

Immunopathology of trachomatous conjunctivitis

AHMED M ABU EL-ASRAR¹, JOOST J VAN DEN OORD,² KAREL GEBOES,²
LUC MISSOTTEN,³ MOHAMED H EMARAH,¹ AND VALEER DESMET²

From the ¹Department of Ophthalmology, Mansoura University Hospital, Egypt; and the ²Department of Ophthalmology and ³Laboratory of Histochemistry and Cytochemistry, University Hospital St Rafael, Leuven, Belgium

SUMMARY Upper palpebral conjunctival biopsy specimens obtained from eight patients with active trachoma were examined by routine histological and immunohistochemical methods. The epithelium expressed class I major histocompatibility complex (MHC) products throughout and class II MHC products in the superficial layers. The epithelial inflammatory infiltrate consisted of polymorphonuclear leucocytes, macrophages, T lymphocytes, and dendritic cells. In the underlying stroma the inflammatory infiltrate was organised as B lymphoid follicles, and there was also a diffuse infiltrate consisting of plasma cells and scattered B lymphoid cells, dendritic cells, T cells, macrophages, and polymorphonuclear leucocytes. Each type of cell has its special location in the tissue. Plasma cells were located on a subepithelial band and as a dense infiltrate round the acini of accessory lacrimal glands. IgA⁺ plasma cells outnumbered IgG⁺ cells, whereas IgM⁺ and IgE⁺ cells were few. Our data provide good evidence for the presence of both humoral and cell mediated immune responses and a possible role for autoimmune mechanisms in the conjunctival tissues of trachoma patients.

Trachoma is a chronic keratoconjunctivitis caused by *Chlamydia trachomatis* serotype A, B, or C.¹ It is one of the leading causes of blindness in underdeveloped countries and affects an estimated 500 million people.²

C. trachomatis is essentially a pathogen of mucosal surfaces, infecting and replicating within epithelial cells.³ Ocular infection with the agent of *C. trachomatis* induces both humoral immunity^{4,7} and cell mediated immunity.⁸⁻¹⁰ The immune response seems to confer partial protection against subsequent infection, yet appears also to be responsible for much of the observed pathology and tissue destruction seen in trachoma.¹¹

Analysis of the components of the local immune response occurring in the conjunctival tissues of patients with active trachoma has so far received relatively little attention. The disease is characterised histologically by the formation of large lymphoid follicles with prominent follicular centres in the stroma.¹² In the conjunctiva of monkeys experiment-

ally inoculated with *C. trachomatis* these follicles were found to consist of B cells, T cells, and macrophages,¹³ but no data are available from human material. Therefore, using the monoclonal antibody technique for the identification of cells involved in the immune response we analysed the in-situ immune reaction in trachomatous conjunctivitis in humans in order to examine more precisely the pathogenesis of this condition.

Subjects and methods

SUBJECTS

Eight patients presenting with active trachoma seen in the Ophthalmic Outpatient Clinic of Mansoura University Hospital were included in the study. All were examined by a slit-lamp, and corneal and conjunctival changes were recorded.

The patients comprised four males and four females. Their ages ranged from 3 to 9 years, average 5 years. Four patients were diagnosed clinically as trachoma stage 2a, three as trachoma stage 1, and one as trachoma stage 2b according to MacCallan's classification.¹⁴ All the patients had upper limbal active pannus.

Correspondence to Professor L. Missotten, Department of Ophthalmology, University Hospital St Rafael, Kapucijnenvoer 7, B3000 Leuven, Belgium.

METHODS

Upper palpebral conjunctival biopsy specimens were obtained from all the patients after informed consent. The specimens were immediately fixed for two to three hours in B_5 fixative composed of: (A) 90 ml distilled water, 6 g mercuric chloride, 2.074 g sodium acetate ($CH_3COONa \cdot 3H_2O$); and (B) 37% formaldehyde solution, pH 5.7. 9 ml of A and 1 ml of B were mixed immediately before use.

