Progress report

Antitrypsin and the liver

While genetic influences may be of considerable importance in determining susceptibility to liver disease only rarely is a simple direct genetic cause identifiable. At most only some 2-3% of patients with cirrhosis can be shown to have single-gene disease such as Wilson’s disease or haemochromatosis. With rapidly progressive disease in infancy, however, the proportion is very much higher. Recently an inherited trait—deficiency of alpha-1-antitrypsin (α1AT)—has been shown to be an important cause of neonatal hepatitis and cirrhosis and also a cause of adult cirrhosis. In addition, partial deficiency may increase susceptibility to cirrhosis associated with other causes.

Protease Inhibitors

Human plasma is known to contain at least six inhibitors of enzymes acting on proteins (proteases) whose wide-ranging functions are not yet completely defined but include, among others, inhibition of trypsin and other serum proteases as well as forming an integral part of the fibrinolytic and kallikrein/kinin systems. Jacobsson showed nearly 20 years ago that the trypsin inhibitory capacity of serum related predominantly to the alpha-1-globulin band of the electrophoretic strip. This band consists almost entirely of a glycoprotein of molecular weight approximately 54000 called variously ‘alpha-1-trypsin inhibitor’, ‘alpha-1-glycoprotein’, and finally ‘alpha-1-antitrypsin’. It constitutes some 3% of the plasma proteins and is produced in the liver. Several methods of estimating the trypsin inhibitory capacity exist, most of them estimating the inhibition of a given amount of trypsin by a given amount of the test serum by ‘back-titrating’ the uninhibited trypsin with an artificial substrate such as BAPNA (benzoyl-d-l-arginine-p-nitroanilide). Approximately 90% of the trypsin inhibitory capacity is due to alpha-1-antitrypsin (α1AT). Estimation is routinely carried out using a radial immunodiffusion method. Levels of α1AT and therefore of trypsin inhibitory capacity tend to vary somewhat in health. They rise in many inflammatory states, in pregnancy, and also while taking ‘the pill’; as might be expected they disappear following hepatectomy. In many liver diseases the levels of α1AT are normal or raised.

Antitrypsin and Lung Disease

By an almost chance observation Laurell in 1963, while scanning routine electrophoretic strips from a sanatorium, noted two patients with emphysema who lacked the alpha-1-globulin band. The association of almost complete absence of α1AT and panacinar emphysema of early onset rapidly became established first in Sweden then throughout Europe, America, South Africa, and elsewhere. With increasing awareness the frequency of the deficiency in emphysema is seen to be rising in different surveys and is now recognized in children as well as in young adults. The lung bases are predominantly
affected and the disease is usually severe. Lung involvement in α1AT deficiency has recently been reviewed by Lieberman.

**Genetics of the Pi System**

Hard on the discovery of the association with emphysema came the observation that the deficiency state was a genetic disease which appeared to behave as an autosomal recessive trait. Numerous instances of affected siblings were recorded. Homozygotes had a mean trypsin inhibitory capacity of 0.24 mg trypsin inhibited per ml of serum compared with the normal of approximately 1.0 mg. Obligate heterozygotes were found to have an intermediate range of activity, about 0.67 mg, but were felt to be free of disease. Recently they also have been shown to be more susceptible to both emphysema and liver disease. Unfortunately, owing to the fluctuation in levels of the trypsin inhibitory capacity under various physiological and pathological situations heterozygotes are not reliably detected by determining the serum trypsin inhibitory capacity. Eriksson calculated the gene frequency to be 0.024 (1 in 42) in Sweden with a carrier (heterozygote) frequency of 0.047 (1 in 21). The homozygote frequency would therefore be approximately 1 in 1800. In Britain the homozygote frequency is thought to be about 1 in 3410 which would mean something like 230 homozygote births per year.

Concepts changed radically in 1965 when Fagerhol and Braend discovered by using acid starch gel electrophoresis, which gives higher resolution, that not one but eight separate bands were detectable in the pre-albumin zone. These can be defined and quantitated by antigen–antibody crossed electrophoresis into several patterns corresponding to the products of several genes. Subtypes have been designated by letters according to their electrophoretic mobility, as Pi M (medium), Pi F (fast), Pi S (slow), Pi Z (ultra slow), and so on. Since each fully penetrant gene determines physicochemical properties of a protein the genetic behaviour is really codominant rather than recessive. Each Pi Z gene consistently codes about 7% of the normal amount of α1AT and the homozygous genotype Pi ZZ corresponds to the originally described ‘homozygous’ deficiency state. Most people (about 86%) are Pi MM, ie, normal, and about 3% are heterozygous Pi MZ. Approximately 9% in Britain have the genotype Pi MS although frequencies of these genotypes vary in different communities. So far some 19 genes, most of them very rare, have been identified with 171 possible combinations although only about 35 have been recognized. The evidence suggests that the genes are all alleles, ie, share the same locus on a chromosome, and recently evidence for linkage with the GM (immunoglobulin heavy chain) locus on the same autosome has been reported. A gene coding no α1AT—Pi O—has recently been described.

