Alpha-1-antitrypsin Deficiency:  

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ABSTRACT

Alpha-1-antitrypsin (A1AT) deficiency originally was recognized as a biochemical abnormality in patients with pulmonary emphysema. Studies of the protein nature of A1AT during attempts to help explain the disease, led to the recognition of a protein polymorphism which expressed itself in several different phenotypes. As investigative work progressed, the spectrum of diseases associated with a deficiency of A1AT increased. Methods for determination of quantitative levels have been developed but these have proved lacking for the discrimination of the various phenotypes, for which specialized testing is necessary.

Of the clinical states associated with deficiencies of A1AT, pulmonary emphysema and hepatic disease are the best defined. Pathogenetic implications, however, remain elusive. As a consequence, preventive aspects are debatable and the usefulness of the determination of A1AT levels as screening measures is controversial.

Introduction

Alpha-1-antitrypsin (A1AT) deficiency was first noted by Laurell and Eriksson in 1963, when they were reviewing serum protein electrophoresis patterns. When examining the patients whose sera exhibited a pattern of decreased to absent alpha1 globulin, it was noted that all were emphysematous and that, in expanded studies, family members showed similar clinical protein pattern findings, suggesting an inherited characteristic. Work was begun to characterize the protein as well as to investigate the cause for its deficiency, its genetics and the reason(s) why its deficiency produced pulmonary disease.

Protein Nature and Function

Alpha-1-antitrypsin is a plasma glycoprotein with a molecular weight of approximately 54,000. It derives its designation “alpha-1” from its migration in the standard serum protein electrophoresis on paper or cellulose acetate. The “antitrypsin” part of the name is a partial misnomer as its inhibitory action is not confined to trypsin but rather includes elastase, skin collagenase, chymotrypsin, plasmin, thrombin, kallikrein, various plant and bacterial proteases and human leukocyte proteases.

A1AT is made in the hepatocyte but complete isolation and characterization of its protein structure has not been mapped out. Amino acids thus far determined include aspartic acid (9.8 percent), glutamic acid (12.9 percent), leucine (9.9 percent) and cysteine (2 moles per mole A1AT). A1AT is known to contain galactose, mannose, N-acetyl glucosamine and sialic acid. It is thought that the proper synthesis of the protein chain
with sialic acid is necessary for appropriate release of A1AT from the hepatocyte.4 Recently, a deficiency of sialyltransferase has been demonstrated in the serum of a patient homozygous for the deficiency and in her heterozygous parents.34 In addition, however, recent studies utilizing neuraminidase enzymatic removal of sialic acid have still left a complex electrophoretic banding pattern suggesting that additional variations in protein structure are present and are biochemically analogous to the amino acid substitutions in the various hemoglobin variants.60

The function of A1AT lacking in the clinical disease state of pulmonary emphysema is its inhibitory action against leukocyte proteases thereby protecting pulmonary tissue from damage during an inflammatory process. It has been shown that leukocyte proteases extracted from purulent sputum can digest both human and hamster lung but this action can be prevented by human serum in direct proportion to the A1AT levels in the serum.40 Without A1AT, the supporting connective tissue fibers are destroyed, leading to disruption of alveolar walls and the development of emphysema.67

Genetic Polymorphism

As attempts progressed to characterize the protein physically and chemically, variations in starch-gel electrophoretic mobility were noted, suggesting a polymorphic group of proteins to which the name "proteinase-inhibitor" (Pi) system was given.16 Twenty-four molecular variants are known,42 theoretically capable of producing approximately 200 genotypes.10 In actuality, many types are so rare in some populations that only 25 have been identified in England (in 10,000 samples) with 11 more detected elsewhere.10

The molecular variants or alleles are given letter designations depending on their relative speeds of migration in starch-gel electrophoresis. M is the medium speed allele; F is faster than M, S, slower and Z, the slowest.51 A "null" variant with no discernible A1AT has been described.59 Nomenclature, as recommended by an International Pi Committee, designates PiM, PiZ, etc. for those in whom the presence of the Pi-(null) has not yet been ruled out. PiMM or PiZZ should be used only for those in whom family studies produce evidence of homozygosity. PiZ-, PiM- will, as expected, have low levels of A1AT on quantitative testing.37

The inheritance of the Pi allele is autosomal codominant with the majority of alleles resulting in normal levels of normally functioning A1AT. Several combinations result in phenotypes associated with low serum levels of A1AT. These include PiZZ which results in levels 10 to 20 percent of normal; PiSS, PiWW and PiPP which produce levels about 60 percent of normal.10 PiNull(Pi blank) as mentioned, produces no detectable A1AT.

