Alpha₁-antitrypsin deficiency, complement activation, and chronic liver disease

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Abstract

Activation of the complement system, the main humoral mediator of inflammation, is restrained by the action of enzyme inhibitors including α_1 -antitrypsin. Deficiency leads to chronic liver disease in about one in five children with this genetic defect. Complement activation was investigated in 34 children with α_1 AT deficiency (12 with minimal, 10 with moderate, and 12 with severe liver disease) and in 38 sex and age matched normal children by measuring the complement parent molecules C3, C4, the C3d fragment and by calculating the C3d:C3 ratio. C3 and C4 were lower in children with severe liver disease compared with controls, indicating impairment of hepatic protein synthesis or complement consumption. The C3d activation fragment was higher in all the patient groups when compared with controls while the C3d:C3 ratio, a measure of activation independent of the concentrations of the parent molecule, was higher in patients than in controls and increased with the degree of disease severity.

These results suggest that complement may have a role in the pathogenesis of the chronic liver disease associated with α_1 AT deficiency.

for liver function which may persist throughout the first decade of life, but only 17% show important clinical features of liver disease.^{4,5} The pathogenic mechanism of liver damage remains unknown, although it has been suggested that the liver disease is caused by the intrahepatocellular accumulation of α_1 AT. But this does not explain why only a minority of those with the PiZZ phenotype develop clinically important liver disease.

Deficiency of α_1 AT may affect the complement system, the main humoral mediator of inflammation.^{6,7} α_1 AT is the principal inhibitor of the powerful serine protease, neutrophil elastase, which has a role in the activation of the complement system.⁸ α_1 AT can inhibit other serine protease activities, including those acquired by components of the complement cascade when activated.^{9,10} Thus deficiency of α_1 AT could result in tissue damage due to unrestrained activation of the complement system whose effects include cell lysis, opsonisation, and chemotaxis of phagocytes.

To investigate the possible association of complement activation with liver disease in α_1 AT deficiency we measured plasma concentrations of C4, the C3d fragment, its parent molecule C3, derived the C3d:C3 ratio as an index of complement activation¹⁰ in children with liver disease of different severities associated with the PiZZ phenotype.

Over 75 alleles have been identified of the codominantly expressed protease inhibitor (Pi) gene which codes for α_1 -antitrypsin (α_1 AT), a protein produced mainly by hepatocytes.¹ The Z allele causes a defect in secretion of α_1 AT and hence its intracellular accumulation with decreased blood concentrations.² The PiZZ phenotype is associated with liver disease in childhood and emphysema in adults.³

About half of all infants born with the PiZZ phenotype have abnormal biochemical tests

Methods

We studied 34 children (17 male, 17 female, median age 6.2 years, age range 0.8-14.6 years) with the PiZZ phenotype who had presented with liver disease in infancy. Liver biopsy specimens were available for 33 of them, performed between 0 and 149, median 25 months, before this study. The patient who was not biopsied had only minor abnormalities of liver function and was the sibling of a child with minimal liver disease. The patients were divided into three groups (table 1): group 1

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Table 1 Mean (SD) liver function test values in patients with α_1 AT deficiency and liver disease and normal controls

	Controls (n = 28)*	Minimal disease (n = 12)	Moderate disease (n = 10)	Severe disease (n = 12)
Bilirubin (μ mol/l)	Not tested	10.1 (7.8)	5.8 (2.8)	19.8 (12.8)
Aspartate transaminase (IU/l)	29.0 (6.2)	47.0 (14.5)	91.0 (78.7)	143.2 (62.0)
Albumin (g/l)	44.5 (4.6)	44.1 (2.2)	43.8 (1.9)	36.9 (6.0)
Gamma glutamyl transpeptidase (IU/l)	10.4 (3.2)	45.0 (74.0)	54.1 (45.0)	204.1 (125.4)
Alkaline phosphatase (IU/l)	304.5 (70.0)	298.9 (82.7)	335.8 (112.2)	528.3 (205.0)

*Liver function tests were available on 28 normal children.

Figure Mean (SEM) values of α_1 -antitrypsin, C3, and C4 expressed in g/l of C3d% expressed as percentage of a 100% reference standard and of the C3d:C3 ratio in normal children (C) and in children with α_1 -antitrypsin deficiency and minimal (I), moderate (II), or severe (III) liver disease.

minimal liver disease (12 children with normal or mildly abnormal liver function tests and no clinical signs of liver disease at the time of this study, comprising 11 with mild histological changes and the child in whom liver biopsy had not been performed); group 2 moderate liver disease (10 children with histological features of fibrosis without cirrhosis and without clinical signs of portal hypertension at the time of study); group 3 severe liver disease (12 children with histological evidence of cirrhosis). At the time of this study, none of the children had clinical evidence of emphysema.

Thirty eight healthy children (21 male, 17 female, median age 6.0 years, age range 0.7–14.7) were studied as controls after the informed consent of their parents and of the older children had been obtained. Peripheral blood was collected by venepuncture into a final ethylene diamine tetra-acetic acid (EDTA) concentration of 10 mmol/l. Following centrifugation at $1000 \times g$ for 15 minutes at 4°C , the plasma was separated and stored at -70°C until used.

