Immunological abnormalities in patients with untreated retinal vasculitis

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SUMMARY Peripheral blood immunological features were assessed in 21 patients with clinical and angiographic evidence of retinal vasculitis (RV). Abnormalities of humoral and cellular immunity were frequent in this group of patients. Lymphopenia was the most common immunological abnormality, being present in 76% of patients at presentation ($p < 0.05$). Peripheral blood T and B cells were decreased with a normal helper (OKT4) to suppressor (OKT8) T cell ratio in 11 patients tested (five with Behçet's syndrome and six with idiopathic RV). Significantly increased concentrations of serum immune complexes were present in 55% of patients ($p < 0.05$). Results of the present study indicate the frequent association of peripheral blood immunological abnormalities in patients with active RV and indicate the possible role of immunological mechanisms in its pathogenesis.

The retina is involved in several systemic autoimmune diseases in which the basic pathology is a vasculitis. Retinal vasculitis (RV) is the basic histopathological lesion of Behçet's syndrome¹ and also occurs in polyarteritis nodosa,² giant cell arteritis,^{3,4} and less frequently in SLE.⁵⁻⁸ The clinical similarity between RV in this group of diseases and the experimental animal model of RV produced in rabbits^{9,10} suggests that immune complexes may be involved in the pathogenesis.^{11,12}

There has been limited opportunity for the study of immune mechanisms in retinal vasculitis owing to the rarity of the condition and difficulties associated with studying a large group of patients with untreated disease. We present here the results of a study of 21 patients with RV investigated at the onset of their disease before beginning treatment. The results highlight the frequency of immunological abnormalities in untreated patients with RV.

Material and methods

PATIENT SELECTION

Twenty-one patients referred to the Uveitis Research Clinic at Sydney Eye Hospital or the Department of

Immunology, St Vincent's Hospital, were investigated. All patients were seen independently by at least two ophthalmologists and had fluorescein angiograms that were independently reported to be consistent with the diagnosis of RV.

IMMUNOLOGICAL INVESTIGATIONS

Using standard radial immunodiffusion techniques we determined levels of the following serum proteins: IgG, IgM, IgA, C3, and C4. IgE levels were measured by radioimmunoassay (Pharmacia). Functional assessment of the classical and alternative pathways of complement were done by measuring the reciprocal of the titre of serum lysing 50% of sensitised sheep red blood cells (CH50)¹³ and sensitised rabbit erythrocytes (PH50)¹⁴ respectively. Complement assays were performed by the laboratories of Dr J Zeigler and Dr J Charlesworth at Prince of Wales and Prince Henry Hospitals, Sydney.

Serum autoantibodies (antinuclear (ANA), gastric parietal cell (GPC), smooth muscle (SM), and reticulin) were determined by an indirect immunofluorescence technique with a composite block of rat liver and stomach and mouse kidney and stomach as the substrate. DNA binding was determined by the anti-DNA assay kit (Amersham). Serum immune complexes (C1q binding assay and quantitative

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cryoglobulin) were determined by the methods of Andrews *et al.*¹¹ All patients were examined for the presence of serum cryoglobulinaemia, while 11 patients were tested in the C1q binding assay.

Peripheral blood T cell numbers and the percentage of T cells in the two major subsets were analysed by indirect immunofluorescence using monoclonal antibodies (OKT3, 4, and 8; Ortho Diagnostics Systems).¹⁵

Retinal autoantibodies were detected by indirect immunofluorescence on fresh human retina, removed from previously normal eyes within 12 hours of death. The retina was carefully dissected free from the choroid, folded on itself several times, placed in OCT (Ames Co.), snap frozen in isopentane and solid CO₂, and processed as previously described.¹⁶

AETIOLOGICAL INVESTIGATIONS

All patients had the following examinations: a full blood count, erythrocyte sedimentation rate, serum and urine biochemical screen, liver function tests, calcium and angiotensin converting enzyme estimations, serological tests for syphilis, toxoplasma, toxocara, cytomegalovirus, herpes simplex, influenza A and B, and adenovirus. Routine chest radiology was performed.

