British Journal of Ophthalmology, 1988, **72**, 83–87

Activated T lymphocytes in uveitis

JEAN DESCHÊNES, DEVRON H CHAR, AND SHAWNYA KALETA

From the Ocular Oncology Unit, Department of Ophthalmology and the Francis I Proctor Foundation, University of California, San Francisco, USA

SUMMARY Two colour flow cytometry techniques were used to assess the activation stages of peripheral and intraocular T lymphocytes in uveitis. Increased numbers of T lymphocytes bearing the interleukin-2 (IL-2) receptors were found in intraocular fluids or peripheral blood or both of 35/51 patients with uveitis. This increased expression of IL-2 receptors on lymphocytes correlated with increased expression of other early T lymphocyte activation markers, HLA-DR and L-35. Both T helper cells (Leu-3A+) and suppressor cells (Leu 2A+) were activated in vivo. A positive correlation was seen between lymphocyte activation and clinical uveitis activity. In idiopathic uveitis activation of Leu-3A lymphocytes (helper/inducer) was significantly increased, and intraocular activation of the Leu-2A lymphocytes (cytotoxic/suppressor) was significantly decreased. These data show that some patients with idiopathic uveitis have a perturbation of T helper cells. Twenty-two of 31 patients with idiopathic uveitis, not associated with systemic disease, had increased peripheral T lymphocyte activation. This finding indicates that in some inflammations believed to be restricted to the eye an abnormal systemic immune activation exists.

Immunological abnormalities important in the pathogenesis and pathophysiology of uveitis are poorly defined.[1] We and others have used flow cytometry to characterise the nature of intraocular lymphocyte subpopulations in uveitis patients.[2-4] The role of intraocular lymphocytes in uveitis is not clear. We have previously shown that many intraocular lymphocytes are present secondary to blood ocular barrier breakdown.[4] However, whether these cells are passive, or actively involved in the pathophysiology of the disease, is uncertain.

Newer techniques can be used to distinguish activated lymphocytes capable of mediating immunological events from resting T cells. Activation of human T lymphocytes results in the expression of interleukin-2 receptors and HLA-DR on their surfaces; these antigens are present in minute quantities on resting T cells.[5-8] In some immunologically mediated diseases activated T cells are markedly increased at the site of inflammation.[8,9] In both rheumatoid arthritis and spondylitis significantly more activated T cells are present in synovium than in the blood.[8] In multiple sclerosis an increase of activated T cells in the peripheral blood is consistent with systemic immune activation.[9]

Correspondence to Devron H Char, MD, Ocular Oncology Unit, Science 315, University of California, San Francisco CA94143, USA.

In this study we examined uveitis patients with known systemic syndromes or idiopathic intraocular inflammation for the presence of interleukin-2 receptors and other early activation markers on T cells from intraocular fluids and peripheral blood. These studies were performed to determine the frequency of in-vivo T lymphocyte activation in uveitis and its correlation with disease activity.

Subjects and methods

Patients were examined and studied in the Uveitis Survey Unit of the Francis I Proctor Foundation. Ocular diagnoses were made on the basis of history, clinical examination, and routine laboratory tests. A diagnosis of idiopathic uveitis was made in the absence of a known clinical uveitis syndrome, systemic disease, or laboratory abnormalities. The diagnosis of HLA-B27 related uveitis was made in anterior uveitis patients who were HLA-B27 positive.

The severity of inflammation was graded by summing numerical values assigned to the various clinical factors of uveitis[10,11]: anterior chamber cells and flare (0 to 4), keratic precipitates (0 or 1), vitreous reaction (0 to 4), cystoid macular oedema, optic nerve oedema, and vasculitis (0 to 4).

