Presence of Langerhans Cells in the Cornea of Klebsiella Keratoconjunctivitis Mice

Enrique García-Olivares,* Buenaventura Carreras,† and José M. Gallardo†

Frozen sections of normal Balb/c corneas and corneas from Balb/c mice with Klebsiella keratoconjunctivitis were examined for the expression of class I, class II H-2 antigens and MAC-1 antigens using monoclonal antibodies in an immunoperoxidase technique. Class I antigens were readily detected, in both normal and diseased corneas, mainly in the epithelium. Class II (Ia) and MAC-1 antigens were not detected in the normal corneas. However, these two antigens were found mainly in the epithelium and to a lesser extent in the stroma of corneas from keratoconjunctivitis mice. Both normal and diseased corneas were furthermore shown to be peroxidase-. Since Langerhans cells (LC) are Ia+, MAC-1+, and peroxidase- cells, we conclude that although the normal mouse cornea is devoid of these cells, under bacterial infection LC infiltrate the corneal epithelium. Invest Ophthalmol Vis Sci 29:108–111, 1988

The expression of major histocompatibility complex (MHC) antigens by the cornea has been studied in different species: (1) indirectly, by immunizing the animals with corneal allografts and testing the immune response against MHC antigens; and (2) directly, by monoclonal antibodies directed toward MHC antigens using immunohistochemical methods or immunofluorescence. Most authors, employing indirect or direct techniques, found that class I antigens were expressed mainly by the corneal epithelium. However, the detection of class II (Ia) antigens is controversial, though the general opinion is that these antigens do not appear or are very scant in normal cornea. Nevertheless, under inflammation and irritation, class II antigens are found in the cornea. In these cases, these antigens seem to be expressed by Langerhans cells (LC) which infiltrate the corneal epithelium, unlike normal conditions in which the cornea has very few or is devoid of LC. LC belong to the monocyte-macrophage lineage and are involved in the antigen presentation to lymphocyte, contributing to the defence against infection in epithelial tissues.

In this paper we have chosen a bacterial keratoconjunctivitis as a model to study the influence of infection on the expression of MHC antigens and on the presence of LC in the mouse cornea.

Materials and Methods

Mice

Adult mice of inbred strain Balb/c were used in these experiments. Studies using these animals conformed with the ARVO Resolution on the Use of Animals in Research.

Keratoconjunctivitis

Since bacterial keratoconjunctivitis is a very frequent disease in laboratory mice, mice eyes were stained with fluorescein and observed with a slit-lamp biomicroscope. Eight out of 24 Balb/c mice showed a superficial keratitis with punctate epithelial erosions and punctate epithelial keratitis associated with a mucopurulent conjunctivitis. Klebsiella spp were identified in bacterial cultures. The infection was, therefore, spontaneous and not experimentally induced. Infected eyes were immediately enucleated and washed extensively in PBS before use.

Antibodies

The following rat anti-mouse monoclonal antibodies (Mab) were used: the M1/42 (Hybritech, La Jolla, CA), directed at class I H-2 antigens; the M5/114 (Hybritech), directed at class II (Ia) mouse antigens; and the M1/70.15 (Sera-lab, Sussex, UK) which recognizes the MAC-1, a mouse antigen identified with the C3bi receptor and expressed by cells of the monocytic-macrophage lineage, including LC.

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From the *Department of Physiology and Biochemistry, Immunology Unit, and the †Department of Ophthalmology, Faculty of Medicine, Granada, Spain.

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Reprint requests: Enrique García-Olivares, Department of Physiology and Biochemistry, Immunology Unit, Faculty of Medicine, 18012 Granada, Spain.
Immunohistology

After being fixed in acetone for 5–10 min, 4 μm frozen sections of normal and *Klebsiella* keratoconjunctivitis corneas were stained by a four-step peroxidase anti-peroxidase (PAP) method. Sections were first treated with rabbit anti-mouse Ig in order to block possible Fc receptors and Ig of mouse cornea. Samples were then incubated with the selected antibody for 60 min at room temperature, followed by a 30 min incubation with goat anti-rat IgG, rabbit anti-goat IgG and finally peroxidase-conjugated goat anti-peroxidase reagent (Nordic, Tilburg, The Netherlands). Each incubation was followed by repeated washing with PBS. Staining was done by incubation of sections in a Tris-Buffer solution (Ph 7.6) that contained 0.05 mg/ml diaminobenzidine and hydrogen peroxide. As control, the first antibody was replaced by non-immune rat serum. Immunoperoxidase-positive cells were counted in central cornea and results expressed as number of positively stained cells ± standard deviation.