Results

The clinical features of patients with untreated RV are summarised in Table 1. The mean age of onset was 35.0±11.3 years, range 17–54 years. There were

12 females in the study population with a mean age of 38.1 (SD 11.5) years (range 20–54 years) and nine males with a mean age of onset of 31.0 (SD 10.3) years (range 17–44 years). The control population consisted of 161 healthy subjects.

CLINICAL FEATURES

Diagnosis was based on the clinical features with supporting angiographic findings. The clinical features used to diagnose RV were based on those described by ffytche.¹⁷ Patients were considered to have central RV if they had evidence of oedema of the optic nerve head and adjacent retina with or without macular oedema and decreased visual acuity.¹⁸ Patients with peripheral RV had evidence of vascular inflammation without optic nerve head involvement and usually confined to veins more than two disc diameters away from the optic nerve. Fourteen patients (eight males and six females) had peripheral RV with a mean age of onset of 32.9 (SD 11.0) years (range 17–47 years). In two cases there was combined central and peripheral RV (one male and one female). Five patients (all of whom were female) had central RV with a mean age of 39.0 (SD 11.5) years (range 21–54). The fundal appearance in central RV was characterised by disc oedema with venous dilatation, perivenular haemorrhages, and vascular sheathing confined to the region of the optic disc. Macular oedema with decreased visual acuity was present in all five patients. Hypertension and raised intracranial pressure were excluded. All cases of central RV cases (9 and 18) had associated uveitis

Table 1 Clinical features in 21 patients with retinal vasculitis

Patient	Sex	Age	Duration (months)	Bilateral	Peripheral or central	VA <6/12	Associated uveitis	Associated systemic disease
1	M	37	18	+	P	+	PU	Sarcoidosis
2	M	40	6	—	P	—	PU	Idiopathic
3	M	26	13	—	P	—	*PU	Idiopathic
4	M	17	24	+	P	+	*PU	Idiopathic
5	F	32	27	—	C	+	*PU	Idiopathic
6	F	36	49	+	C+P	+	*PU	Behçet's
7	F	52	22	+	P	+	AU+PU	Idiopathic
8	F	30	15	—	P	+	*PU	Idiopathic
9	F	54	53	—	C	+	AU+PU	Behçet's
10	M	28	16	+	P	+	AU+PU	Behçet's
11	F	20	7	—	P	+	PU	Behçet's
12	M	43	24	—	P	—	AU+PU	Behçet's
13	M	44	3	+	C+P	+	*PU	Idiopathic
14	M	18	3	—	P	—	*PU	Idiopathic
15	F	41	21	—	C	+	*PU	SLE
16	F	45	11	—	P	+	*PU	SLE
17	F	48	8	+	P	+	PU	Idiopathic
18	F	47	25	—	C	+	AU+PU	Behçet's
19	M	26	2	—	P	—	*PU	Idiopathic
20	F	31	36	+	P	+	AU+PU	Behçet's
21	F	21	15	—	C	+	*PU	Idiopathic

AU=anterior uveitis. PU=posterior uveitis. SLE=systemic lupus erythematosus. *PU=chorioretinal lesions.

with vitreous inflammatory cells. One patient with Behçet's syndrome (case 18) developed severe thrombotic glaucoma requiring enucleation. All patients with central and central combined with peripheral RV had decreased visual acuity at presentation (Table 1).

DISEASE ASSOCIATIONS

Relevant associated diseases were present in 10 of 21 patients with RV (Table 1). Behçet's syndrome was the most common disease association, being present in seven patients (five females). It was diagnosed by the presence of oral and genital ulcers and the absence of any other cause.¹⁹ RV was confined to the peripheral veins in four patients with Behçet's syndrome (Fig. 1), with two patients having central RV and one patient having panretinal involvement (Fig. 2).

Two patients had SLE diagnosed on the basis of American Rheumatism Association criteria²⁰ and the presence of significantly raised titres of DNA antibodies. Both patients were females and had symptoms suggestive of SLE for the previous five years. In each case RV was severe, with decreased visual acuity.