Aqueous and vitreous specimens were obtained as previously described after informed consent was

received.[4] Peripheral blood lymphocytes (PBLs) were partially purified with Ficoll-Hypaque density gradient (Pharmacia, Piscataway, NJ). PBLs were washed twice with cold phosphate buffered saline (PBS). Vitreous samples were filtered through nylon mesh before analysis. Cell counts were performed before and after filtration to ensure adequate recovery of lymphocytes. One million PBLs were placed in ten 12×75 mm plastic tubes (Falcon, Oxnard, CA), vitreous cells were distributed in seven 12×75 mm BSA coated plastic tubes (Falcon, Oxnard, CA), and aqueous cells were distributed in seven 400 µl BSA coated Eppendorf microtubes (Brinkman Instruments, Inc.). Cells were washed once with PBS containing bovine serum albumin 1%, sodium azide 0·1%, and heparin 1 unit/ml (staining buffer) and labelled in a volume of approximately 35 µl at 4° C for 30 minutes.

Cytofluorometric analyses of cell populations were performed in two colours with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) conjugated commercial monoclonal antibodies to human leucocyte antigens (a gift from Becton Dickinson Corporation). Antibodies selected were: Leu-2A (FITC) (T cytotoxic/suppressor), Leu-3A (FITC) (T helper/inducer), Leu 4 (FITC) (T cells mitogenic), Leu-12 (FITC) (B cells), Leu-15 (PE) (T suppressor cells, NK cells, monocytes, granulocytes), anti-interleukin-2 receptors (PE), anti-HLA-DR (PE), and two experimental early activation markers for T lymphocytes L-35 and L-36 (unpublished data from Becton Dickinson Corporation). Background fluorescence activity was determined with non-specific immunoglobulins G_1 (FITC) and G_2 (PE).

Exclusion of small monocytes from the isolated lymphocytes population was verified with Leu-M3 (macrophages). Dilutions of monoclonal antibodies were 1:5 for PBLs and 1:50 for intraocular lymphocytes.[3]

Cells and antibodies were incubated for 30 minutes, at 4° C, washed, and resuspended in 30 µl of staining buffer. Fixation was obtained by addition of 30 µl of a fresh 1% paraformaldehyde solution in PBS. Samples were analysed with a dual channel fluorescence activated cell sorter (FACS IV) (Becton Dickinson Corporation). Wide angle forward light scatter, volume, green fluorescence (FITC), and red fluorescence (PE) were measured and analysed in list mode. Volume and scatter were set on the lymphocyte peak. The amplification of the red and green photomultiplier tubes was adjusted so that signals generated from the FITC positive cells did not appear over background in the red detection system and vice versa for the PE positive cells. Non-specific fluorescence (red, green, or both) in the control samples was subtracted from values for red, green or dual fluorescence in the positive samples. The percentage of activated T cells was calculated by dividing the number of activated T cells by the total number of that respective T lymphocyte total subset population.

STATISTICAL ANALYSIS

Correlations between clinical uveitis activity, steroid treatment, and intraocular and peripheral blood lymphocyte activation were calculated by Kendall's τ B statistical analysis.[12] This particular test was chosen because it can accommodate the fact that ocular samples were not obtained from patients with mild disease. Tukey's range test was used to calculate p values between lymphocyte activation in normal controls and uveitis patients.

Results

Intraocular and/or peripheral blood lymphocytes were analysed in 51 uveitis patients. Peripheral blood lymphocytes were analysed in 14 control volunteers. Fig. 1 shows a representative two-colour fluorescence histogram of IL-2 receptor and Leu-4 staining. Similar histograms were obtained with other antibodies. Each analysis was done on 500000 cells for PBLs and at least on 1000 cells for ocular samples. Table 1 shows the percentage of IL-2 positive cells in patients with uveitis. Normal control peripheral

