancini radial immunodiffusion technique

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with normal values of about 200 mg/dl. The most widely used functional assay measures the capacity of serum to inhibit a trypsin-substrate interaction. BAPNA (benzoyl-arginine *p*-nitroanilide) is the usual substrate. The anilide is converted to a yellow aniline dye measured spectrophotometrically. One milliliter of serum normally inhibits 1.1–2.0 mg of trypsin. Phenotyping, the definitive laboratory determination is carried out with the Laurell-Fagerhol 2 step immunoelectrophoretic procedure. Other techniques include isoelectric focusing at pH of 3.0–6.0, agarose electrophoresis at a pH of 8.6, and immunofixation.

On liver biopsy, diastase-resistant, PAS-positive globules are typically observed among those with ZZ, MZ, S, and possibly other phenotypes. Electron microscopic examination shows these as amorphous material distending the cisterna of endoplasmic reticulum of the mitochondria (*1*).

Pathogenesis

What is the evidence for a pathogenetic role of α_1-antitrypsin deficiency in pulmonary disease? The principal concept is that a homeostatic balance exists between proteolytic enzymes located within polymorphonuclear leukocytes (PMNs) or pulmonary alveolar macrophages (PAMs) and circulating α_1-antitrypsin, or comparable enzyme inhibitors within the lungs themselves. There is no question that proteolytic enzymes can induce various lung diseases, at least under certain conditions. For example, emphysema has been induced in hamsters using papain and in dogs with PMN derived enzymes (*2*). Newly developed household detergents contain maxatase and alcalase, enzymes derived from *B. subtilis*. Their inhalation has led to the development of asthma-like conditions among some housewives and industrial workers. There are numerous other examples of enzyme-induced lung disease.

It is known that AAT and related proteins form complexes with various enzymes. It is also known that α_1-antitrypsin can be localized to the membranes of PAM cells by immunofluorescent technique (Dr. Allen Cohen) (*3*). Among individuals with serum inhibitor deficiency, those with normal levels of PMN esterase tend to have emphysema, in contrast to those with subnormal concentrations of PMN esterases who are spared the disease (*4*).

There may be a synergistic effect between the number of cigarettes smoked, elastase-like activity in PMNs, and decreased AAT levels in the development of chronic obstructive pulmonary disease (COPD) (*5*). Moreover, cigarette smoke reduced AAT inhibition of pancreatic and leukocytic elastase (*6*). A recent report (*7*) however, suggested that the absolute level of proteolytic enzyme in pulmonary alveolar macrophage is not in itself a determinant of emphysema. Administration of aerosolized AAT may prevent papain-induced emphysema in hamsters (*8*). It has been shown that leukocytes are selectively sequestered in the basal portions of the lung, which is the site of maximum involvement in COPD associated with AAT deficiency. That is, perfusion and ventilation are particularly reduced at the lung bases. Cigarette smokers have more PAM cells and each cell has more proteolytic capacity than is the case for nonsmokers. Therefore, numerous types of experimental data support the view that α_1-antitrypsin deficiency is a cause of COPD, although none provide absolute proof. Moreover, pathogenetic mechanisms operative for diseases of other organ systems (intestinal, hepatic, collagen vascular) are not elucidated by the studies mentioned.

AAT is an acute-phase reactant and is increased in various infections, tumors, myocardial infarctions, pregnancy, estrogen administration, and typhoid vaccine in similar fashion to the erythrocyte sedimentation rate. There is a nongenetically determined deficiency in serum AAT levels in respiratory distress syndrome of newborns (*9*). A similar decrease may precede renal homograft rejection.

Phenotypic Variants and Lung Disease

The association of the ZZ deficiency state and COPD is well described. A familial form of emphysema involving males and females equally, with onset in early adult life has been identified. However, even in this case, the time of onset and severity of disease are widely variable. In some cases, no lung disease at all develops and the individual lives into the sixth or seventh decade, especially if not exposed to respiratory irritants (*10*). About 80% of ZZ individuals will develop COPD, 10–15% develop cirrhosis during infancy, and an occasional child, or adult will have both lung and liver disease.