IMMUNOLOGICAL INVESTIGATIONS

Serum IgG levels were significantly reduced in five patients (range 6.1–6.8 g/l ($p < 0.05$)). One patient had an increased serum IgA, while four had significantly raised serum IgM levels ($p < 0.05$), and six patients had raised serum IgE levels compared with the control population ($p < 0.05$).

Serum complement levels (C3 and C4) were

normal or slightly increased in all patients with RV (Table 2). Increased C4 levels were detected in the serum of eight patients tested, while C3 levels were raised in only one patient, who also had an increased C4 level. The mean C3 level was 1.3 (SD 0.3) g/l (range 0.9–2.0) and for C4 the mean was 0.3 (SD 0.1) g/l (range 0.1–0.6). Primary complement deficiency was excluded, as all patients had a normal or raised CH50 (mean 286 (SD 64) units, range 200–400).

Autoantibodies were present in the serum of nine patients (Table 2). Three patients (Nos. 1, 7, and 13) had low titre reticulin antibodies (1/10), while an additional three patients (Nos. 3, 4, and 17) had low titre smooth muscle antibodies ($\leq 1/40$). Significantly raised serum autoantibody titres were present in three patients, two with SLE (cases 15 and 16) who had raised antinuclear antibodies and increased DNA binding before treatment, one of whom also had a high titre of gastric parietal cell antibodies and a third patient (case 8) with idiopathic RV had a rheumatoid factor titre of 1/80. None of the five patients or nine controls tested had autoantibodies to human retina by indirect immunofluorescence.

A comparison of the immunoglobulin abnormalities in different aetiological groups of patients with RV is summarised in Table 3, which demonstrates the increased frequency of peripheral blood immunological abnormalities in patients with idiopathic RV and Behçet's syndrome. Seven patients had significantly raised cryoglobulin levels when compared with controls ($p < 0.005$). Of these, three had Behçet's syndrome, three had idiopathic RV, and one SLE (Table 3). The mean C1q binding level was 12.4 (SD 7.3)% (range 5–40%) and this was significantly increased in comparison with the control group (mean 6.5 (SD 5.6)% , range 1–18%). Six patients had raised C1q binding levels, three with Behçet's

Fig. 1 Patient 11. A 20-year-old female with recurrent mouth and genital ulcers. Fundus photograph of peripheral retinal vasculitis involving the superior temporal veins.

Fig. 2 Patient 6. A 36-year-old female with Behçet's syndrome. The patient has panuveitis with central and peripheral retinal vasculitis.

Table 2 Immunological findings in 21 patients with retinal vasculitis

Patient	ESR (mm/h)	Lymphocytes absolute no. (cells/mm ³)	Immunoglobulin level				Complement			Autoantibodies
			IgG (g/l)	IgA (g/l)	IgM (g/l)	IgE (U/ml)	C3 (g/l)	C4 (g/l)	CH50 (units)	
1	5	1742	15.0	1.1	1.5	120	0.9	0.4	260	Retic 1/10
2	12	1380	8.7	2.7	0.9	60	1.5	0.3	240	—
3	1	1420	6.6	0.9	3.0	30	1.1	0.2	200	SM 1/10
4	13	1346	12.0	1.6	0.9	317	1.4	0.3	243	SM 1/10
5	6	1506	7.3	1.1	0.8	6	1.2	0.3	375	—
6	21	960	10.0	2.1	3.5	80	1.1	0.4	NT	—
7	12	880	6.1	1.6	1.1	70	2.0	0.6	400	Retic 1/10
8	3	840	10.0	1.2	1.3	68	1.2	0.4	234	RF 1/80
9	12	1125	6.8	1.2	1.1	220	1.0	0.3	NT	—
10	4	670	16.0	4.0	2.0	218	0.9	0.2	367	—
11	1	1820	8.5	1.5	2.7	18	1.2	0.3	246	—
12	7	1230	12.5	1.5	1.7	108	1.3	0.2	>400	—
13	5	1170	7.6	2.4	0.8	730	1.2	0.4	225	Retic 1/10
14	1	1112	11.2	1.6	1.3	240	1.4	0.2	280	—
15	4	1300	8.3	2.4	1.4	140	1.0	0.1	200	ANA 1/640, DNA 30, GPC 1/640
16	14	2240	9.3	1.4	1.0	146	1.5	0.4	340	ANA 1/160, DNA 24
17	21	1600	13.0	3.1	0.9	35	1.3	0.2	294	ANA 1/40, SM 1/10
18	12	260	6.7	2.0	0.9	120	1.4	0.2	340	—
19	2	860	12.0	1.3	1.2	80	1.6	0.3	NT	—
20	6	1260	6.7	1.1	0.8	334	1.5	0.4	280	—
21	18	840	11.0	1.2	2.3	125	1.5	0.4	320	—
mean±2SD	9 ±6	1217* ±438	9.8 ±2.8	1.8 ±0.8	1.5 ±0.8	174* ±175	1.3 ±0.3	0.3 ±0.1	286 ±64	
Controls (n=161)	11 ±9	2920 ±765	10.9 ±3.6	2.0 ±1.8	1.3 ±0.8	45 ±103	1.1 ±0.6	0.2 ±0.1	300 ±100	