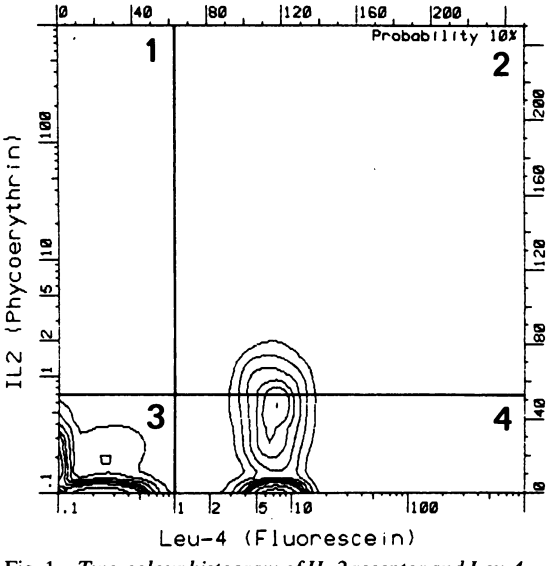

Fig. 1 *Two-colour histogram of IL-2 receptor and Leu-4 staining. Cells in quadrant 2 are Leu-4+ IL-2+; these cells are activated T cells. Cells in quadrant 3 are Leu 4− IL-2−, and therefore are non-activated lymphocytes of non-T cell lineage. Cells in quadrant 4 are Leu-4+ IL-2−, which are non-activated T cells.*

Table 1 *Percentage of aqueous (AQ), vitreous (VI), and peripheral blood (PB) IL-2 (+) T lymphocytes*

Diagnosis	*Uveitis clinical intensity*		*% IL-2 + lymphocytes*		
			2a	*3a*	*4*
Sarcoidosis	12	AQ	100·0	91·0	86·0
		PB	97·5	70·8	97·2
Pars planitis	10	PB	0·5	4·9	4·0
Toxoplasmosis	9	AQ	7·9	5·6	3·4
		PB	8·1	4·0	4·9
Idiopathic ant. uveitis	8	PB	0	2·5	2·2
Idiopathic panuveitis	8	VI	0	0	0
		PB	1·8	0·1	1·1
Pars planitis	7	PB	0·8	2·7	3·7
HLA-B27 iridocyclitis	7	AQ	9·7	9·0	5·7
		PB	2·6	5·0	1·6
Bilateral idiopathic retinal necrosis	7	VI	14·3	16·6	11·1
Idiopathic ant. uveitis	7	AQ	—	22·0	13·0
		PB	0·8	3·1	2·6
Eales' disease	6	PB	6·6	6·1	3·2
Idiopathic panuveitis	6	PB	9·2	3·1	2·6
Idiopathic panuveitis	6	PB	4·8	7·3	4·5
Idiopathic post. uveitis	6	VI	29·6	34·8	14·3
		PB	3·3	6·5	5·8
Idiopathic post. uveitis	6	VI	62·4	56·5	22·6
		PB	4·0	8·5	6·3
Idiopathic panuveitis	5	AQ	1·4	1·3	2·0
		PB	0	8·8	0
Syphilis	5	VI	2·2	58·6	24·0
		PB	0	0	0
Idiopathic panuveitis	5	PB	4·5	8·7	6·5
Fuchs's heterochromic cyclitis	5	PB	0·2	4·7	2·6
Pars planitis	5	PB	7·6	7·1	3·0
Idiopathic panuveitis	5	PB	2·1	7·4	4·0
Sarcoidosis	5	PB	44·7	13·8	10·5
Idiopathic panuveitis	5	AQ	—	—	34·8
		VI	—	0	0
		PB	5·3	9·5	7·5
HLA-B27 iridocyclitis	5	AQ	—	14·7	9·8
		PB	4·0	16·3	12·9
Idiopathic post. uveitis	4	VI	9·0	12·0	8·0
		PB	4·4	5·0	4·8
Idiopathic panuveitis	4	AQ	1·0	0	1·0
		PB	5·5	4·7	4·5
Idiopathic panuveitis	4	AQ	—	39·1	12·6
		PB	3·6	4·3	2·7
Pars planitis	4	AQ	—	35·7	19·6
		PB	0·7	7·0	5·7
Beçhet's syndrome	4	AQ	—	—	26·2
		PB	2·9	12·1	8·4
Sarcoidosis	4	PB	7·4	14·4	14·0
Iridocyclitis ankylosing spondylitis	4	AQ	—	—	15·2
		PB	9·7	8·4	7·2
Toxoplasmosis	4	AQ	—	22·6	25·2
		PB	3·6	3·3	6·0
HLA-B27 iridocyclitis	4	AQ	31·3	11·5	12·9
		PB	14·8	6·5	25·7
HLA-B27 iridocyclitis	4	PB	0	0	0
Idiopathic iridocyclitis	4	PB	4·0	6·1	3·9
Reiter's	3	PB	3·9	4·7	3·2
HLA-B27 iridocyclitis	3	PB	0·8	1·4	1·3
Pars planitis	3	PB	7·5	6·0	3·0
Idiopathic post. uveitis	3	VI	0	0	0
		PB	0	0	0
Idiopathic post. uveitis	3	PB	1·6	2·5	2·9
Idiopathic panuveitis	3	PB	0	1·7	4·3
Pars planitis	3	PB	1·6	3·5	1·8
Uveal effusion	2	PB	0·3	5·4	4·8
Birdshot choroidopathy	1	AQ	0	4·3	0
		PB	0	3·4	1·2
Idiopathic iridocyclitis	1	PB	1·2	0·6	0·7
Idiopathic panuveitis	1	PB	2·6	21·3	16·5
Idiopathic panuveitis	1	PB	0	0	0·3
HLA-B27 iridocyclitis	1	PB	0	0	1·9
Sarcoidosis	1	PB	5·3	4·4	3·6
Pars planitis	1	PB	4·5	6·7	1·9
Pars planitis	1	PB	2·4	3·2	2·1