The relationship of the most common heterozygote state, MZ, and lung disease is controversial. In evaluating each study concerning this problem, one must consider the nature and size of the patient population and of the laboratory methods used. In some investigations, patients in hospitals were studied, others focused on people in the community. The age range of the subjects, their smoking habits, their degree of exposure to environmental pollutants varied widely in different investigations. Burrows (*11*) has called attention to the importance of childhood lung disease as a major determinant of

adult lung disease. None of the studies reported thus far has considered this variable. Attempts to find an association of variant phenotypes with asthma or cystic fibrosis in children have been unsuccessful. However, studies of subsets of asthma, such as steroid-dependent or nonatopic groups have shown a relationship. This suggests that AAT deficiency states might be related to certain subtypes of a disease even though there was no association with the overall condition. Another problem is that the range of pulmonary function testing is not uniform among the various reports. Some have been limited to simple tests of large airway obstruction, others have included more extensive studies. Several reports have been based on quantitative measurements of serum AAT only. The authors assumed that those subjects with levels below a certain value had a genetically determined deficiency. This assumption cannot be supported by data, including our own, which show substantial overlap of values in heterozygous subjects with levels seen in MM subjects. Conversely, numerous conditions are associated with elevated AAT levels, and hence, a heterozygote could at least temporarily have a value in the normal range. The concentration of elastase and other enzymes in PMNs and PAMs may also be under genetic control and may contribute to the development of emphysema. Measurement of concentrations of these enzymes has not been included in previous population studies. It is therefore difficult to judge the role of these confounding variables in evaluating the possible association of heterozygote deficiency with lung disease.

One can readily divide the published investigations into two categories. Those which demonstrate an association of MZ, or other heterozygote state with lung disease, or abnormal lung function, and those which show no such association. For example, of three abstracts submitted to the American Thoracic Society in 1977, dealing with industrial workers in Canada, two showed no differences between heterozygotes and MM subjects regarding abnormal lung function tests (*12–14*). The third showed an association of increased risk of COPD with prolonged employment in a granary among cigarette smokers (*15*).

There have been several investigations which strongly support the association of the MZ phenotype with obstructive lung disease. An excessive proportion of this phenotype was observed among two groups of patients in West Germany with chronic obstructive lung disease. There were either hospitalized patients, or those retired from work because of this disease. The control group was comprised of blood donors. The prevalence of the MS phenotype was also higher among the patients but the trend was not statistically significant (*16*). An earlier study by Kueppers showed that heterozygotes comprised 25.5% of patients with COPD (25 of 98) but only 22 of 188 healthy controls ($p < 0.01$) (*17*). Lieberman was perhaps the first to suggest such an association, finding 17–18% incidence of heterozygotes in COPD compared with only 4% in controls (*18*). However, that initial study was based on quantitative determinations of trypsin inhibitory activity without phenotyping. In a later study he suggested that cigarette smoking enhanced the risk. A study from Toronto suggested an increased risk of COPD in hospitalized cases related to the MZ phenotype. By combining several series with their own, they found this trend to be highly significant (*19*). Decreased vital capacity and electrocardiographic changes, suggesting right ventricular abnormalities, were found in a small group, 11 subjects in Warsaw, Poland who were identified as heterozygotes by quantitative testing only (*20*). Abnormalities in MZ subjects with ventilation scanning have been shown by other groups such as Fallat (*21*) and Kanner (*22*).

Heterozygote (MZ) children have shown statistically significant differences in forced expiratory flow rates, and increased frequency dependent characteristics of total pulmonary resistance when compared with MM controls (*23*). These findings are comparable to those observed in MZ adults.

In contrast, Talamo found no difference in MZ and MS prevalence in patients with COPD compared with controls (*24*). This was confirmed in several subsequent studies by other investigators. For example, a long-term, large-scale community survey in Tucson showed no difference in respiratory symptoms, disease, or ventilatory function (FEV_1/FVC, and FEF_{75}) related to three major phenotype groups, M, MS, and MZ. Similarly there were no differences in the rates of deterioration of function with age or smoking (*25*). Similar results were reported by this group using only quantitative analysis of α_1-antitrypsin (*26*). In the intervening years, other groups likewise concluded that there was no correlation demonstrable between the MZ phenotype group and COPD. In a survey of 500 adults in Rochester, Webb, et al. (*27*) showed no significant abnormalities of pulmonary function among those with variant phenotypes compared with controls. A similar observation had been made by Welch's group in Oklahoma (*28*).