Only the Pi Z gene has been unequivocally associated with lung and liver disease. Its racial prevalence seems to vary and to be higher for instance in north European stock such as English, Irish, and German and much lower in some ethnic groups such as Jewish, Italian, and Negro.

**Liver Involvement**

The first report of liver disease associated with α1AT deficiency seems to have been in 1967 by Ganrot and his colleagues who noted, without particular
Antitrypsin and the liver

comment, that two of their series had died of cirrhosis and another of liver carcinoma. Liver disease had not been previously expected or detected and indeed Eriksson (1964) had performed bromsulphalein tests on some of his patients with normal results. In 1968 Sharp and his coworkers first drew attention to the association with infantile cirrhosis. As with the lung disease discovery four years earlier the observation was fortuitous—while surveying electrophoretic patterns in all, this group discovered six families with seven affected individuals plus a further three children with liver disease who had died before studies were begun. Johnson and Alper described four infants with low antitrypsin levels and liver disease (neonatal hepatitis). In two of these the hepatitis was progressive and led to cirrhosis. Additional cases of infantile liver disease were reported by Sharp in 1971 and by Porter and his colleagues in 1972. The latter detected five α1AT-deficient infants out of a total of 28 with neonatal hepatitis picked up in a survey of the South East Metropolitan Hospital Region, England. Two of the five infants appeared to recover completely. Aagenaes and his colleagues reported a further five infants with hepatitis and stressed the cholestatic characteristics. Thus the hepatic manifestations of α1AT deficiency appeared to be predominantly neonatal hepatitis and cirrhosis but recently an association with adult disease is becoming recognized, both cirrhosis and hepatoma.

In the light of early experience the view became general that in homozygous α1AT deficiency (Pi ZZ) the patient, if not entirely normal, develops either liver disease or obstructive lung disease but probably not both. This is now known to be erroneous. Aminotransferases have been noted to be elevated in patients with emphysema. In addition there are now a number of well documented cases with both lung and liver disease. Nevertheless the fact that they occur more often in isolation suggests that the mechanisms for development of liver and lung disease may be totally unrelated (vide infra).

Both lung and liver disease in α1AT deficiency is essentially associated with the homozygous Pi ZZ phenotype. It seems possible, however, that heterozygous Pi MZ individuals may be more susceptible to emphysema and neonatal hepatitis. Furthermore, individuals heterozygous for another gene and with associated disease have been reported such as Pi SZ with emphysema and cirrhosis. Other genotypes have been looked at, such as FZ, but no disease pattern has yet emerged.

NEONATAL HEPATITIS

Instances of familial hepatitis have been reported for many years although in many of these no cause is demonstrable. The evidence suggests that antitrypsin deficiency may be a major cause of neonatal hepatitis. In the small series reported by Aagenaes and his colleagues from Oslo, 40% of infants with intrahepatic cholestasis were of Pi ZZ phenotype. In 1971 the King's College Hospital Group began a survey in south east England, which has a birth rate of 40 thousand yearly in a population of approximately 2.5 million. At the latest tally, two and a half years later, there were 13 cases of α1AT deficiency among some 100 cases of neonatal hepatitis. Results of other surveys throughout the world will be awaited with interest.

The clinical picture of α1AT hepatitis is not distinctive. Jaundice is usually evident in the first week or two of life although sometimes as late as two months. Cholestasis may be marked with pale stools and dark urine and biliary atresia may be suspected. Bleeding may be prominent.
The histological features are not uniform but variable\(^4\). Necrosis and infiltration with plasma cells, lymphocytes, and eosinophils are usual; cholestasis with bile ductular proliferation and increase in connective tissue common\(^6,4,15\). In contrast giant cell formation is not common\(^87,60\). Brownish-grey pigment may be seen in the periportal cells\(^4\).

Most infants are of low birth weight suggesting a pathogenesis \textit{in utero}\(^60\). Cholestasis gradually subsides in some three weeks to three months\(^66\). The prognosis is said to be poor with cirrhosis a usual outcome\(^50,60\). However, only four of the 13 King’s College series\(^66\) have thus far developed cirrhosis, nine remaining well, although it will be interesting to follow their progress to adult life. In other series infants have subsequently been shown to develop hepatosplenomegaly and portal hypertension some years after the initial jaundice has cleared\(^27,48,50\).

**ADULT LIVER DISEASE**

In adults \(\alpha\)1\,AT deficiency produces a less well defined picture. Liver changes vary from mild to moderate fibrosis\(^50,67\) through to advanced cirrhosis which is not infrequently found incidentally or only at necropsy\(^44,51,52,53,54,67,68\). In contrast to the infant disease cholestasis is not a feature of adult disease although a biliary cirrhosis type of histological picture has been described\(^54\) and also cholangiocellular carcinoma\(^57\). A history of previous infantile hepatitis is strikingly lacking in these adult patients but this may simply reflect, in part at least, the inadequacy of retrospective information.