blood T cell IL-2 receptor, HLA-DR, L-35, and L-36 expressions are shown in Table 2.

Uveitis patients had significantly more activated T cells (IL-2R positive) in the blood than healthy controls ($p<0·05$). Thirteen of 17 patients with systemic uveitis syndromes and 22 of 31 patients with idiopathic uveitis had increased IL-2R expression on Leu-4+ cells (Fig. 2).

The majority of cells in aqueous and vitreous of uveitis patients were T cells. Table 3 enumerates the total T, total B, and T cell subsets in both the intraocular components and peripheral blood in uveitis patients.

Using a Kendal τ B analysis we found a statistically significant correlation between clinical uveitis activity and the percentage of activated T cells in both intraocular fluids and peripheral blood (Table 4). A positive correlation was seen between aqueous T lymphocyte activation and peripheral blood T lymphocyte activation (Leu-2A correlation=0·714;

Table 2 *Control population*

Lymphocyte surface antigens	*% IL-2 +*	*HLA-DR +*	*L-35 +*	*L-36 +*
2A	0·6±1·1	6·7±4·4	0·8±0·4	0·9±0·4
3A	0·7±0·9	4·0±1·7	0·5±0·2	0·5±0·2
4A	1·8±1·3	ND	ND	ND

ND=not done.
± is followed by standard deviation.

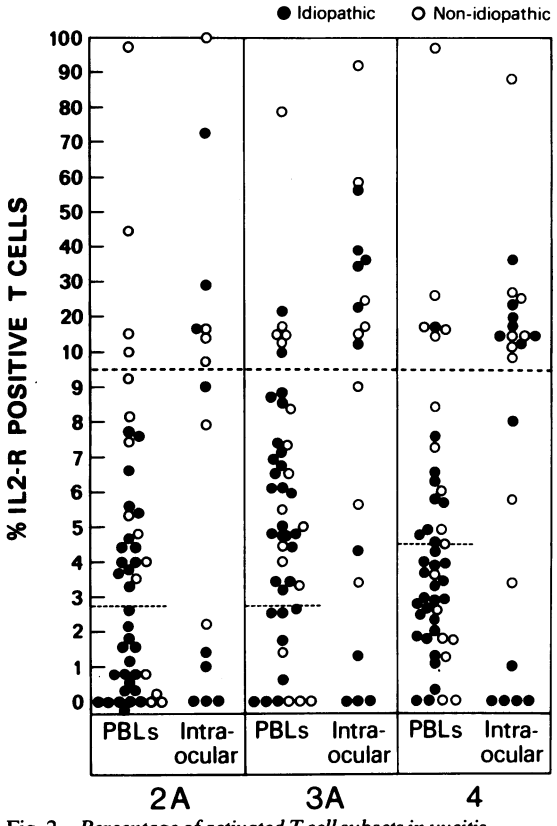

Fig. 2 *Percentage of activated T cell subsets in uveitis patients. The dotted lines represent 2 standard deviations above the mean percentage of activated T cells in controls.*

