

α_1 Antitrypsin serum levels and phenotypes in patients with retinal vasculitis

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SUMMARY α_1 antitrypsin is an important immunoregulatory protein, the serum level of which is genetically determined. Deficient phenotypes of this ubiquitous protease inhibitor are associated with a variety of inflammatory diseases including anterior uveitis. In order to investigate the role of this protease inhibitor in the pathogenesis of retinal vasculitis (RV) 25 patients were investigated. Diseases associated with RV included Behçet's syndrome (8), SLE (2), and sarcoidosis (1). Deficient phenotypes of α_1 antitrypsin were not associated with RV. However, the serum α_1 antitrypsin level was significantly increased in patients with active RV and paralleled disease activity in patients studied prospectively.

Immunogenetic factors, especially HLA antigens, have been shown to be associated with a variety of inflammatory eye diseases, particularly those affecting the uvea. There is increasing evidence that other immunogenetic markers such as deficient phenotypes of the α_1 protease inhibitor Pi (α_1 antitrypsin) are important markers of ocular disease susceptibility. Severe α_1 AT deficiency (PiZZ) was originally reported by Laurell and Eriksson¹ to be associated with emphysema. Subsequent investigations suggest that even mild degrees of Pi deficiency are associated with many inflammatory and immunologically mediated conditions. Among such disorders are chronic liver disease, rheumatoid arthritis, juvenile chronic arthritis, systemic lupus erythematosus, fibrosing alveolitis, ankylosing spondylitis, and anterior uveitis.^{2,3}

The serum concentration of Pi is genetically determined.¹ The genes regulating Pi are linked to that governing the Gm marker on IgG,⁴ are not in the HLA region, and have recently been shown to be coded for by codominant alleles on chromosomes 14.⁴ The serum level of Pi does not accurately reflect the underlying Pi phenotype, and these proteins act as acute phase reactants, their level being increased

by a number of drugs as well as infection and other inflammatory disorders.^{5,6} Thus people with mildly deficient phenotypes PiMS or PiMZ may have serum levels in the normal range, and electrophoretic techniques are required to detect these phenotypes.⁶

Recent studies in our department have indicated that retinal vasculitis (RV) is associated with the HLA-DR4 antigen.⁷ In order to examine further the role of immunogenetic factors in RV a group of 25 patients with well characterised RV were investigated.

Material and methods

Patient selection. Twenty-five patients referred to the Uveitis Research Clinic at Sydney Eye Hospital or the Department of Immunology, St Vincent's Hospital, were investigated. Retinal vasculitis is defined as inflammation confined predominantly to the retinal vessels. The criteria for the diagnosis of RV is based on the presence of typical ocular signs as outlined by Ffytche⁸ and Sanders⁹ and supported by fluorescein angiographic evidence of RV with focal areas of vascular leakage, local dilatation, and narrowing or occlusion of veins with associated retinal oedema.^{8,10} All patients were seen independently by at least two ophthalmologists and a physician. Retinal fluorescein angiograms were performed on all patients and were reported indepen-

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dently to be consistent with the diagnosis of RV. Control subjects for serum α_1 antitrypsin determination consisted of 161 normal persons. A population of 339 blood donors had previously been studied to determine the normal distribution of α_1 antitrypsin phenotypes (Table 2).

Aetiological investigations. All patients had a chest x-ray done, full blood count, erythrocyte sedimentation rate, and biochemical screen including liver function tests, calcium and angiotensin converting enzyme, serological tests for syphilis, toxoplasma, and viruses (cytomegalovirus, herpes simplex, and hepatitis B), for autoantibodies, and a urine analysis.

α_1 Antitrypsin serum determination. Serum levels of α_1 antitrypsin levels were determined by standard radial immunodiffusion plates (Hyland Diagnostics).

α_1 Antitrypsin phenotyping. This was carried out by the technique of flat bed isoelectric focusing on polyacrylamide gels at pH 3.3–5.0.¹¹ Where necessary, resolution was enhanced by immunofixation techniques.^{12,13}

Results

RETINAL VASCULITIS PATIENTS

The disease associations in the 25 patients with RV are summarised in Table 1, while Table 2 details the serum α_1 antitrypsin phenotypes of individual patients with RV. The mean age of onset was 35 years SD 11.1, range 17–54. There were 13 females in the study population, with a mean age of 38 years, SD 11, range 21–54, and 12 males with a mean age of onset of 31 years, SD 10, range 17–44.