Abnormal results are printed in bold face.

* $p \leq 0.05$ (patients with RV compared with controls, Student's *t* test).

NT=not tested. RF=rheumatoid factor. GPC=gastric parietal cell. Retic=reticulin antibody. ANA=antinuclear antibody. SM=smooth muscle antibody. DNA=DNA binding.

SI conversion: lymphocytes/mm³ $\times 10^6$ =lymphocytes/l. U/ml $\times 1000$ =U/l.

syndrome (cases 11, 12, and 18), two with idiopathic RV (cases 3 and 13), and one SLE (case 16). Five patients had both increased cryoglobulins and C1q binding. Four of the seven RV patients with Behçet's syndrome had increased circulating immune complexes compared with controls ($p < 0.05$). Serum

immune complex levels paralleled disease activity and fell to normal levels in seven of the RV patients repeatedly tested after the institution of effective treatment.

The mean peripheral blood lymphocyte count was 1.22 (SD 0.44) $\times 10^9$ /l (range 0.26 – 2.24×10^9 /l) and

Table 3 Comparison of immunological abnormalities in retinal vasculitis according to disease association

Disease associations	Patient no.	Immunoglobulin levels				Immune complexes	(Lymphopenia (<1500 cells/mm ³))
		↓ IgG	↑ IgA	↑ IgM	↑ IgE		
Idiopathic	2,3,4,5,7,8 13,14,17,19,21	*2	0	2	3	5	9
Behçet's syndrome	6,9,10,11,12 18,20	3	1	2	3	6	6
SLE	15,16	0	0	0	0	2	1
Sarcoidosis	1	0	0	0	0	0	0

*Figures refer to the number of patients having a particular immunological abnormality in each group. SI conversion: cells/mm³ $\times 10^6$ =cells/l.

this was significantly lower than the value for controls ($p < 0.005$), with 16 patients having lymphopenia (< 1500 cells/mm³) at presentation ($p < 0.005$). T cell subset numbers were measured in six patients with idiopathic RV and five with Behçet's syndrome by means of monoclonal antibodies. The percentage of T cells (OKT3) was 72% (SD 10%) for patients with idiopathic RV, 78% (SD 15%) for patients with Behçet's syndrome, and 77% (SD 12%) for controls. The T cell ratios of OKT4 to OKT8 cells were 2.0 and 1.8 respectively, which were similar to the value for the control group (1.9).

Discussion

Although a large variety of systemic diseases have been described in patients with retinal vasculitis, recent information suggests that only a small proportion of these diseases occur with a significant frequency in association with RV. In agreement with the findings of Dumonde *et al.*¹² and Andrews *et al.*¹¹ Behçet's syndrome was the most frequent disease seen in patients with RV, together with SLE and sarcoidosis. These diseases are idiopathic, with prominent immune abnormalities and a presumed immunological basis. The results of the present study support the concept that RV is an immunologically mediated disease.