Estimates of the frequency of variants (from MM) in the population differ and not all variants are associated with disease. In a study of newborns in England and Wales only 10 to 20 percent of those born with the ZZ genotype had disease. Most of their parents were MZ heterozygotes.10 In American studies, the PiZZ gene appears most common in individuals derived from Northern and Central European countries—viz.: those of Irish, English, German and French-Belgian extraction with a heterozygote prevalence varying between 4.7 and 9 percent.40 There is some variation in these figures in consideration of the various ethnic backgrounds and size of study,51 but a lower prevalence (0 to 3 percent) of Z heterozygotes is noted for the American Negro, the Mexican American, the American Indian and those subjects of Jewish and Italian heritage.40,51 American, Scandinavian and English investigators agree that the PiMM allele is the normal allele for all populations studied. Subjects from Spain and Portugal show a higher gene frequency of PiS than does a Norwegian control group.40,51
Laboratory Methods

Multiple methods are available to determine quantitative levels of A1 AT in serum, including electroimmunodiffusion, nephelometry, and radial immunodiffusion. These techniques use A1 AT as the antigen against known amounts of antibody in an antigen-antibody system determining A1 AT directly as a protein. Other assays are functional and use the enzyme inhibitory activity of A1 AT in a trypsin or chymotrypsin system as a measure of serum content. Polyacrylamide gel electrophoresis has also been described but may be beyond routine laboratory determinations of multiple samples. A method utilizing guinea pig ileal homogenates also lacks clinical practicality.

Inter- and intramethod comparisons of some of these methods have been reported and all are clinically valid, once normals are established within a given laboratory, for the determination of homozygote deficient individuals of ZZ type. Selection of a given method is more a function of personnel, space and available equipment than any avowed superiority of a suitable method.

For the correct determination of genetic variants and phenotypes not homozygous deficient, electrophoresis of serum on acid-starch gels followed by antigen-antibody crossed electrophoresis is essential. Lieberman noted that quantitative measurements of serum-trypsin-inhibitory capacity (STIC) or A1 AT by radial immunodiffusion (RID) gave low normal levels for approximately 15 percent MZ heterozygotes and 60 to 80 percent of MS heterozygotes, as determined by phenotyping. In that same study, 13 of 250 individuals with MM normal phenotype had STIC and RID levels below the normal range, thus quantitative values alone would have resulted in misclassification. Some heterozygote levels can be elevated by therapeutic estrogen stimulation, thus causing a further overlap in quantitative values. Lieberman has utilized this phenomenon as a screening procedure in association with quantitative studies.

<table>
<thead>
<tr>
<th>TABLE I</th>
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<tr>
<td>Factors Affecting Serum Alpha-1-antitrypsin Levels</td>
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<tr>
<td>Increased Level</td>
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<tr>
<td>Acute or chronic infections</td>
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<tr>
<td>Pregnancy</td>
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<td>Therapy with estrogens, including contraceptive preparations</td>
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<tr>
<td>Steroid therapy</td>
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<td>Malignancy</td>
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<td>Post-operative state</td>
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Homozgyous ZZ individuals will not respond to this exogenous estrogen stimulation. "False positive" elevations of A1 AT levels occur (table I) and, if not appreciated, can cause a heterozygote to be classified as "normal." Interestingly, A1 AT levels in women do not vary during the menstrual cycle.

Factors causing "false low" levels of A1 AT are listed in table I.

Phenotyping methods are basically those described by Fagerhol and Laurell, with subsequent modifications introduced in order to obtain better separation, clearer definition or more reproducible patterns. Interpretation of phenotyping electrophoretic patterns requires some skill and practice. Before conclusions can be drawn about prevalence of given phenotype in a sample, one must remember to consider the ethnic variation within the sample. Isoelectric focusing on polyacrylamide gel slabs has been proposed as a method for phenotyping which is faster, easier and as sensitive as the time consuming acid starch gel, followed by antigen-antibody crossed electrophoresis.

Pulmonary Disease

In spite of the fact that emphysema was the first disease associated with A1 AT deficiency and thus has had the longest time span for work to be done elucidating an interrelationship, many questions are still unanswered.

The clinical picture of a homozygous
A₁AT deficient (ZZ) patient which has emerged is a young person, as often female as male, with thin habitus, no bronchitis (early in course), overinflated lungs, low, immobile diaphragm and small heart on X-ray. Respiratory failure and cor pulmonale, if they occur, are late, but there is an early onset of progressive dyspnea with labored respirations. Frequently, there is selective destruction of lower lobe regions leading to decreased perfusion at the bases, detectable by lung scan or angiography.

