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T-lymphocyte subpopulations in uveitis

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SUMMARY Following an inconclusive study of differential lymphocyte counts in uveitis in which the peripheral blood was examined only once in the course of each case a longitudinal study has been carried out in patients with acute anterior uveitis. Venous blood lymphocytes were examined at intervals throughout the course of the illness, from presentation until six months later. No changes in E-rosetting T cells or total lymphocyte values have been found, nor any variations from normal in the helper (OKT4)/suppressor (OKT8) T-cell ratio. Random studies performed in a sample of patients with heterochromic cyclitis have also failed to reveal consistent abnormalities in peripheral blood lymphocyte parameters.

Disturbances of immune mechanisms have long been suspected of playing a central role in ocular inflammation. Ophthalmologists are now taking as profound an interest in immunology as their colleagues in internal medicine long have done, and there is the distinct possibility that primary advances may be made in the field of ocular immunology which shed light over the whole science.

Our endeavours in this field have left behind mountains of contradictions, rejected theories, and literature resulting from well-intentioned but profitless work in which the blessings of intuition have not directed the search in the right direction. For example, at different times and from different standpoints phenomena revealed by research have been regarded first as the cause of disease, then as mere epiphenomena, and finally as protective mechanisms.

The immune system is an intricate network homoeostatically balanced by positive and negative internal signals or messages passing between the different subsets of lymphocytes. Regulation of the immune response appears to involve a subset of peripheral blood T lymphocytes known as suppressor cells. A quantitative or qualitative deficiency of supressor cells, that is, a reduction in their number or their inability to produce sufficient amounts of suppressor factor(s) may therefore be responsible for chronic inflammation[1] and autoimmune disease.[2]

In various types of uveitis we found a wide variety of immunological abnormalities,[3] but it was clear to us that endless estimation of immunoglobulins was likely to indicate neither the cause nor the appropriate treatment. This conclusion has been reached by others.[4] We likewise drew no firm conclusions from our early work on lymphocyte subpopulations[5] and moved as others have done towards greater refinements in characterisation of lymphocyte subpopulations by the use of monoclonal antibodies. It was also very apparent to us that longitudinal studies of the same patients would be necessary, similar to those of Byrom *et al.*[6]

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Materials and methods

The first of the uveitis syndromes we chose to investigate was acute anterior uveitis. Venous blood lymphocytes were examined in 25 patients with acute anterior uveitis (15 male, 10 female) at presentation and one, two, three, and six months later. Their ages ranged from 19 to 55 years (mean 33 years). All cases were of unknown aetiology, but two patients had associated ankylosing spondylitis. At no time were the patients taking corticosteroids systemically. All patients were free of symptoms and signs three months after presentation. On each occasion the total and differential leucocyte counts were performed, and the percentage of lymphocytes forming E rosettes and the number of helper and suppressor T lymphocytes were measured, the latter by means of the OKT4 and OKT8 (Ortho Diagnostics) monoclonal antibodies respectively. Twenty-five apparently normal healthy control subjects of age and sex distribution similar to that of the patients were also investigated.

15 ml of venous blood collected in a preservative-free heparinised container was mixed with equal quantities of RPMI 1640 tissue culture medium (Flow Laboratories) containing 2 g/l sodium bicarbonate. Lymphocytes were isolated by a standard density gradient technique using Ficoll-Hypaque. The lymphocytes obtained were washed three times and the number adjusted to 3×10^6/ml (mean viability of the lymphocytes>98% as assessed by 0·1% trypan blue exclusion). Two drops of neuraminidase-treated sheep red blood cells, which had been stored in fetal calf serum containing antibiotics, were then added to 200 μl of the lymphocyte suspension, and after spinning for 5 minutes at 300 *g* this suspension was then incubated at 4°C for one hour. The cells were then gently resuspended and at least 200 E rosettes (lymphocyte with at least three red cells attached to it) were counted in a haemocytometer. A total and differential leucocyte count was also performed.

Subpopulations of T lymphocytes were estimated by an indirect immunofluorescence technique. Monoclonal antisera recognising helper (OKT4) and suppressor (OKT8) subpopulations were used. 5 μl of the monoclonal reagents or phosphate buffered saline was added to 200 μl aliquots of lymphocyte suspension. The cells were incubated at 4°C for 20 minutes, after which excess antibody was removed by washing twice with cold phosphate buffered saline and sodium azide (to prevent capping). 100 μl of a second layer fluorescein-labelled rabbit anti-mouse Ig was then added to each aliquot and left to incubate for a further 20 minutes at 4°C. After two washes wet slide preparations were made. Positively stained cells were counted under a Zeiss epifluorescence microscope, at least 200 cells being counted per slide. The results were expressed as a percentage after subtraction for non-specific staining.

