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Complement activation in uveitis

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SUMMARY To determine whether complement is activated in uveitis we have measured plasma levels of C3d, a sensitive indicator of complement activation. Increased levels of C3d were found in 11 of 15 patients with idiopathic uveitis, 13 of whom had circulating immune complexes containing complement components. Since during complement activation potent mediators of inflammation are generated, it is suggested that the activation of complement, possibly triggered by uveal deposition of immune complexes, has an important role in the pathogenesis of uveitis.

The pathogenesis of uveitis has not yet been established, but clinical and experimental data strongly implicate immune mechanisms.[1-14] The condition may be associated with connective tissue disorders such as juvenile rheumatoid arthritis, ankylosing spondylitis, and, though rarely, systemic lupus erythematosus,[2-4] where immune complexes are thought to have a pathogenetic role.[5] Circulating immune complexes have also been found in patients in whom uveitis is the only detectable lesion.[6] Circulating immune complexes, however, might also represent an immune reaction secondary to ocular tissue destruction. A primary role for immune complexes in the pathogenesis of uveitis is suggested by the evidence obtained in experimental animal models.[7-10] Wong *et al.*[10] have shown that the administration of heterologous serum to rabbits previously sensitised with the same serum produces uveitis which becomes progressively more serious at every subsequent injection, suggesting that the increasing levels of immune complexes are closely related to the eye injury. Furthermore an increase in vascular permeability, a typical sign of chronic uveitis, can be reproduced in experimental animals by the direct administration of preformed immune complexes.[9] In experimental models of lens induced uveitis the deposition of immunologbulin and complement factors has been detected by immunofluorescence,[11] suggesting complement fixation by deposited immune complexes.

So far no study on complement activation in uveitis in man has been performed in spite of the fact that circulating immune complexes and hypocomplementaemia have been repeatedly reported in this condition.[1 6 12-14] We therefore decided to investigate complement activation occurring in vivo in such patients by measuring plasma levels of C3d, a fragment derived from the activation of C3. Simultaneously we have investigated the presence and composition of circulating immune complexes, titres of autoantibodies, and immunoglobulin levels.

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Patients and methods

PATIENTS

Fifteen patients suffering from idiopathic uveitis were studied: seven (four females, three males, age range 14–64, median 53 years) had anterior uveitis (chronic iridocyclitis), and eight (six females, two males, age range 18–68, median 54 years) had chronic panuveitis. Thirteen patients had symptoms for over six months, while two patients with iridocyclitis had had symptoms for three to six months. Six patients were on systemic corticosteroid treatment (prednisone 10–30 mg daily).

Thirty-nine normal controls, 19 males and 20 females with an age range of 10 to 70 years, median 48 years, were also studied.

Table 1 *Analysis of immune complex composition after PEG precipitation in 15 patients with chronic panuveitis (P) or iridocyclitis (I)*

Patient no.	*Sex*	*Age*	*Diagnosis*	*IgG*	*IgM*	*IgA*	*C3*	*C4*	*Clq*
1	M	43	P	+	−	−	++	++	+
2	F	65	P	++	−	+	++	++	+
3	F	61	P	++	−	−	−	+	−
4	F	47	P	++	+	+	+	+	+
5	M	68	P	+	−	+	−	+	+
6	F	18	P	++	−	−	−	+	−
7	F	38	P	−	−	−	−	−	−
8	F	66	P	−	−	−	−	−	−
9	F	14	I	++	+	−	++	++	+
10	F	57	I	+	−	−	−	−	+
11	M	64	I	++	+	−	++	++	+
12	F	49	I	++	−	−	−	−	−
13	M	55	I	++	−	−	−	−	+
14	M	53	I	++	−	−	−	+	−
15	F	25	I	++	−	−	−	−	−

METHODS

Serum was obtained by centrifuging clotted blood at 1000 *g*. Plasma was separated immediately after drawing blood in edetic acid (EDTA) (10 mmol/l). Samples were stored at −70°C till tested.