Panacinar emphysema, especially of lower lobes, is present, which may be complicated by secondary bronchitis or bronchiectasis. Depending on the selection of the patient group, a family history of early onset emphysema may be elicited in up to 25 percent of the patients.8-64

In the heterozygote population the picture is less clear cut. There is a male predominance, and there is a greater history of smoking, chronic bronchitis, bronchiectasis and bronchospasm. If there is a strong environmental exposure to pulmonary irritants, heterozygotes may have the more severe clinical presentation of homozygotes.61

Anatomic changes in the lungs, as seen at necropsy, are those of severe panlobular emphysema, most marked in the basal segments of the lower lobes. Bullae are rare and no changes are noted in the pulmonary vasculature. The emphysema consists of both dilatation and destruction of alveoli without accompanying fibrosis. In some subjects anatomic changes of early bronchitis are seen.22 Similar clinical and morphologic findings have been reported in children with the disease.21 The reason postulated for the primarily lower lobe involvement is that there is greater vascular perfusion and hence, sequestration of white cells thereby allowing greater proteolytic destruction by enzymes.33

The principal significance of heterozygosity (whether of Z or S allele) for A₁AT deficiency is in the development of emphysema. What can be done, if anything, to minimize this effect is the subject of a voluminous literature. In an extensive review by Kueppers and Black,33 their discussion of obstructive lung disease, and its relationship to genetics, cigarette smoking and environmental agents, surveys almost 70 references examining different aspects of pathogenesis. Difficulties in comparisons arise because of variability of patient samples, uncertainty as to phenotypes and differences in experimental methods, especially with the use of pulmonary function tests. Their conclusions, however, are that homozygous ZZ individuals carry a high risk of developing early onset chronic obstructive pulmonary disease. With advancing age, as many as 80 percent of these individuals may be affected. Heterozygotes for Z and also MS may be similarly predisposed, but to a lesser degree. Cigarette smoking accelerates the pulmonary function deterioration in those either homozygous or heterozygous for the Z allele.

Hepatic Disease

About five years after the recognition of a lack of alpha₁ globulin in the serum electrophoresis of the adults with emphysema, Sharp and Freier noted a similar absence in the serum electrophorogram from a child with unexplained cirrhosis.57 That child had a younger sibling, also with liver disease, and no discernible A₁AT.

Initially, it was thought that a deficiency of A₁AT was associated with cirrhosis in children and emphysema in adults. As the clinical syndromes became better defined, all combinations of liver-lung disease, in heterozygous and homozygous individuals were reported.5,7,27,52 The hepatocytes of subjects who are heterozygous or homozygous for Z all contain PAS positive, diastase resistant amorphous intracytoplasmic inclusions. These inclusions are seen, albeit in varying sizes and quantities, even in asymptomatic heterozygous relatives of affected patients.1 They can also be found in livers of patients with pulmonary emphysema, even if they do not have any liver disease.40 "False positive" PAS positive
granules in the hepatocyte include lipofuscin, lipochrome pigment and copper. Electron microscopic examination shows numerous globules up to three μm in diameter which are of a uniform medium electron density with an amorphous texture. Each globule is limited by a single membrane in continuity with (or close approximation to) the endoplasmic reticulum.

By immunofluorescence microscopy, the globules within the hepatocytes stain with specific fluorescein isothiocyanate conjugated (FITC) antisera to A1 AT. Recently, the PAS positive globules have been isolated from the livers in three adults and have been shown to contain a glycoprotein identical to normal A1 AT by molecular weight and immunologic determinations. This glycoprotein, however, manifested a different electrophoretic mobility, which the authors showed to be due to a sialic acid deficiency, thereby confirming Bell's earlier work.

The clinical picture for the liver disease associated with A1 AT is relatively non-specific. Fifty percent of the children who eventually develop cirrhosis have an icteric episode in the neonatal period, which clears spontaneously in a few months. After a variable period of time, the children develop cirrhosis with its clinical symptoms. Growth and development are either normal or slow.

Forty percent of children seen with neonatal cholestasis over a three year period had A1 AT deficiency. In this group, all with cholestasis or cirrhosis were homozygotes for A1 AT deficiency. Related heterozygotes had no disease, a finding similar to that reported by Sharp. If these children have liver biopsies during their jaundiced neonatal period, the findings may be those of the non-specific "neonatal hepatitis." The inclusions must be looked for specifically.

