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 suppressor 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 and autoimmune disease.

In various types of uveitis we found a wide variety of immunological abnormalities, 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. We likewise drew no firm conclusions from our early work on lymphocyte subpopulations 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.

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.

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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 bicar-
bonate. 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 neu-
roaminidase-treated sheep red blood cells, which
had been stored in fetal calf serum containing anti-
biotics, 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 haemo-
cytometer. 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 epifluor-
escence 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 investiga-
ted 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
heterochromatic 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
elicted 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 func-
tions of lymphocytes in humoral and cell-mediated
immunity. With the advent of monoclonal anti-
bodies, 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](https://bjo.bmj.com/first-published-as-10.1136/bjo.68.10.746-on-1-october-1984)
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. stated that during the active stage of acute anterior uveitis the function of the suppressor cells was abnormal.

Nussenblatt et al. 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, herpes simplex keratitis, Graves' ophthalmopathy, Behçet's syndrome, and Mooren’s ulcer.

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

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malities have already been detected in these patients. Dernouchamps et al.10 have reported high levels of immune complexes in the aqueous humour of patients with heterochromic cyclitis, and Hammer and Olah11 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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References