C3d was measured by a recently described nephelometric technique[15] using commercially available anti-C3d antiserum (DAKO). Immune complex analysis[16]: to separate immune complexes 200 µl of serum was mixed with 200 µl of 4% polyethyleneglycol (PEG) 6000 dissolved in borate buffer (pH 7·4, 0·1 mol/l). This mixture containing 2% PEG was incubated at 4°C for 60 minutes and then centrifuged at 4°C for 20 minutes at 1500 *g*. The supernatant was discarded and the precipitate was resuspended in 50 µl of borate buffer and analysed by means of bidimensional immunodiffusion. Specific antisera directed to IgG, IgM, IgA, and complement factors C3, C4, Clq were used (Behring). Owing to the semiquantitative nature of this technique, immune precipitates are reported in Table 1 as absent (−), detectable (+), or heavy (++).

Serum levels of immunolglobulin G, A, M and of complement factors C3 and C4 were measured by a standard nephelometric technique.[17] Non-organ-specific autoantibodies and IgM rheumatoid factor were measured by standard techniques.[18] Statistical analysis was by Student's *t* test and χ^2.

Results

Eleven out of 15 (73%) patients had increased levels of C3d. The mean of the patients' values was significantly higher (1±0·1, mean±SEM) than that of the controls (0·499±0·017; t=7·3, p<0·001) (Fig. 1). Circulating immune complexes were detected in 13 out of 15 patients, and details on their composition are summarised in Table 1. IgG could be detected in 13 of the 15 precipitates (87%) but IgM in only three cases (20%) and IgA in three cases (20%). C3 was found in five of 15 precipitates (33%), C4 in nine out of 15 (60%), and Clq in nine out of 15 (60%).

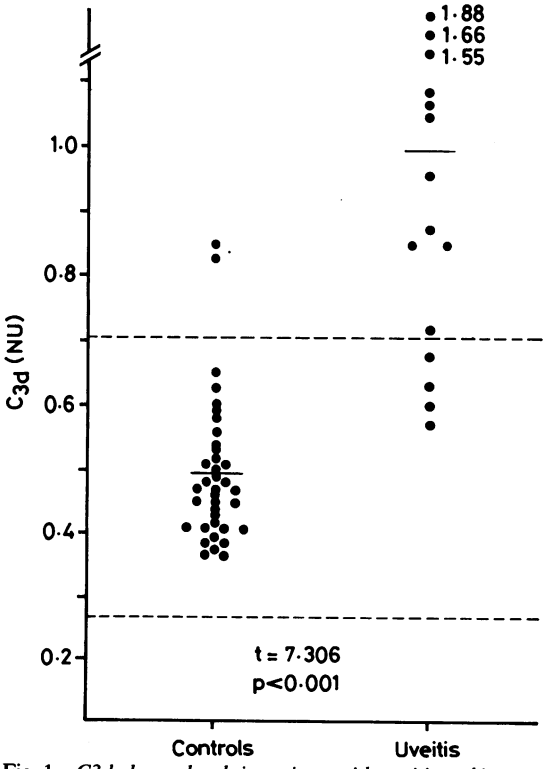

Fig. 1 *C3d plasma levels in patients with uveitis and in normal controls. C3d values are expressed in nephelometric units (NU). The dotted lines represent the upper and lower limit of normal obtained as 2SD above and below the mean of results in controls.*

Table 2 *Blood level of immunoglobulin G, A, M, complement factors C3 and C4, fragment C3d, and titres of rheumatoid factor (RF) and autoantibodies (AA) in the 15 patients with chronic uveitis*