Reports are now beginning to appear of an additional associated morphological finding, i.e., of hepatocellular carcinoma, with or without cirrhosis, in patients with A1 AT deficiency (homo- and heterozygous).

Other Diseases

Alpha-1-antitrypsin levels have been studied in other disease states with varying results. Levels are normal or elevated in biliary atresia, tyrosinemia and familial cystic disease of the liver and elevated in acute hepatitis, pointing to a potential value of the test in the differential diagnosis of jaundice in the neonate. Malabsorption may be associated with A1 AT deficiency as may thyroiditis. Increased levels have been noted in sarcoidosis and have been proposed as a laboratory index of disease activity. Increased serum and synovial fluid levels are found in rheumatoid arthritis, presumably on an inflammatory basis.

An association with membrano-proliferative glomerulitis has been postulated following the post-mortem demonstration of A1 AT on the glomerular basement membranes in a child with PiZ cirrhosis and renal disease. The authors postulate that this is more than fortuitous and that inadequate amounts of protease inhibitor (A1 AT) allow for bacterial or white cell proteases to damage the glomerular basement membrane.

Several investigators have examined the role of A1 AT in the idiopathic respiratory distress syndrome and have shown that serum levels of A1 AT are low only during the acute stage of the process and that A1 AT seems to be deposited within the hyaline membranes during their formation. This deposition may explain the decreased serum levels. None of these children have had liver inclusions.

At present, all of these associations may be nothing more than coincidental and additional work is required to determine a cause and effect relation.

Pathogenesis and Projections on the Problem

There is a sufficient lack of knowledge about the A1 AT question, at present, to provide for a deluge of information for a
number of years. Numerous questions still need to be answered.

Are the PAS positive globules in the liver A1AT? Are they normal A,AT or do they represent A1AT which lacks sialic acid? Is the deficiency of sialyltransferase an isolated one or does it represent a manifestation of defective glycosylation in general? Does the defective (?) A1AT accumulate because it cannot be excreted or is it more rapidly cleared from the serum? Given the fact that the A1AT accumulates in the hepatocyte, how does it cause fibrosis, progressing to cirrhosis? In the lung, tissue damage is attributed to uncontrolled proteolytic digestion by cellular enzymes. Could a similar mechanism be at work in the hepatocyte? Some have speculated that it requires an additional insult, such as hepatitis antigen for the liver to be damaged. Why do the majority of children, homozygous for Z, not have clinical liver disease even though inclusions are present? Why does a given individual get lung disease and not liver disease, or vice versa? Why do some people get both while others have neither?

Neuraminidase treatment of adult sera gives a migration of A1AT like that of an infant: Is an infant’s liver too immature to add sialic acid appropriately? The incidence of emphysema in affected adults is much greater than that of liver disease in children. Are there inciting factors in lung disease which are more ubiquitous? Does one survive subclinical liver disease, only to develop emphysema years later?

Does cigarette smoking accelerate the frequency and increase the severity of pulmonary emphysema? The current answer to this depends on how completely one studies the prospective group (subclinical pulmonary function changes and severity of the A1AT deficiency). Some feel the correlation between cigarette smoking is positive; others feel it is negative. In considering this last point, one comes to the question of clinical applicability of the knowledge we now possess. The genetic defect cannot be prevented. Treatment for the disease is non-specific and supportive. Should wide scale population screening be attempted in order to counsel those with abnormal A1AT variants to avoid hepatic or pulmonary toxins? The immediate crux of this matter is the necessary time-consuming phenotyping procedure. As discussed previously, this is not a method for screening large groups. Estimates of the abnormal gene in the population (including heterozygotes) vary from 3.5 percent to 6 to 14 percent. Population screening for such a small return would be prohibitively expensive. A possible alternative proposed by Lieberman results from a fortuitous observation that samples of variants produced a “double ring” on certain immunodiffusion plates. The patterns were reproducible by denaturing the protein. It was suggested that an antibody to this material for variants would be an effective screening device. Large scale studies need to be done to verify this. In the meantime, family members of an index case, brought to medical attention because of symptoms, certainly ought to be evaluated.

For the future, work is progressing on isolating and purifying A1AT from patients with normal and abnormal variants. With these tools in hand, pure antisera can be made for investigative studies and other protein characteristics elucidated. The recently described linkage between Pi and Gm, the locus for the H chain of the IgG molecule provides another relationship for study to aid in expanding understanding of both systems.

References