Liver function tests were carried out when the blood samples were taken (table 1).

COMPLEMENT

A C3d standard was derived from normal human serum by incubation with 3 mg/ml of inulin at 37°C for one hour, producing exhaustive activation of the alternative pathway C3 convertase.¹¹ After centrifugation at $1500 \times g$ for 30 minutes the supernatant was aliquoted and used as a C3d standard.

The laser nephelometric method used has been described previously.¹¹ Briefly, test plasma

and, to obtain a standard curve, C3d standard, were mixed with an equal volume of 22% polyethylene glycol (PEG) 8000 in phosphate buffered saline (PBS) and incubated at 4°C for two hours. After centrifugation at $1500 \times g$ at 4°C for 20 minutes supernatants were diluted in PBS as follows. The supernatant obtained from the C3d standard was double diluted from 1 in 5 to 1 in 160 to provide a reference curve. Supernatants obtained from test samples were assayed at a dilution of 1 in 5. After incubation with an equal volume of rabbit anti-human C3d (Dakopatts, code A063, lot 017), diluted 1 in 5 in PBS, for 50 minutes at room temperature, results were read in a Boehringer nephelometer and expressed as a percentage of the C3d standard.

Plasma concentrations of C3 and C4 were measured using a nephelometric technique with specific antisera according to the manufacturer's instructions (Boehringer, Marburg, Germany). Results were expressed in g/l.

The C3d:C3 ratio was calculated to provide an index of C3 activation that is independent of the initial concentration of the C3 molecule.

α_1 -ANTITRYPSIN

The patients, Pi phenotype had been determined by isoelectrofocusing.¹²

Plasma concentrations of α_1 AT were measured by radial immunodiffusion (The Binding Site, Birmingham, England) and results were expressed as g/l.

C-REACTIVE PROTEIN

C-reactive protein was measured using a nephelometric technique with specific antisera according to the manufacturer's instructions (Boehringer, Marburg, Germany).

Mean results were compared by Student's *t* test; relations between variables were analysed using the coefficient of correlation *r*. Data were also analysed using the non-parametric equivalents of the tests used, yielding similar levels of significance.

Results

The results of α_1 AT, C3, C4, C3d and C3d:C3 are presented in the figure and in table 2. As expected, α_1 AT blood concentrations in the patients with the PiZZ phenotype were significantly lower than in the controls. Concentrations in patients with severe liver disease tended to be lower than in patients with moderate liver disease.

C4 and C3 concentrations were significantly lower in patients with severe liver disease compared with those with moderate or minimal liver disease and in normal controls. C3 concentrations were also significantly higher in patients with moderate or minimal liver disease than in controls, the highest concentrations being in those with moderate disease. C4 concentrations, although following a similar pattern, were not significantly higher in patients with minimal liver disease than in controls.

In the entire patient group C3 and C4 concentrations correlated positively with each other ($r = 0.85$, $p < 0.0001$) and with α_1 AT

Table 2 Levels of significance expressed as *p* values for comparisons of mean values of α_1 AT C4, C3, C3d, and C3d:C3 between patient and control groups

	α_1 AT	C4	C3	C3d	C3d:C3
Controls <i>v</i> patients	$p < 0.0001$	$p = 0.33$	$p = 0.018$	$p < 0.0001$	$p = 0.003$
Minimal disease <i>v</i> controls	< 0.0001	0.24	0.003	0.002	0.24
Moderate disease <i>v</i> controls	< 0.0001	0.06	< 0.0001	< 0.0001	0.03
Severe disease <i>v</i> controls	< 0.0001	0.0004	0.04	< 0.0002	< 0.0001
Minimal <i>v</i> moderate disease	0.40	0.30	0.07	0.09	0.19
Minimal <i>v</i> severe disease	0.171	0.006	0.001	0.44	0.02
Moderate <i>v</i> severe disease	0.08	0.0004	0.0001	0.09	0.13

concentrations ($r = 0.57$, $p = 0.00038$ and $r = 0.35$, $p = 0.039$, respectively). The values of both C3 and C4 correlated positively with albumin ($r = 0.69$, $p = 0.0001$ and $r = 0.43$, $p = 0.02$) and negatively with bilirubin ($r = -0.58$, $p = 0.002$ and $r = -0.42$, $p = 0.04$), alkaline phosphatase ($r = -0.44$, $p = 0.03$ and $r = -0.34$, $p = 0.009$), and gamma glutamyl transpeptidase (GGT) ($r = -0.53$, $p = 0.009$ and $r = -0.45$, $p = 0.03$). C3 values also correlated negatively with aspartate aminotransferase (AST) ($r = -0.41$, $p = 0.004$).

C3d concentrations in the entire patient group were significantly higher than in the controls, the concentrations in the group with severe liver disease being similar to those in the other disease groups.