As already stated, primary liver cell carcinoma has been reported in several instances\(^44,61\) and in Lund a large survey of hepatoma has revealed nearly 10\% with evidence suggestive of \(\alpha\)1\,AT deficiency\(^51\). Whether this is a primary feature of the disease or simply a consequence of chronic liver disease is not yet clear.

**Pathogenesis of Antitrypsin Liver Disease**

It seems that only two organs show pathological changes in \(\alpha\)1\,AT deficiency— the lungs and the liver—and other tissues are strikingly spared\(^60\) although glomerulonephritis has been described in association\(^68\). Patients with severe deficiency (Pi ZZ) fall into one of three pathological categories: (1) no clinical disease; (2) emphysema in early adult life, without and with liver disease; (3) childhood hepatitis and cirrhosis.

Aagenaes\(^4\) has estimated the ‘risk’ for these three groups of the Pi ZZ phenotype as 10-20, 50-60, and 20-30\% respectively. The pathological mechanisms involved are still not understood but the fact that some people escape clinical disease altogether suggests that some additional factor or ‘trigger’ is involved. For emphysema, infection and smoking have been suggested\(^24,28\). For neonatal hepatitis and cirrhosis viral hepatitis B has been postulated, notably by Porter and his colleagues\(^3\), three out of five of whose patients had detectable Australia antigen in the acute phase. It should be noted, however, that in other series this was reportedly absent\(^47,69\).

An important fact that has emerged in the last two years is that the livers of virtually all individuals carrying the Pi Z gene have a detectable microscopic abnormality. Globules staining with periodic acid Schiff (PAS) and resistant to diastase (therefore not glycogen) were first reported in 1971\(^70,71\). Numerous studies since then have confirmed these findings both in heterozygotes and
homozygotes, with or without frank liver disease. This suggests that the finding is a primary feature of the deficiency state. There is ample evidence from the studies mentioned to suggest that the globules are in fact accumulations of αlAT itself. Although not proven to be chemically or enzymatically identical, they do seem to be antigenically identical as can be shown by specific immunofluorescence using fluorescein-tagged antihuman alpha-l-antitrypsin. This latter is now available commercially. The globules vary in size up to 30 μ diameter and are often multiple within cells or coalescent. They tend to lie within dilated endoplasmic reticulum. Groups of globule-containing cells tend to congregate in the periportal areas. They are best identified by immunofluorescence and can easily be missed on light microscopy with haematoxylin and eosin. The amount of fluorescence correlates with the gene dose (one or two Pi Z genes) but not with the severity of the liver disease which again suggests some additional trigger factor at play. Such specific fluorescence has not been found in normal controls or in patients with other liver diseases. The reason for this cytoplasmic inclusion of αlAT is not known although a recent theory ties this to abnormality or deficiency of the sialic acid radical of the glycoprotein which in turn may influence membrane transport and thus impair release. The gene Pi Z may thus be a structural gene coding for the sialic acid component. Clearly, however, failure of release of αlAT cannot, of itself, explain the liver disease since it occurs in otherwise normal livers. It may, however, account for the emphysema component of this disease. There is evidence that, in addition to trypsin, αlAT also inhibits collagenase, elastase, chymotrypsin, and leucocyte proteases. Serum deficient in αlAT may thus fail to counteract destructive proteases produced during pulmonary infection. The same arguments could clearly not apply for the hepatocytes. One possible explanation is that intracellular accumulations of excess and active αlAT may inhibit the normally protective proteolysis of absorbed intestinal toxins. Another possibility advanced by Gans and based on animal models is that defective intestinal barriers allow toxins to reach the Kupffer cells where during detoxification lysozomal enzymes are released; deficiency of circulating αlAT may thus allow these enzymes to effect liver damage. Further studies in these areas are currently under way.

Treatment

Other than general measures specific treatment of αlAT deficiency liver disease has thus far proved of no avail. The administration of exogenous αlAT would require such large plasma volumes as to be totally impracticable. The half-life of injected αlAT is only approximately six days. Attempted enzyme induction (using phenobarbitone) has failed. An unsuccessful liver transplant has been carried out in one homozygous child with cirrhosis; it is of interest that his serum trypsin inhibitory capacity levels rose to normal within two days after operation and remained so until his death.

Conclusions

Alpha-l-antitrypsin deficiency is an important predisposing cause of infantile liver disease. In some individuals it may act in conjunction with some additional factors such as viral hepatitis. Enzyme levels are readily measurable by
immunodiffusion methods, which are now available in commercially prepared kits (Behringwerke Partigen plates, Hoechst Pharmaceuticals), and simple electrophoresis. It should be considered in all infants with hepatitis, prolonged cholestasis, or cirrhosis.

Partial deficiency of the enzyme is less easily measured and ideally demands determination of the Pi phenotype by special electrophoretic methods. The importance of its role in predisposing the liver to other toxic agents is not yet known. It is, for instance, possible that it may be one of the factors explaining why only a small proportion of alcohol abusers develop cirrhosis. So far only sporadic cases of partial deficiency with liver disease have been reported but larger surveys are currently underway.

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References


**Addendum**

Since preparation of this review further homozygous and heterozygous (FZ) patients with liver disease have been described.