Immunoglobulin abnormalities in RV were first reported by Koliopoulos *et al.*²¹ and their observation of a raised serum IgM level in this disease was subsequently confirmed by Chilman²² and is similar to the findings of the present study. In contrast to previous studies, this study showed a significantly increased frequency of raised serum IgE levels and decreased IgG levels in patients with RV. These changes have not previously been reported in patients with RV. Raised IgE levels could be attributed to associated allergic diseases in two patients; the remaining four patients had no obvious cause for a raised IgE level and it was not associated with a peripheral blood eosinophilia.

The presence of circulating immune complexes (CIC) in a high proportion of patients with RV was first reported by Andrews *et al.*¹¹ and subsequently by Dumonde *et al.*¹² The results of the present study confirm these earlier studies with 55% of patients tested having significantly increased serum immune complexes as detected by the C1q binding assay and 33% having cryoglobulinaemia. Immune complexes were more frequently detected in the sera of patients with associated diseases, particularly Behçet's syndrome, and were more often present in the sera of patients with associated uveitis and peripheral RV. No definite aetiological agent was identified in patients with RV, and there were no antigens which

could be incriminated in immune complex formation, with the possible exception of DNA antibodies in patients with SLE.

Serum complement levels (C3 and C4) and total haemolytic complement were either normal or increased in all patients with RV. The raised levels may reflect the role of these proteins as acute phase reactants, similar to the observed increased α -1 antitrypsin levels recently reported by us in patients with active RV.²³ Primary complement deficiency has been reported in several forms of vasculitis, including SLE.²⁴ Andrews *et al.*¹¹ reported decreased C3 levels in 24% of 17 patients with RV, and Dumonde *et al.*¹² reported complement abnormalities of 'non-specific type' in the majority of their patients. These discrepant results may be due to several factors, including the timing of serum sampling, treatment regimen and the presence of an associated systemic vasculitis.

Other retinal autoantibodies have recently been reported in patients with RV.¹² This is of considerable interest in view of the fact that experimental RV may be induced in certain primates following immunisation with retinal S antigen and the demonstration of subsequent development of serum retinal antibodies in these animals.²⁵ Five patients with untreated RV were tested at presentation for the presence of retinal antibodies by an indirect immunofluorescent technique with human retina as the substrate. Retinal antibodies were not detected in this assay. The difference between these results and those of Dumonde *et al.*¹² may be due to differences in methodology or may reflect the fact that such antibodies develop only after prolonged or severe ocular inflammation. Autoantibodies, including smooth muscle (SM), antinuclear (ANA), and gastric parietal cell (GPC) antibodies, have been reported previously in patients with RV.^{11,12} With the exception of the presence of antinuclear antibodies, which suggest an underlying connective tissue disease such as SLE, the significance of other autoantibodies in RV is obscure. They may be an epiphenomenon or reflect ocular tissue damage, for example to vascular smooth muscle. Alternatively these immunological findings may reflect an immunoregulatory abnormality with increased B cell activity secondary to change in regulatory T cell subsets (T_H and T_S), as has been proposed in other vasculitides such as SLE.^{24,26}

This concept is supported by the observation that lymphopenia was the most common immunological abnormality at presentation, being detected in 16 patients. In the subsets of patients tested both T and B lymphocytes were decreased, and there was no change in the ratio of helper to suppressor T cells. Although lymphopenia is a common manifestation of certain other vasculitic syndromes, such as SLE,²⁴ it has not previously been reported in patients with RV.

Lymphopenia was observed in 55% (6/11) of patients with circulating immune complexes and the same proportion of patients with autoantibodies, while all patients with raised serum IgE levels were lymphopenic. Changes in T cell subsets may help to explain these observations, which may be secondary to a decrease in specific immunoregulatory T cells. Several mechanisms may be envisaged to explain this observation, including the presence of lymphocytotoxic antibodies, and this possibility is being examined.

The recent realisation of the association of RV with the HLA DR4 antigen²⁷ indicates an immunogenetic predisposition to this disease, while the results of the present study in a small group of untreated patients with RV confirm the frequency of peripheral blood immunological abnormalities, especially immune complexes and lymphopenia, in this previously enigmatic disease and indicate the possible role of autoimmune mechanisms in the pathogenesis of retinal vasculitis.

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