Values of the C3d:C3 ratio, the index of C3 activation, were significantly higher in the entire patient group than in the controls, with values being highest in the group with severe disease, intermediate in those with moderate disease, and similar to the controls in the group with minimal disease.

In the entire patient group the C3d:C3 ratio correlated negatively with albumin concentration ($r = -0.62$, $p = 0.00008$) and positively with alkaline phosphatase ($r = 0.45$, $p = 0.008$), GGT ($r = 0.45$, $p = 0.01$), and aspartate aminotransferase concentrations ($r = 0.32$, $p = 0.07$).

α_1 AT concentrations did not correlate with C3d:C3 ratio within either the entire patient group, the group with severe liver disease, or the control group.

All but one of the 37 normal controls and all 31 patients with α_1 AT deficiency tested had C-reactive protein values below the upper limit of normal of 1 mg/dl. C-reactive concentrations below the detection limit of 0.25 mg/dl were seen in 32 of 37 controls and in 24 of 31 patients. There was no difference between patients and controls.

Discussion

This study provides evidence that liver disease associated with α_1 AT deficiency is associated with an increase in complement activation, as shown by the C3d:C3 ratio being significantly higher in patients than in controls. Our data also suggest an association between increasing severity of liver disease and increasing degrees of complement activation, as shown by the C3d:C3 ratio being highest in patients with severe liver disease, and by the correlations of C3d:C3 values with other markers of liver damage: albumin, alkaline phosphatase, GGT and aspartate aminotransferase activities.

The C3d:C3 ratio is an index of C3 activation independent of initial concentrations of the parent C3 molecule, and hence the higher C3d:C3 ratio in patients with severe liver disease indicates that there was greater activation of the parent C3 molecules whose concentration was dwindling. The low concentrations of C3 in these patients may reflect complement consumption or reduced production due to a decline in the number of functioning

hepatocytes, the latter hypothesis being supported by the simultaneous decrease in the concentrations of C4, albumin, and α_1 AT, which are all produced in the liver.¹³ An alternative explanation for the lowest concentrations of α_1 AT being observed in patients with cirrhosis may be that the more severely impaired the production of α_1 AT, the more serious the liver damage. Why patients with minimal or moderate liver disease have increased C3 concentrations is unclear. Although it is tempting to attribute this increase to the known behaviour of C3 as an acute phase reactant,³ this explanation is questioned by the finding that C-reactive protein concentrations in the patients were similar to control values.

There are several possible mechanisms by which α_1 AT deficiency might lead to complement activation. Trypsin-like enzymes such as elastase activate both the classical and alternative pathways⁸ which merge at the stage of C3. It is conceivable that elastase or trypsin, which are normally inhibited in the blood by α_1 AT, may cause complement activation in those with α_1 AT deficiency. Previous reports have also shown that α_1 AT could inhibit the serine protease activity of classical and alternative pathway components, including C1s, C1r, and factor D.⁹ This suggests that α_1 AT deficiency may lead to a decrease in inhibition and hence an increase in activation of the complement system, with consequent production of mediators of inflammation. Although these mediators were not directly estimated in this study, we did measure the concentrations of the activation fragment C3d. This is produced on an equimolar basis with the biologically active C3a but has a prolonged half-life, leaving a longer lasting trace of complement activation.¹⁴

Complement activation may not merely be associated with liver disease associated with α_1 AT deficiency but it may be a major effector of the liver damage, perhaps through tissue inflammation, cell lysis, or recruitment of phagocytes. The PiZZ phenotype, causing intracellular accumulation of α_1 AT and deficient blood concentrations, predisposes an individual to develop liver disease in infancy, but the fact that only a minority of children born with the PiZZ phenotype show clinical features of liver disease suggests that α_1 AT accumulation is not the prime or sole effector of liver damage.

The absence of a correlation between α_1 AT activity and the C3d:C3 ratio may mean that α_1 AT is not directly associated with C3 activation. The behaviour of C3¹⁵ and α_1 AT³ as acute phase reactants, however, and the difference in the half-lives of C3d¹⁴ and α_1 AT³ could conceal any direct correlation between α_1 AT and the C3 activation ratio. Furthermore, α_1 AT concentrations may be associated with activation of the complement system at an earlier point in either the classical or alternative pathways of the cascade.

Activation of the complement system is under the control of other inhibitors, stimulators, and genetic and environmental

factors,¹⁶ and differences in any of these could determine who of those with the PiZZ phenotype develops liver disease. If complement activation does have a role in the liver damage associated with α 1AT deficiency then the reason why the liver and not other organs are affected in infancy remains to be established. One can speculate that environmental factors, such as toxins or hepatotropic viruses, or additional genetic factors, such as the possession of the HLA DR3-Dw25 allele¹⁷ may be implicated in this liver specificity.

This study, which is based on a cross-section of patients with α 1AT deficiency and liver disease, suggests that there is an association between the degree of complement activation and the severity of liver damage. Whether the association is due to complement activation causing liver damage, or to the liver damage causing complement to be activated, remains to be elucidated.

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