The specimens were then kept in absolute methanol until embedded in paraffin. Semiserial sections were cut and stained with haematoxylin and eosin for routine histology. Serial 6 μ m sections were stained by a three-step indirect immunoperoxidase

procedure¹⁵ with the following monoclonal antibodies: MT_1 , MB_2 , LN_2 , $TAL-1B_5$, and $3MA134$.

MT_1 defines all T lymphocytes¹⁶; MB_2 defines all B cells, excluding mature plasma cells¹⁶; LN_2 identifies B cells, macrophages, interdigitating reticulum cells, and Langerhans cells¹⁷; $TAL-1B_5$ is directed against major histocompatibility complex (MHC) class II (HLA-DR) α chains¹⁸; and $3MA134$ defines mononuclear phagocytes (Pulford *et al.*, unpublished data).

An unlabelled, three-step peroxidase-anti-peroxidase (PAP) procedure¹⁵ was applied for polyclonal antibodies directed against immunoglobulins A, G, M, and E as well as β_2 -microglobulin

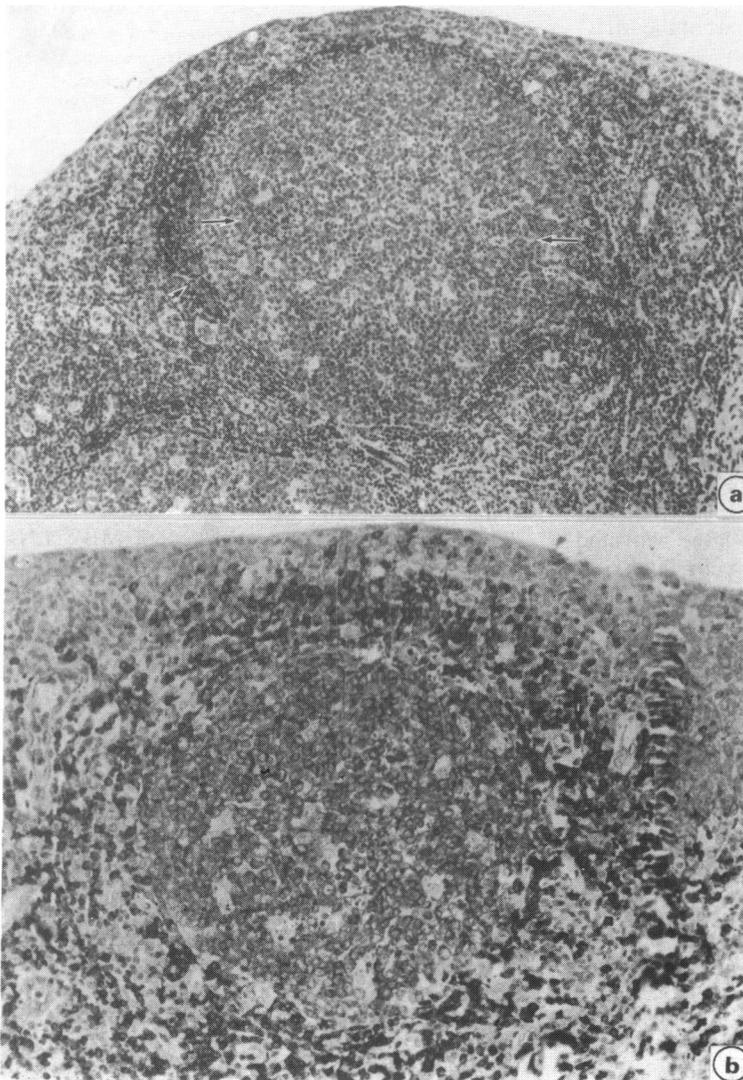


Fig. 1 (a) Conjunctiva obtained from a patient with trachoma stage 2a showing secondary lymphoid follicle containing a large pale follicular centre (arrows) surrounded by a thin lymphocytic mantle (arrowheads). Haematoxylin and eosin, $\times 85$. (b) MB_2 staining. Note that the lymphoid follicle and surrounding mantle are composed mostly of MB_2^+ B lymphocytes. Three-step indirect immunoperoxidase for MB_2 , $\times 135$.

