

# Identification of T and B lymphocytes in the human conjunctiva and lacrimal gland in ocular diseases\*

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**SUMMARY** The presence and localisation of T and B lymphocytes in biopsy specimens of human conjunctiva and lacrimal gland from 34 patients were studied in frozen sections. Eighteen patients had follicular conjunctivitis, 13 patients had Sjögren's syndrome, and 3 were normal. All cases of follicular conjunctivitis showed a similar picture. B cells were in higher number than T cells, but both types of lymphocytes were present in the infiltrates. In the developed follicles T cells occupied the periphery and B cells the central part of the infiltrates. In patients with Sjögren's syndrome the conjunctival biopsies showed a higher number of B lymphocytes. The number of T cells was higher in the lesions from Sjögren's syndrome than in cases of follicular conjunctivitis. The infiltrate in lacrimal gland biopsies showed T cells which were scattered while B cells were more numerous and formed clusters close to vessels. Normal conjunctiva and lacrimal gland showed no adherence of either sheep erythrocytes or human erythrocytes sensitised with antibody and complement.

Recent advances in immunology now permit the distinction between human thymus-dependent (T) and thymus-independent (B) lymphocytes based on different membrane receptors (see Mendes, 1977).

Human T cells bear receptors for sheep erythrocytes (E) and human B cells bear receptors for C3b. These receptors enable the identification of T and B cells in suspension or in tissue sections by rosette formation or by adherence of the corresponding indicator. Human erythrocytes sensitised with antibody and complement (HEAC) or zymosan-C3 complexes (ZC) have been used as indicators of B cells (Mendes *et al.*, 1974b; Kajdacsy-Balla and Mendes, 1976).

We have applied these methods to identify T and B cells in inflammatory infiltrates in biopsies obtained from patients with follicular conjunctivitis of different aetiologies and Sjögren's syndrome in order to verify the nature of the infiltrates in several eye diseases. Since certain nonlymphoid tissues may bear C3b receptors as found in renal glomeruli (Gelfand *et al.*, 1975), we also looked for these receptors in normal ocular conjunctiva and lacrimal gland.

\*Presented at the Third International Symposium on Ocular Immunology and Immunopathology, San Francisco, 1978.

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## Patients and methods

### PATIENTS

We studied 31 patients with the following diagnoses: 18 with follicular conjunctivitis (comprising 9 caused by adenovirus, 3 by chlamydia, 1 by herpes simplex, 1 by molluscum contagiosum, 1 secondary to the use of 5-iodo-2'-deoxyuridine (IDU), 1 with chronic microbial allergic conjunctivitis, and 2 with chronic folliculosis, and 13 patients with Sjögren's syndrome.

All patients with follicular conjunctivitis were submitted to conjunctival biopsy. In the group with Sjögren's syndrome a conjunctival biopsy was performed in 11 and a lacrimal biopsy in 4 patients. Three normal volunteers were also submitted to conjunctival and lacrimal gland biopsy.

### IDENTIFICATION OF T AND B LYMPHOCYTES IN TISSUE SECTIONS

We have used the method described in detail by one of us elsewhere (Silveira *et al.*, 1972; Mendes *et al.*, 1974a, b).

Our current technique uses 6  $\mu$ m thick cryostat sections which are dried at room temperature for 10 minutes and then covered with one drop of either 0.5% E suspension in Hanks's balanced salt solution or 0.5% HEAC in phosphate buffered

saline pH 7.2, containing EDTA ( $1.7 \times 10^{-3}$  M). The sections were incubated at  $37^\circ\text{C}$  for 15 minutes and then for 1 hour at  $4^\circ\text{C}$  in a moist chamber. The excess indicator was removed by placing the sections over saline (0.15 M sodium chloride) at  $4^\circ\text{C}$  for 20 minutes, thereby allowing the nonadherent erythrocytes to settle by gravity. The slides were then dried, fixed with absolute methanol, and stained with haematoxylin-eosin.

The degree of adherence of the markers to the lymphocyte infiltrate was classified from 0 to \*\*\*.

## Results

**Follicular conjunctivitis.** The results in Table 1 show that, independently of the aetiology, the follicles were constituted predominantly by B cells as detected by the adherence of HEAC. In well developed follicles T lymphocytes as detected by the adherence of E showed a peripheral disposition, while B cells occupied the central area (Figs. 1 and 2).

**Sjögren's syndrome.** The conjunctival biopsies showed a higher number of B cells. T lymphocytes were present, and their number was higher than in cases of follicular conjunctivitis (Table 2). The lacrimal gland biopsies also revealed a greater proportion of B cells forming large clusters, while T cells were scattered. A large number of lymphocytes did not show adhesion either to HEAC or to E.

## Discussion

For the first time the methods used to identify T and B cells have been applied to the study of the nature

of the mononuclear cell infiltrate in the conjunctiva and lacrimal gland in several diseases.

Our findings have shown that different aetiologies of follicular conjunctivitis lead to the same kind of infiltrates, which are constituted mainly of B cells, with T cells present in the periphery of the follicle.