Table 3 *Uveitis patients: aqueous (AQ), vitreous (VI), and peripheral blood (PB) lymphocyte surface antigens*

	2AQ		3AQ		4AQ
(AQ)	32·2±22·7	(AQ)	36·8±21·5	(AQ)	46·6±25·5
(VI)	20·5±21·3	(VI)	18·3±20·1	(VI)	31·9±29·4
(PB)	28·3±10·6	(PB)	43·5±11·7	(PB)	72·0±11·3
	12		15		
(AQ)	8·6±7·8	(AQ)	3·2± 3·5		
(VI)	2·9±3·3	(VI)	9·6±11·8		
(PB)	7·2±6·0	(PB)	18·2±16·3		

Table 4 *Kendall's τ B correlation between uveitis clinical activity and the percentage of IL-2 (+) lymphocyte subsets*

Subsets	*Coefficients*	*Probability*
Intraocular Leu-2A	0·569	P=0·003
Intraocular Leu-3A	0·573	P=0·009
Intraocular Leu-4	0·542	P=0·014
Peripheral Leu-2A	0·269	P=0·009
Peripheral Leu-3A	0·370	P=0·0003
Peripheral Leu-4	0·275	P=0·006

p=0·0057, Leu-3A correlation=0·473, p=0·029). No correlation was seen between intravitreal T lymphocyte activation and peripheral blood T lymphocyte activation.

Increased expression of the other early activation markers correlated well with increased expression of IL-2 receptors on T lymphocytes (HLA-DR: correlation=0·412, p=0·0008; L-35: correlation=0·567; L-36: correlation=0·411, p=0·0024) (Table 4).

The diagnosis of idiopathic uveitis correlated with increased expression of the IL-2 receptors on peripheral Leu-3A+ lymphocytes (correlation= 0·247, p=0·033). No correlation was seen between increased IL-2 receptors expression on peripheral Leu-2A+ lymphocytes (correlation=0·08, p=0·5) and the diagnosis of idiopathic uveitis; a negative correlation was seen between increased IL2+Leu 2A lymphocytes in aqueous and the diagnosis of idiopathic uveitis (correlation: −0·572, p=0·049).

Discussion

We demonstrated activated T cells in aqueous, vitreous, and peripheral blood of uveitis patients using an anti-interleukin-2 receptor monoclonal antibody. Expression of IL-2 receptors on lymphocytes correlated with the expression of other early activation markers: HLA-DR, L-35, and L-36. The degree of T cell activation correlated with clinical uveitis activity.

In peripheral blood both patients with systemic syndromes associated with intraocular inflammation and patients with idiopathic uveitis had significantly more activated T cells than healthy controls. These data show systemic T cell activation in at least a subset of idiopathic uveitis patients.

We and others have previously found evidence of systemic immune response in some idiopathic uveitis patients. Kauhn *et al.*[13] found elevated systemic immunoglobulin levels in idiopathic uveitis, and we have observed that some patients have circulatory immune complexes.[14] Nussenblatt *et al.*[15] reported increased OKT8+ (suppressor cells) T lymphocytes in patients with active posterior uveitis. Snyder *et al.*[16] found elevated levels of IgG and antiganglioside antibodies in pars planitis. Murray and Rahi[17] demonstrated systemic deficits of suppressor cells in various ophthalmological conditions, including idiopathic uveitis.

Our findings suggest that some idiopathic uveitis may be systemic diseases with mainly ocular signs and symptoms. The aetiology for systemic lymphocyte activation in ocular inflammatory disease is uncertain. Lymphocytes could be activated locally, and the systemic lymphocyte activation observed might represent spillover from the eye. However, it seems

improbable that a small organ like the eye would be able to generate the amount of activated lymphocytes seen in the systemic circulation. Probably this perturbation of the immune system is due either to an abnormality of immunoregulatory homoeostasis or to altered immunological reactivity towards uveogenic antigens. Our findings indicate that a perturbation of the systemic T helper cells is present in some patients suffering from idiopathic uveitis. Recently, Caspi and coworkers established a uveogenic T helper lymphocyte line capable of transferring experimental autoimmune uveoretinitis (EAU) in the rat.[18] With regard to these findings it is tempting to postulate that some idiopathic uveitis could be a 'T helper mediated disorder'. Further studies are necessary to delineate the relative importance of these mechanisms as well as to determine the relative contribution of intraocular versus systemic lymphocyte activation in uveitis.