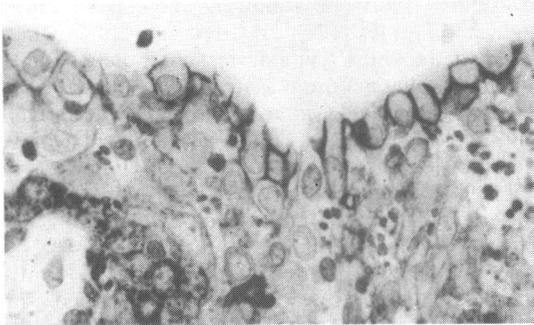


Fig. 2 *TAL-1B₅ staining shows membranous HLA-DR expression in the superficial epithelial layer. Three-step indirect immunoperoxidase for TAL-1B₅, ×385.*

(b₂-m), which is a non-polymorphic part of the MHC class I.

Results

LIGHT MICROSCOPIC FINDINGS

All biopsy specimens showed comparable changes. The overlying epithelium showed mild to moderate hyperplasia, and was infiltrated by a mixed inflammatory infiltrate consisting of mononuclear and polymorphonuclear leucocytes. In the superficial epithelial layers variable numbers of Halberstaedter Prowazek inclusion bodies were seen.

In the underlying stroma, the inflammatory infiltrate was organised as lymphoid follicles and as a diffuse infiltrate. Variably sized secondary lymphoid follicles (Fig. 1a) containing a large, pale follicular centre surrounded by a lymphocytic mantle were seen throughout the biopsy specimens. The follicular centre was composed of small and large activated cleaved and non-cleaved lymphoid cells, and contained many stainable body macrophages. The surrounding lymphocytic mantle was thinned, and contained small darkly stained lymphocytes and lymphocytes of variable size and shape, indicating a progressive transformation.

In the area between the follicles and the epithelium, as well as in areas of diffuse inflammation, a mixed population of lymphocytes, polymorphonuclear leucocytes, macrophages, and some mast cells and eosinophils was observed. The blood vessels in these areas were distended and lined by prominent endothelium.

Finally, a band of plasma cells was situated directly underneath the epithelium, and a dense infiltration by plasma cells was observed also around the acini of accessory lacrimal glands.

IMMUNOHISTOCHEMICAL FINDINGS

All epithelial cells expressed β_2 -microglobulin;

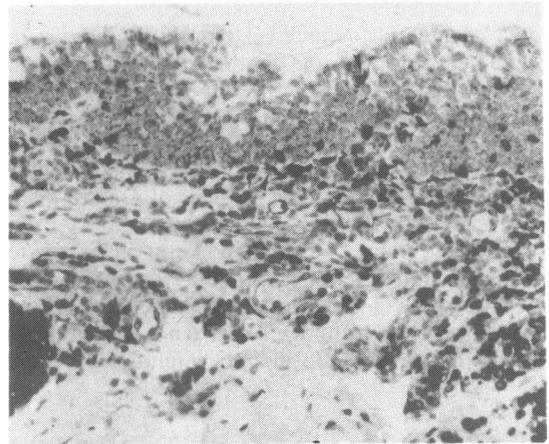


Fig. 3 *LN₂ staining. Note the presence of LN₂+ dendritic cells in the epithelium, in the underlying stroma, and at the interface between epithelium and stroma (arrow heads). Three-step indirect immunoperoxidase for LN₂, ×130.*

membranous HLA-DR expression was noted mainly in the superficial epithelial layers (Fig. 2). The inflammatory infiltrate in the epithelium consisted of large numbers of 3MA134⁺ macrophages, as well as variable numbers of MT₁⁺ T lymphocytes and polymorphonuclear leucocytes. LN₂⁺ HLA-DR⁺ dendritic cells were observed in the deeper epithelial layers as well as in the underlying stroma (Fig. 3), where they were admixed with MT₁⁺ T lymphocytes, 3MA134⁺ macrophages, and scattered MB₂⁺ lymphoid cells. Occasional dendritic cells were situated at the interface between epithelium and stroma.