AETIOLOGY AND DISEASE ASSOCIATIONS

Relevant associated diseases were present in 11 of the 25 patients with RV (Tables 1 and 2). Behçet's syndrome was the most common disease association, being present in eight patients (five females). It was diagnosed clinically by the presence of oral and genital ulcers together with eye disease. Two patients had SLE diagnosed on the basis of ARA criteria¹⁴ and the presence of significantly raised titres of DNA antibodies. The clinical features of this group of patients have been previously reported.⁷

α_1 ANTITRYPSIN SERUM LEVELS AND PHENOTYPES

The mean serum α_1 antitrypsin level in RV patients was 2.5 g/l (SD 0.5), and this was significantly higher than in controls (mean 1.6 g/l, SD 0.5); $p < 0.05$, Student's *t* test (Table 1). The serum α_1 antitrypsin level decreased with the introduction of steroid therapy and remained at normal levels in patients whose disease was in remission. In three persons who experienced a relapse in their RV the clinical deterioration was accompanied by an increase in

Table 1 α_1 Antitrypsin serum levels and phenotypes in 25 patients with retinal vasculitis

Patient	Diagnosis	α_1 AT (g/l) serum level	α_1 AT phenotype
1	Sarcoidosis	2.1	M1M2
2	Idiopathic	3.3	M1M1
3	Idiopathic	1.6	M M
4	Idiopathic	2.1	M1M1
5	Behçet's syndrome	2.7	M1M1
6	Idiopathic	2.4	M1M2
7	Behçet's syndrome	1.8	M1M3
8	Behçet's syndrome	2.8	M1M1
9	Behçet's syndrome	2.2	M2M3
10	SLE	2.1	M1M2
11	SLE	3.6	M1M1
12	Idiopathic	2.5	M1M1
13	Behçet's syndrome	2.7	M1M1
14	Idiopathic	2.3	M3M3
15	Behçet's syndrome	1.7	M1M1
16	Behçet's syndrome	2.6	M1M3
17	Behçet's syndrome	3.0	M1M3
18	Idiopathic	2.5	M1M1
19	Idiopathic	2.8	M1M1
20	Idiopathic	1.7	M1M1
21	Idiopathic	2.8	M1M1
22	Idiopathic	2.3	M1S
23	Idiopathic	1.9	M1M2
24	Idiopathic	3.1	M1M2
25	Idiopathic	2.7	M1M1

Table 2 α_1 Antitrypsin phenotypes in 25 cases of retinal vasculitis

Aetiology	No. of patients	% Phenotype			
		MM	MS	MZ	MS and/or MZ
Idiopathic	14	93	7	0	7
Behçet's syndrome	8	100	0	0	0
SLE	2	100	0	0	0
Sarcoidosis	1	100	0	0	0
Controls	339	89	7	4	11

serum α_1 antitrypsin levels.

Twenty-six alleles have been described for Pi. Each phenotype is described by a letter of the alphabet with M, S, and Z being the most common. Each phenotype has a distinct pattern on isoelectric focusing with polyacrylamide gel. Heterozygous individuals—for example, PiMZ—have both M and Z bands, while homozygous individuals such as PiMM or PiZZ have only a single M or Z band. PiMM is the commonest, occurring in 90% of the population. The remaining 10% of phenotypes are associated with a variable decrease in Pi levels.¹⁵

There were no deviations from the normal frequency of deficient phenotypes in 25 patients examined (Table 2). One patient had the MS phenotype. There were no patients with homozygous deficient phenotypes.

Discussion

α_1 antitrypsin is an important regulatory protein, acting as an inhibitor of serine proteases with activity against a large range of enzymes such as trypsin, leucocyte neutral proteases, collagenases, elastin, thrombin, kallikrein, and sperm acrosin.¹⁶ It has the highest serum concentration of any protease inhibitor, and this combined with its low molecular weight (55 000) allow it to achieve very high concentrations in both intra- and extracellular compartments. Pi also is an important acute phase reactant, and it is this aspect of its activity that may be reduced in patients with deficient Pi phenotypes.⁶

The results of the present study indicate the capacity of serum α_1 antitrypsin to act as an acute phase reactant, with raised serum levels being present in the majority of patients with active RV. Serum α_1 antitrypsin may provide a useful marker of disease activity in patients with RV, as other parameters such as the ESR are usually normal in patients with this disease.⁷

Previous investigations have revealed an association between deficient phenotypes of α_1 antitrypsin and another important inflammatory eye disease, anterior uveitis.^{3,17} This finding has not been universal, and several groups have failed to substantiate this observation.^{18,19} These discrepant findings may be the result of a number of variables including Pi phenotyping techniques, population characteristics, or the failure of several authors to correlate the Pi phenotype with disease severity. Similarly, we have previously reported the lack of association between patients with deficient phenotypes of α_1 antitrypsin and posterior uveitis and a separate and smaller group of patients with RV.³

In keeping with observations in other immune mediated inflammatory disease and vasculitis syndromes such as SLE it was expected that patients with retinal vasculitis would show an increased frequency of α_1 antitrypsin deficiency, but this was not the case. A deficient α_1 antitrypsin phenotype was present in only one patient in the present study and in contrast to earlier findings in patients with anterior uveitis this subject did not have a more severe disease. Similarly, in contrast to previous studies, neither patient with SLE had a deficient α_1 antitrypsin phenotype. α_1 Antitrypsin deficiency has not been reported in association with Behçet's syndrome, and the results in this small group of patients show a lack of association with this disease. The results of the present study indicate significantly increased serum

levels of α_1 antitrypsin in patients with RV but a normal distribution of α_1 antitrypsin phenotypes.

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