Other Pulmonary Diseases

There are three other possible areas of association of pulmonary disease with AAT deficiency: byssinosis, cadmium exposure, and tuberculosis.

Studies in these areas are, however, limited.

Cadmium chloride given to mice reduces plasma antitryptic activity (*29, 30*). Industrial workers exposed to cadmium over a long period of time have an increased incidence of emphysema (*31*). Cadmium concentrations in emphasematous lungs are higher than those of normal lungs. It is also known that cadmium accumulates in the human body as a consequence of cigarette smoking. However, there is no direct evidence for a role of AAT deficiency in this condition.

In unpublished studies of small numbers of cotton mill workers with byssinosis in South Carolina a high prevalence of MZ and ZZ phenotypes have been found (Dr. Robert Allen, personal communication).

AAT levels were significantly decreased in cases of active pulmonary tuberculosis in Athens, Greece (*32*). There was a trend toward increased prevalence of S and Z alleles, but the sample size was too small to permit analysis. Prior reports showed either normal or elevated values of AAT in tuberculosis.

Future Studies

The epidemiologic studies reviewed have not led to definitive conclusions regarding the possible interaction of variant phenotypes of AAT, industrial pollutants and the prevalence of airway disease. The number and range of relevant variables which must be considered in designing future studies are formidable. The size of the populations required to establish significant differences in the occurrence of pulmonary disease may also be substantial. Studies of chronic disease in adults are necessarily complicated by the existence of prior illnesses, and this is especially so with lung disorders. Childhood and even neonatal disease, may contribute to pulmonary problems in later life. Hence, populations to be studied would require screening for these factors. The age, sex, and height of the subjects, extent and duration of exposure to pollutants, including cigarette smoke, assays of various inhibitors in serum and of enzymes in PAM and PMNs, tests of pulmonary function, and extent of lung disease are examples. Some of these are difficult to express in quantitative terms. For example the number of "pack-years" of cigarettes smoked, and recollection of prior health history may be inaccurate.

An alternative strategy may be provided by prospective studies of prevalence of pulmonary and other disease among various phenotypic groups starting with the neonatal period. This would reduce the number of variables but necessarily would exclude the role of industrial exposure. In any case, an epidemiologic approach may be expensive, time consuming, complex and may not add further insights. In contrast, basic investigations continue to yield valuable information regarding the nature of the proteolytic enzymes and their interaction with inhibitors. Studies at this level may be more productive than epidemiological approaches.

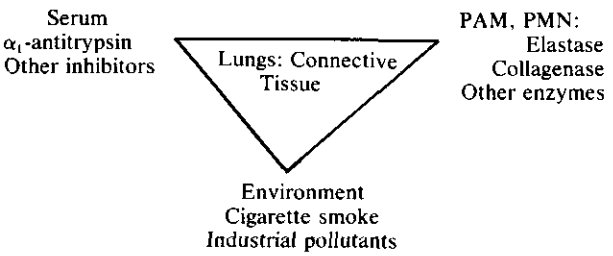

FIGURE 1. Relationship of inhibitors, enzymes, and environment.

Summary

This subject concerns the complex interrelationship of a genetically determined protein deficiency, enzymes which are inhibited by that protein, environmental challenges such as cigarette smoke and industrial pollutants, and the occurrence of obstructive lung disease (Fig. 1). Unequivocal establishment of an etiological role for AAT deficiency, expecially of intermediate degree, has proven to be difficult. Confounding variables such as enzyme concentration in PMN and PAMs, duration of exposure to potential environmental hazards, differences in laboratory methods utilized in measuring AAT and in studying pulmonary function all require investigation. The definitive study, incorporating all of these and other factors, has yet to be conducted.

No single, clear-cut conclusion can be drawn from analysis of present studies. In those circumstances in which heterozygotes appear to be predisposed to COPD, phenotypic screening of the population at potential risk, such as industrial workers, may be appropriate. Conversely, in conditions in which no association is demonstrated, such screening would not be justified. Perhaps, the best one can do is to suggest a "Scotch verdict"; that is, the issue of causation is not proven.

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