The lymphoid follicles consisted of MB₂⁺ LN₂⁺ HLA-DR⁺ B lymphocytes (Fig. 1b). In the follicular centre large 3MA134⁺ stainable body macrophages (Fig. 4a), and some MT₁⁺ T cells were observed (Fig. 4b).

In the subepithelial band of plasma cells (Fig. 5a) IgA⁺ cells outnumbered IgG⁺ cells, whereas IgM⁺ and IgE⁺ plasma cells were rare. A similar Ig distribution was found in the plasma cells populating the accessory lacrimal glands (Fig. 5b).

Discussion

Using in-situ immunohistochemical techniques and a panel of monoclonal antibodies we have analysed the cell populations involved in the immune response in the conjunctiva of patients presenting with active trachoma. Distinct compartments involved in humoral and cell mediated immune responses were observed.

The humoral immune response was represented by

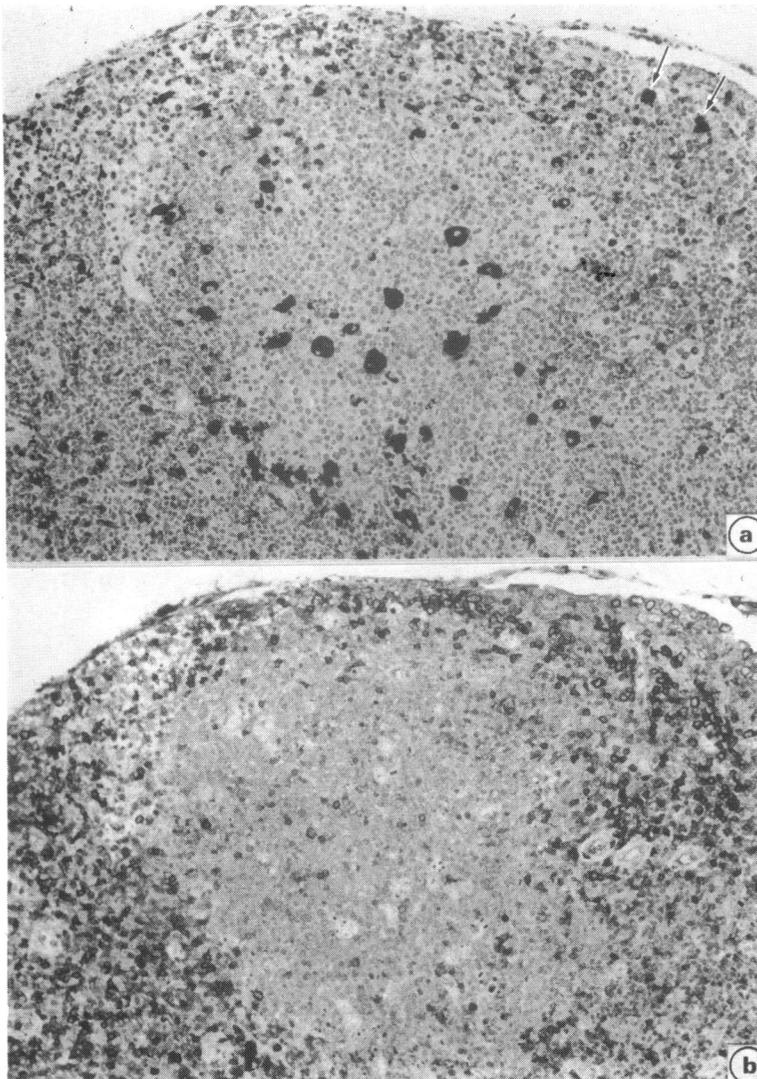


Fig. 4 (a) 3MA134 staining shows large immunoreactive macrophages in the follicular centre. Smaller 3MA134⁺ macrophages are present also in the surrounding infiltrate and in the epithelium (arrows). Three-step indirect immunoperoxidase for 3MA134, $\times 135$. (b) MT₁ staining. Few MT₁⁺ T lymphocytes are present in the follicular centre, whereas many T lymphocytes are present in the surrounding infiltrate. Three-step indirect immunoperoxidase for MT₁, $\times 135$.