Previous studies on salivary gland biopsies have shown that B cells constitute the major population

Table 1 *Degree of adherence of E and HEAC in conjunctival biopsies from patients with follicular conjunctivitis*

Diagnosis	Degree of adherence	
	E	HEAC
Adenovirus	*	**
	**	***
	*	***
	*	***
	*	**
	*	**
	*	**
	*	***
Chlamydia	*	***
	0	***
	*	*
Chronic folliculosis	*	**
	**	***
IDU	0	*
Molluscum contagiosum	**	**
Herpes simplex	*	***
Chronic microbial allergic	*	**

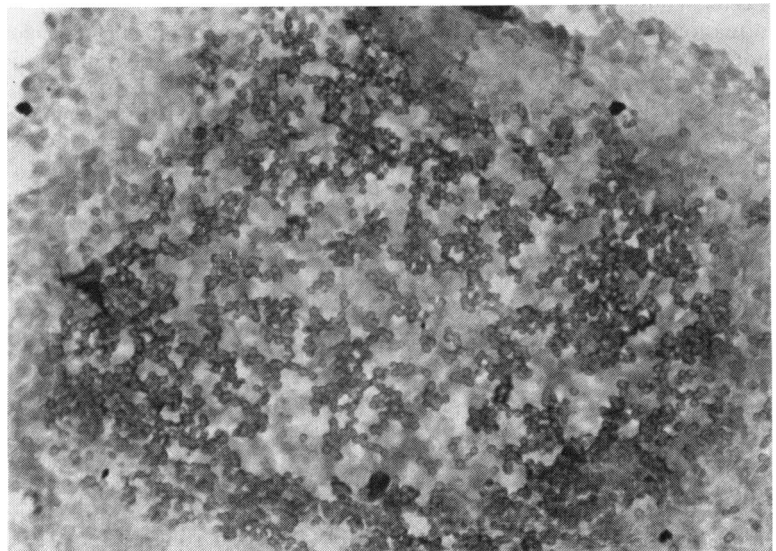


Fig. 1 *Follicular conjunctivitis: adherence of HEAC to B lymphocytes (haematoxylin and eosin)  $\times 100$*

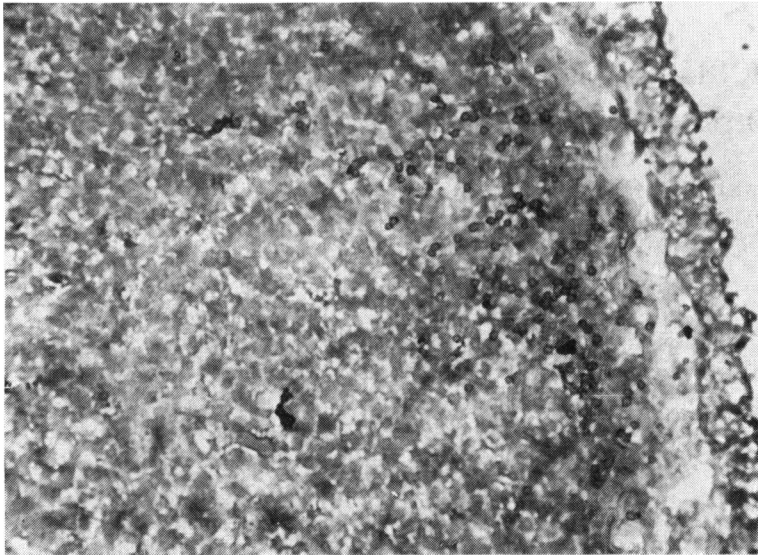


Fig. 2 Follicular conjunctivitis: adherence of E to T lymphocytes (haematoxylin and eosin) × 100

Table 2 Degree of adherence of E and HEAC in conjunctival and lacrimal gland biopsies from patients with Sjögren's syndrome

E	HEAC
<i>Conjunctival biopsy</i>	
*	**
*	***
**	**
**	**
**	***
*	**
*	*
*	*
<i>Lacrimal gland biopsy</i>	
*	***
**	***
**	**
**	***

of lymphocytes in lesions from patients with Sjögren's syndrome (Scheinberg *et al.*, 1976). Our results have also revealed a greater number of B cells in conjunctival and lacrimal gland infiltrates in this syndrome.

The normal conjunctival and lacrimal gland biopsies which were examined by the same method

revealed no adherence of either E or HEAC, indicating that nonlymphoid cells in these tissues do not bear receptors for E or for complement.

Supported by FAPESP, CNPq, CAPES, and FINEP.

**References**

Gelfand, M. C., Frank, M. N., and Green, I. (1975). A receptor for the third component of the complement in the human renal glomeruli. *Journal of Experimental Medicine*, **142**, 1029-1034.

Kajdacsy-Balla, A. A., and Mendes, N. F. (1976). Rosette formation between human B lymphocytes and zymosan-C3 complexes using different complement sources. *Journal of Immunological Methods*, **9**, 205-209.

Mendes, N. F. (1977). Immunological identification of human lymphoid cell populations. *Lymphology*, **10**, 85-93.

Mendes, N. F., Kopersztych, S., and Mota, N. G. S. (1974a). T and B lymphocytes in patients with lepromatous leprosy. *Clinical and Experimental Immunology*, **16**, 23-29.

Mendes, N. F., Miki, S. S., and Peixinho, Z. F. (1974b). Combined detection of human T and B lymphocytes by rosette formation with sheep erythrocytes and zymosan-C3 complexes. *Journal of Immunology*, **113**, 531-536.

Scheinberg, M. A., Mendes, N. F., Kopersztych, S., and Cathcart, E. S. (1976). Clinical applications of T, B and K cells determinations in rheumatic diseases. *Seminars in Arthritis and Rheumatism*, **6**, 1-18.

Silveira, N. P. A., Mendes, N. F., and Tolnai, M. E. A. (1972). Tissue localization of two populations of human lymphocytes distinguished by membrane receptors. *Journal of Immunology*, **108**, 1456-1460.