This work was supported in part by an unrestricted grant from That Man May See, by an unrestricted grant from the Beal Foundation, and by NIH grant EY0 3675. Dr Deschênes is the recipient of a McLaughlin fellowship.

References

1 Char DH. *Immunology of ocular tumors*. New York: Grune and Stratton, 1978.
2 Kaplan HJ, Waldrep CJ, Nicholson JK, Gordon D. Immunologic analysis of intraocular mononuclear cell infiltrates in uveitis. *Arch Ophthalmol* 1984; **102:** 572–5.
3 Freeman WR, Char DH, Garovoy MR, Lyon H. Microtechniques for flow cytometric analysis of aqueous humor lymphocytes. *J Immunol Methods* 1984; **73:** 447–55.
4 Deschênes J, Freeman W, Char DH, Garovoy MR. Lymphocyte subpopulations in uveitis. *Arch Ophthalmol* 1986; **104:** 233–6.
5 Ucehiyama T, Broder S, Waldman TA. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. *J Immunol* 1981; **126:** 1393–7.
6 Leonard WJ, Depper IU, Ucehiyama T, Smith A, Waldman TA, Greene WC. A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor; partial characterization of the receptor. *Nature* 1982; **300:** 267–9.
7 Miyawaki T, Yachie A, Uwadana N, Ohziki S, Najaoki T, Tarijuchi N. Functional significance of Tac antigen expressed on activated human T lymphocytes: Tac antigen interacts with T cell growth factor in cellular proliferation. *J Immunol* 1981; **129:** 2474–8.
8 Burmester GR, Jahn B, Gramatzki M, Zacher J, Kalden JR. Activated T cells in vivo and in vitro: divergence in expression of Tac and Ia antigens in the non-blastoid small T cells of inflammation and normal T cells activated in vitro. *J Immunol* 1984; **133:** 1230–4.
9 Hayler DA, Fox DA, Manning ME, Schlossman SF, Reinherz EL, Weimer HL. In vivo activated T lymphocytes in the peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. *N Engl J Med* 1985; **312:** 1405–11.
10 Hogan MJ, Kimura SF, Thygeson P. Signs and symptoms of uveitis. *Am J Ophthalmol* 1959; **47:** 155–70.
11 Kimura SJ, Thygeson P, Hogan MJ. Signs and symptoms of uveitis II. Classification of the posterior manifestations of uveitis. *Am J Ophthalmol* 1959; **47:** 171–6.
12 Kendall MG. *Rank correlation methods*. 2nd ed. London: Griffin, 1955.
13 Kauhn LS, Schlaegel TF, Weber JC, Biegel A. Serum immunoglobulins and uveitis. *Arch Ophthalmol* 1983; **101:** 458–9.
14 Char DH, Christensen M, Stein P, Massi R. Immune complexes in uveitis. *Am J Ophthalmol* 1979; **87:** 678–81.
15 Nussenblatt RB, Salinas-Carmona M, Leake W, Scher I. T lymphocyte subsets in uveitis. *Am J Ophthalmol* 1983; **95:** 614–21.
16 Snyder DS, Tessler HH, Yokoyama MM. Immunologic studies on Voyt-Koyanagi-Harada syndrome and pars planitis. In: Silverstein AM, O'Connor GR, eds. *Immunology and immunopathology of the eye*. New York: Masson, 1979: 71.
17 Murray PI, Rahi A. New concepts in the control of ocular inflammation. *Trans Ophthalmol Soc UK* 1985; **104:** 152–8.
18 Caspi RR, Roberge FG, McAllister CG, El-Saied M, Kuwabara T, Gery I, Hanna E, Nussenblat RB. T cell lines mediating experimental uveoretinitis (EAU) in the rat. *J Immunol* 1986; **136:** 928–33.

Accepted for publication 15 December 1986.