The second of the uveitis syndromes we investigated was heterochromic cyclitis. As this condition runs a quiet course without remarkable variations in signs of inflammatory activity, we confined this part of our study to once only examination of the venous blood lymphocytes in each case. Ten cases of heterochromic cyclitis were examined (5 male, 5 female); their ages ranged from 14 to 42 years (mean 30 years). The total number of lymphocytes, E-rosetting T cells, and OKT4 and OKT8 subsets were measured as above. Ten apparently normal healthy control subjects of age and sex distribution similar to the patient group were also investigated.

All the above tests were carried out the same day the blood was taken, because stored blood, particularly at 4°C, produces a low yield of E-rosetting and helper T cells.[7]

Results

The values of total lymphocyte count, E-rosetting T cells, and helper/suppressor ratio, measured repeatedly in patients over a six-month period from the onset of acute anterior uveitis, were not found to differ significantly from control values (Table 1). Furthermore no trends in these values could be elicited in individual patients followed up over the six months (Tables 2–5).

In the patients with heterochromic cyclitis again we found no significant difference in total number of lymphocytes, E-rosetting T cells, and OKT4 and OKT8 subsets when compared with controls (Table 6).

Discussion

Developments in the field of cellular immunology are now constantly illuminating the complex functions of lymphocytes in humoral and cell-mediated immunity. With the advent of monoclonal antibodies, the role of T-cell subsets, especially helper and suppressor T cells, have been made clearer. Imbalances of the helper/suppressor ratio have increased our understanding of immune regulation in various disease processes.

As the aetiopathogenesis of acute anterior uveitis is usually unknown, it is not unreasonable to suggest that there is some underlying immunological defect in these patients. Already various immunological abnormalities have been reported. Byrom *et al.*[6]

Table 1 *Acute anterior uveitis*

Group and month studied	*No. of patients*	*Total lymphocyte count* $\times10^9$/l	*% E-rosettes*	*% OKT4*	*% OKT8*	*OKT4/OKT8 ratio*
Acute anterior uveitis						
0 (presentation)	25	2·55±0·69	66·72±4·13	42·08±2·22	24·84±1·86	1·70±0·13
1	25	2·81±0·93	67·32±4·53	41·92±1·87	25·32±2·04	1·67±0·14
2	24	2·63±0·89	66·54±5·81	41·67±1·88	24·25±1·80	1·72±0·11
3	23	2·84±1·05	66·61±3·71	41·65±1·97	24·57±1·41	1·70±0·17
6	19	2·78±0·97	66·95±4·82	41·79±2·02	23·84±1·68	1·76±0·13
Controls	25	2·54±0·68	67·96±5·08	41·76±2·33	25·12±1·62	1·67±0·13

Mean ±1 SD is given. All differences were statistically not significant.

Table 2 *Total lymphocyte count* $\times 10^9/l$ *over a six-month period in 19 patients with acute anterior uveitis*

Patient	Months from presentation (0)				
	0	1	2	3	6
1	2·97	3·52	1·92	3·44	3·33
2	2·79	1·95	2·61	1·80	1·62
3	2·04	3·71	2·94	3·36	3·87
4	2·88	2·07	0·90	1·92	2·10
5	2·03	2·16	2·52	2·45	2·28
6	2·42	3·42	3·60	2·88	2·80
7	1·60	1·74	2·70	1·56	2·52
8	2·96	2·40	1·80	1·95	2·20
9	2·72	3·52	3·12	3·44	3·96
10	1·89	1·80	1·76	2·73	1·26
11	2·17	2·20	3·15	2·70	2·70
12	2·10	2·15	2·10	1·84	1·65
13	2·25	3·96	3·60	3·85	3·78
14	2·66	2·32	3·44	2·40	4·00
15	2·20	3·36	3·00	3·70	3·33
16	4·06	4·62	4·42	5·98	4·32
17	3·50	2·64	1·65	2·28	1·85
18	1·62	1·90	1·85	1·52	1·40
19	3·36	3·51	2·80	4·55	3·60

Table 3 *Percentage of E rosettes over a six-month period in 19 patients with acute anterior uveitis*