In order to test the presence in normal and diseased corneas of any peroxidase-containing cell (macrophages or neutrophils), the endogenous peroxidase of corneal cells was inhibited neither in normal nor in diseased controls.

Results

Expression of MHC Antigens by Normal Mouse Corneas and Corneas From *Klebsiella* Keratoconjunctivitis Mice

The Mab M1/42 directed at class I H-2 antigens reacted with the epithelium of normal and diseased corneas. In both cases, occasional staining was seen in the stroma, mostly in the diseased corneas (Table 1). Class II (Ia) antigens were not detected in the normal cornea by the Mab M5/114. However, this antibody did react with the epithelium of corneas with *Klebsiella* keratoconjunctivitis and also with some cells of the stroma (Fig. 1, Table 1).

Presence of LC in Corneas From *Klebsiella* Keratoconjunctivitis Mice

As expected, normal corneas were not stained when we used the Mab M1/70.15 (Table 1), which recognizes the MAC-1. On the contrary, this antibody reacted with the epithelium of the diseased corneas, and some cells were also stained in the stroma (Fig. 2, Table 1).

Controls in which a non-immune rat serum was used were carried out to test the specificity of our results and, since the endogenous peroxidase had not previously been inhibited (see Methods), also to determine the presence in the cornea of peroxidase-containing cells. Neither in normal nor in diseased controls were peroxidase + cells found (Table 1). Thus, these Ia+, MAC-1+, peroxidase− cells detected in the *Klebsiella* keratoconjunctivitis corneas seem to be LC that infiltrate the corneal epithelium.

Discussion

The results presented in this paper show that, while absent or scant in normal mouse cornea, Ia+, MAC-1+, peroxidase− cells are detected mainly in the epithelium and in a lesser density in the stroma of the *Klebsiella* keratoconjunctivitis corneas (Table 1, Figs. 1, 2). Since LC are Ia+, MAC-1+ peroxidase− cells,13,14 this suggests that chemotactic factors released in bacterial keratoconjunctivitis attract LC from the surrounding tissues (limbus and conjunctiva) to the central cornea, as happens under corneal inflammation6 or irritation.5 The possibility that these infiltrating cells were macrophages and not LC can be excluded. Although macrophages are also Ia+ 15 MAC-1+ inflammatory cells, and from a morphological point of view, are sometimes difficult to distinguish from LC,15,16 they are peroxidase+.17

The presence of LC in the stroma of keratoconjunctivitis corneas suggests that these cells can arrive from the epithelium, where they are detected in a higher density (Table 1, Figs. 1, 2). In the stroma, LC can secrete inflammation mediators18 that could contribute to the inflammation and vascularization of the cornea and facilitate the access of LC to local lymph nodes. Here, as has already been demonstrated in skin,10,19 they could present the antigens to immune-specific cells.

Although LC seem to play, in the cornea as in the rest of epithelial tissues, a defensive role against infection, it is striking that they are absent or scant in...
the normal cornea (Table 1), while the other epithelial tissues, even the limbus and conjunctiva, have a more or less uniform distribution of LC.\textsuperscript{6,20,21} Avascularization of the cornea could explain this absence, since LC are derived from the bone marrow and from there migrate through the bloodstream to the epithelial tissues.\textsuperscript{22} However, it is known that cells that belong to the macrophage lineage also move spontaneously and randomly even without a chemotactical stimulus.\textsuperscript{23} This spontaneous movement would imply that LC progressively populate the cornea until a density equivalent to the limbal or conjunctival is attained. Since this does not happen in normal conditions, we believe that an active mechanism inhibits the infiltration of the cornea by LC. The \textit{raison d’être} of this hypothetical mechanism might be to keep the cornea free of cells that otherwise could produce alterations in refraction. However, under infection, this inhibitory mechanism would probably be blocked and the corneal transparency sacrificed to an ade-
quate immune defence by the LC that enter the corneal epithelium.

From the results shown in this paper we can draw the following conclusions: (1) Since bacterial keratoconjunctivitis is relatively frequent in the laboratory mouse, many of the discrepancies in the expression of class II antigens by the cornea could be due to the fact that some of the experimental animals presented this disease and their corneas were infiltrated by LC. Therefore, we think that the animal corneas should be observed before experimentation. (2) In clinical keratoplasty, human donor corneas should also be observed before transplantation. The presence of Ia+ cells may prejudice the outcome of the graft by virtue of the potent allogenic stimulation of these antigens.

**Key words:** corneal histocompatibility antigens, class II antigens, MAC-1 antigen, Langerhans cells, Klebsiella keratoconjunctivitis

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**References**