large, active lymphoid follicles formed of B lymphocytes, scattered transformed B lymphoid cells in the lymphocytic mantle and area between the follicle and epithelium, and large numbers of plasma cells below the surface epithelium and around the acini of accessory lacrimal glands. Previous in-vitro studies^{19,20} have shown that *C. trachomatis* stimulates B cells to proliferate and differentiate into immunoglobulin-secreting plasma cells. Differentiation to plasma cells is enhanced by T cells, leading to the secretion of large quantities of polyclonal immunoglobulins. Our results indicate that the in-vivo counterpart of this in-vitro response consists of B cells that are stimulated by chlamydial antigens

exposed on the dendritic reticulum cells acting as antigen presenting cells in the follicular centre. Subsequently the B cells undergo transformation, leave the follicular centre, and differentiate into plasma cells, which migrate through the region between the lymphoid follicles and the epithelium. That region was found to consist mainly of T cells and dendritic cells, and it thus appears to represent a suitable microenvironment for the terminal differentiation of B lymphocytes into plasma cells.

IgA⁺ plasma cells outnumbered IgG⁺ plasma cells, whereas IgM⁺ and IgE⁺ plasma cells were conspicuously rare. These findings are in accordance with the detection of type-specific chlamydial IgA

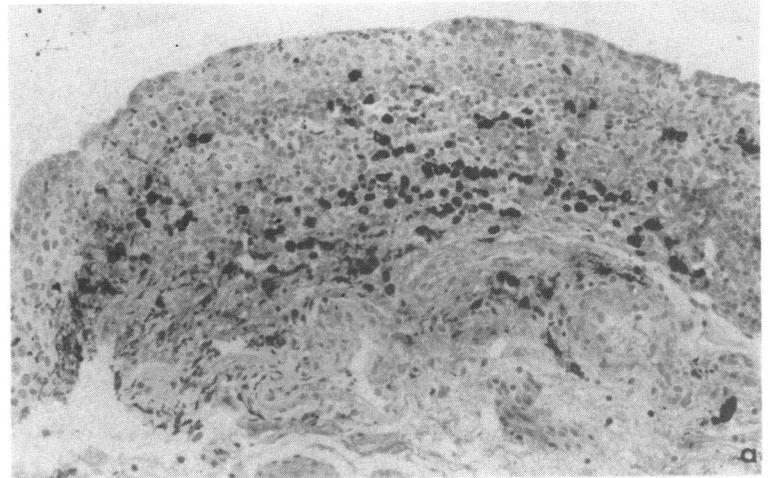
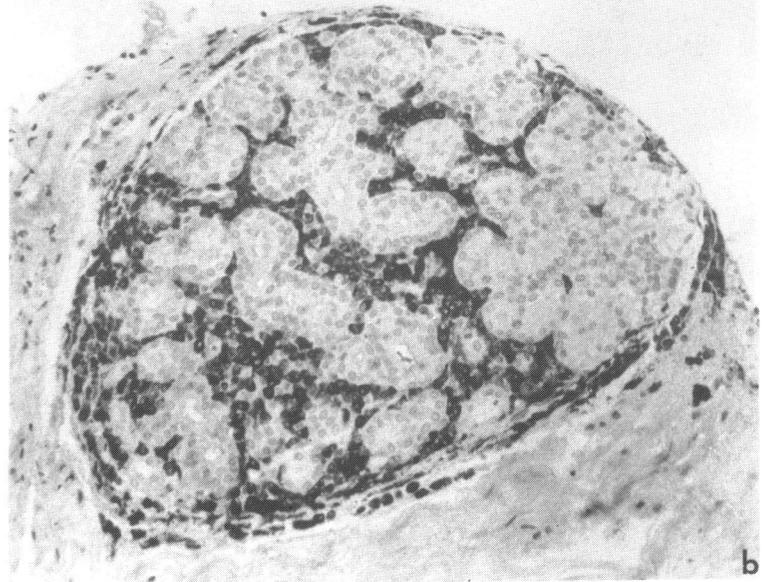