Patient no.	*IgG g/l*	*IgA g/l*	*IgM g/l*	*C3 g/l*	*C4 g/l*	*C3d NU**	*RF*	*AA*
1	10·5	2·7	1·2	0·90	0·24	1·10	—	—
2	13·8	2·4	0·3	1·00	0·35	0·85	—	1:10†
3	20·6	2·5	0·4	1·15	0·37	0·72	1:10	—
4	13·9	3·0	2·4	1·11	0·31	1·55	—	—
5	13·4	1·5	0·6	0·86	0·49	1·88	—	—
6	8·6	2·0	0·7	0·89	0·39	0·96	—	—
7	8·9	1·6	1·3	0·66	0·30	0·68	—	—
8	7·4	1·4	1·4	0·72	0·32	0·85	—	—
9	11·8	1·1	1·3	0·78	0·26	0·57	—	1:10‡
10	11·8	2·9	2·5	0·88	0·26	1·66	—	1:10‡
11	15·7	3·4	2·4	0·96	0·33	0·60	—	—
12	12·0	1·7	1·5	0·86	0·25	1·07	—	—
13	9·7	1·5	0·7	0·65	0·21	1·05	—	1:10†
14	6·0	1·1	0·6	0·74	0·39	0·88	—	—
15	3·7	0·5	0·6	0·41	0·10	0·63	—	—

Normal values= IgG: 5–16 g/l; IgA: 0·5–4 g/l; IgM: 0·5–2 g/l; C3: 0·5–1·2 g/l; C4: 0·2–0·4 g/l; C3d<0·71 NU.
*NU = nephelometric units.
†GPC = gastric parietal cell autoantibody.
‡ANA = antinuclear autoantibody.

Increased blood levels of C3d were significantly associated with presence of circulating immune complexes containing IgG (χ^2=24·5, p<0·0005). Serum levels of immunoglobulins G, A, and M and of the complement factors C3 and C4 were normal in the great majority of cases (Table 2). Non-organ-specific autoantibodies and rheumatoid factor were present in five patients (33%) (Table 2). The findings were not significantly different in treated and untreated patients.

Discussion

Our data confirm the presence of circulating immune complexes in patients with idiopathic uveitis. Analysis of their composition has produced the interesting observation that in addition to immunoglobulin they contain complement components of the classical pathway. This suggests that they are able to fix complement in vivo. Complement-fixing immune complexes have been shown to play a pathogenetic role in other conditions such as experimental glomerulonephritis, where antigen, antibody, and complement factors are found deposited in a typical pattern in injured glomeruli.[19] A similar pattern of immunoglobulin and complement deposition is found in renal biopsies of patients affected by a variety of glomerulonephritides, suggesting similar damaging mechanisms.[20] Although the high morbidity and the technical difficulty associated with uveal biopsy prevent a direct detection of tissue immune reactants in man, animal studies have shown eye deposition of immunoglobulin and complement in experimentally induced uveitis.[11] Furthermore complement depletion has been shown to prevent both experimental uveitis and glomerulonephritis.[21,22]

In the present study we show that IgG-containing circulating immune complexes in patients with uveitis are significantly associated with increased levels of C3d, a sensitive indicator of complement activation. This is not surprising, since immunoglobulin G belonging to IgG1, IgG3, and, to a lesser extent, IgG2 subclasses activate complement when combined with their antigen. During complement activation a series of potent mediators of inflammation are produced.[23] It is thus possible that they are responsible for the phlogistic ocular lesion.

Why the inflammatory process is confined to the eye remains to be established. Deposition of immune complexes has been shown to be influenced by the class of immunoglobulin, the antigen-antibody ratio, antigen adhesiveness, and organ vascularisation.[5] The eye shares with the kidney the ability to filter large amounts of fluid. This plus a specific affinity of immune complexes for the uvea could explain the localisation. Recently, Lowder *et al.* have shown that C3b and Fc receptors are present in capillaries and along the basement membrane of the ciliary body.[24] It is thus possible that uveitis results from the binding of circulating immune complexes for which the uveal tract has appropriate receptors.

The finding of normal serum C3 and C4 but increased C3d in uveitis may reflect a phenomenon similar to that found in rheumatoid arthritis.[25] In this condition normal C3 and C4 levels in the serum contrast with reduced levels in the affected joints, while C3d, increased in the serum, show an even

higher level in the joints: a similar gradient of concentration may occur in uveitis. To determine more directly a possible pathogenetic role of complement-activating immune complexes in uveitis, the presence of immune complexes and complement fragments should be sought in the aqueous humour.

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