Patient	Months from presentation (0)				
	0	1	2	3	6
1	70	63	60	63	62
2	65	68	75	69	70
3	62	70	70	73	71
4	62	60	64	60	57
5	60	67	62	67	66
6	65	65	62	59	67
7	66	75	67	62	74
8	75	74	73	70	75
9	61	64	67	65	60
10	62	64	60	71	70
11	70	65	74	65	72
12	64	67	61	66	65
13	64	70	64	64	62
14	69	66	73	66	64
15	64	66	73	66	63
16	70	69	62	72	70
17	69	70	65	72	69
18	63	68	67	69	68
19	73	62	62	69	66

Table 4 *Percentage of OKT4 cells over a six-month period in 19 patients with acute anterior uveitis*

Patient	Months from presentation (0)				
	0	1	2	3	6
1	42	41	41	43	39
2	43	41	40	41	43
3	43	44	40	41	43
4	37	38	42	40	36
5	40	42	43	40	41
6	42	41	45	43	43
7	46	43	43	40	42
8	45	41	42	40	41
9	44	43	43	40	40
10	40	39	40	45	43
11	46	43	44	41	42
12	40	40	42	46	41
13	44	45	43	42	44
14	43	45	44	42	44
15	44	42	43	42	41
16	42	45	40	42	42
17	39	40	40	42	45
18	42	41	39	44	42
19	42	41	39	45	42

Table 5 *Percentage of OKT8 cells over a six-month period in 19 patients with acute anterior uveitis*

Patient	Months from presentation (0)				
	0	1	2	3	6
1	27	24	24	22	21
2	25	27	27	26	22
3	22	29	23	25	23
4	25	28	27	24	21
5	25	26	24	22	24
6	25	22	24	22	24
7	26	29	26	25	24
8	27	26	26	25	26
9	24	27	25	26	26
10	25	22	23	25	26
11	30	28	25	26	24
12	25	25	23	24	26
13	23	26	24	27	23
14	24	23	26	24	23
15	26	25	24	26	22
16	26	27	24	25	26
17	25	22	25	26	23
18	26	24	22	23	24
19	26	26	21	24	24

made the observation that there was a marked T lymphocytopenia in patients with acute anterior uveitis. This began several weeks after the onset of the attack and persisted well after recovery. They also reported a transient early increase in B lymphocytes. However, we have been unable to confirm these findings in this study. Grabner *et al.*[8] stated that during the active stage of acute anterior uveitis the function of the suppressor cells was abnormal.

Nussenblatt *et al.*[10] have reported increased suppressor T-lymphocyte number and activity in patients with active posterior uveitis. Quantitative and qualitative suppressor T-cell abnormalities have also been demonstrated in other eye conditions, such as retinitis pigmentosa,[11] herpes simplex keratitis,[12] Graves' ophthalmopathy,[13] Behçet's syndrome,[14] and Mooren's ulcer.[15]

Measuring immunological parameters in heterochromic cyclitis is a natural step forward, as little is known about its aetiology. Immunological abnor-

Table 6 *Heterochromic cyclitis*

Group	*No. of patients*	*Total lymphocyte count* $\times 10^9/l$	*% E-rosettes*	*% OKT4*	*% OKT8*	*OKT4/OKT8 ratio*
Heterochromic cyclitis	10	2·72±0·73	65·30±3·65	40·90±1·45	25·10±1·60	1·63±0·08
Controls	10	2·30±0·66	68·50±5·08	41·90±2·73	25·00±2·05	1·68±0·16

Mean ±1 SD is given. All differences were statistically not significant.

malities have already been detected in these patients. Dernouchamps *et al.*[16] have reported high levels of immune complexes in the aqueous humour of patients with heterochromic cyclitis, and Hammer and Olah[17] indicated that cellular immune processes may also play a role in its pathogenesis, since hypersensitivity to lens alpha-crystallin has been demonstrated by lymphocyte transformation and leucocyte migration inhibition tests.

We failed to demonstrate any consistent alteration in T cells and their subpopulations in acute anterior uveitis. Similarly in patients with heterochromic cyclitis the number of E-rosetting cells and the helper/suppressor ratio were within the acceptable normal range.

At this stage of our knowledge it is evident that the relatively simple techniques we have hitherto used are unlikely to contribute greatly to our understanding of the mechanisms of disease production in uveitis. Studies of cellular function are therefore needed, and we have recently begun measuring suppressor T-lymphocyte activity in uveitis in the hope that we may throw some light on the aetiopathogenesis of this puzzling group of eye conditions.

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