Fig. 5 (a) *IgA staining shows many IgA⁺ plasma cells in a subepithelial band. PAP method for IgA, $\times 135$.* (b) *IgA staining. Note the presence of dense accumulation of IgA⁺ plasma cells around the acini of accessory lacrimal glands. PAP method for IgA, $\times 135$.*



and IgG antibodies in the tears of patients with trachoma.⁵ Dense accumulations of plasma cells, showing a similar Ig distribution, were seen around the acini of accessory lacrimal glands, suggesting that these glands constitute part of the conjunctival protective immune system. According to Franklin and Remus²¹ these IgA⁺ plasma cells originate from B cells present in the conjunctiva-associated lymphoid tissue (CALT), and are involved in the formation of secretory IgA, which has been found to play a part in the defence against chlamydial infection by preventing its adherence to epithelial cells.²²

Scattered LN₂⁺ HLA-DR⁺ dendritic cells were observed in the epithelium, in the underlying stroma,

and at the border of both regions. By analogy with the skin²³ these dendritic cells might be antigen-loaded Langerhans cells, previously shown to occur in the conjunctival epithelium,^{24,25} which have migrated towards the underlying stroma, where they present their antigens in an HLA-DR restricted manner to T lymphocytes. In addition to helper T cells involved in B cell differentiation, the antigen-specific proliferation of T cells induces the development of cytotoxic effector T cells.²⁶ The majority of T lymphocytes present in the epithelium are likely to belong to the cytotoxic T cell subset engaged in cytotoxicity of infected cells. For the cytotoxic T cells to mediate cytotoxicity they must recognise class I

MHC antigen displayed by diseased cells.

The epithelial cells not only expressed the class I MHC associated β_2 -microglobulin, but were also found to express HLA-DR antigens at their surface. HLA-DR antigens are present on cells involved in immune responses, including B cells, activated T cells, and antigen-presenting cells. The presence of HLA-DR antigens on these cells is associated with antigen recognition and presentation to T cells and the initiation of specific B and T cell responses.^{27,28} We have shown that the normal conjunctival epithelium does not express HLA-DR antigens.²⁵ Its expression on the conjunctival epithelial cells in trachoma patients is probably due to the release of interferon-gamma by activated T cells present in the epithelium. Interferon-gamma has been shown to induce HLA-DR synthesis and expression by a variety of epithelial cells in vitro^{29,30} and is produced by lymphocytes stimulated with *C. trachomatis*.³¹ HLA-DR expression might allow conjunctival epithelial cells to present chlamydial antigens to T cells and thus to enhance the immune response in trachoma. Moreover, this HLA-DR expression may result in unopposed T cell proliferation and lead to perpetuation of the immune response. In addition, induction of HLA-DR expression on epithelial cells has been associated with autoimmune reactions—for example, autoimmune thyroiditis^{32,33} and diabetes mellitus.³⁴ The epithelial cells expressing HLA-DR might present autoantigens to T lymphocytes which activate effector B and T lymphocytes, leading to induction of an autoimmune reaction.³⁵ In this context the appearance of autoantibodies in patients with chlamydial salpingitis³⁶ may be relevant.

Finally, considerable numbers of 3MA134⁺ macrophages were observed in the epithelium as well as in the underlying stroma. Whereas most of these cells probably serve as scavenger cells involved in non-specific phagocytosis and degradation of cellular debris, a possible role as effector cells in cell mediated immunity to *C. trachomatis*, as suspected previously,³⁷ cannot be ruled out.

In summary, our data provide histological and immunohistochemical evidence for the development of both humoral and cell-mediated immune responses in the conjunctival tissue of patients suffering from trachoma. The role played by autoimmune mechanisms in this chronic infection needs further investigation.

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