Lymphocytes and Langerhans Cells in the Normal Human Cornea

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The distribution of B- and T-lymphocytes, of OKI-a positive cells, and of HLA-ABC antigens in the normal cornea was investigated using monoclonal antibodies. The lymphocytes and Langerhans cells are present mainly in the well-vascularized limbal region but also occur albeit in small number in the center of the cornea. HLA-ABC antigens are strongly expressed on the epithelial cells of the cornea and the limbus. Invest Ophthalmol Vis Sci 26:220–225, 1985

Corneal transplantation has a high success rate if it is performed with a good technique and in appropriate conditions.1–3 The evolution of the corneal graft is influenced by various factors, such as possible disease of the cornea, vascularization of the host cornea, compatibility of donor and receptor tissue antigens, and sex and size of the graft.3 Occasionally immune rejection occurs, most often when the host cornea is vascularized.1,4 Available evidence suggests a primary role for the HLA-D/DR region of the major histocompatibility gene complex in allograft reaction. This is due to the capacity of HLA-D/DR locus antigens to stimulate helper T-lymphocytes.5 The low incidence of allograft rejection in corneal transplantation suggests that the immunologic system of the cornea is developed only poorly, if present at all. The purpose of the present investigations was to study the normal distribution of the elements that play a role in the immunologic defence of the cornea. Therefore, the following components of the immunologic defence mechanisms were studied in the normal human cornea: (1) the presence of T- and B-lymphocytes and of their subsets; (2) the localization and distribution of Langerhans cells; and (3) the localization and distribution of the major histocompatibility antigens, ie, the HLA-ABC antigens and the HLA-DR or la-like antigen in the normal human cornea.

Materials and Methods

In total six eyes were studied. Five were enucleated because of primary malignant melanoma of the choroid. The mean age of these patients was 54 years (range 46 to 67). There were four men and one woman. One eye was obtained post mortem from a 62-year-old man who died of myocardial infarction. The cornea was removed immediately after enucleation of the eye and divided into two equal parts. One part was fixed in Bouin’s solution and embedded in paraffin. Semiserial sections were cut and stained with hematoxylin and eosin for diagnostic histopathology.

The other part was snap frozen in liquid nitrogen cooled isopentane. Five-micron frozen sections were cut, dried at room temperature for 24 hr, and fixed in cold acetone for 10 min. Serial sections were incubated for 30 min with a panel of monoclonal antibodies (Table 1). A 15-min wash in three changes of phosphate buffered saline (PBS), pH 7.2, was followed by incubation for 30 min with peroxidase-conjugated rabbit antimouse IgG (Dako-immunoglobulines, Denmark; diluted 1/20 and containing 5% normal human serum). The sections were washed again in three changes of PBS, pH 7.2, and the reaction product was developed using 0.05% 3-amino-9-ethylcarbazole (Aldrick Co; Beerse, Belgium) and 0.01% H2O2 in a 0.1 M acetate buffer, pH 4.9, for 10 min. Subsequently sections were washed in acetate buffer, counterstained with Mayer’s hematoxylin and mounted.

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Table 1. Characteristics of monoclonal antibodies

<table>
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<th>Monoclonal antibody</th>
<th>Dilution</th>
<th>Cellular distribution</th>
<th>Application</th>
<th>Origin</th>
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<tr>
<td>OKT4</td>
<td>1/10</td>
<td>Peripheral T-lymphocytes, thymocytes, splenocytes</td>
<td>Identification of human helper/inducer T-lymphocyte subclass</td>
<td>ORTHO (Raritan, NJ)</td>
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<td>OKT6</td>
<td>1/10</td>
<td>Thymocytes</td>
<td>Identification of human “common” thymocytes</td>
<td></td>
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<tr>
<td>OKT8</td>
<td>1/10</td>
<td>Peripheral T-lymphocytes, thymocytes, splenocytes</td>
<td>Identification of human suppressor/cytotoxic T-lymphocyte subclass</td>
<td></td>
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<tr>
<td>OKT11</td>
<td>1/10</td>
<td>Peripheral T-lymphocytes, thymocytes</td>
<td>Identification of peripheral T-lymphocytes</td>
<td></td>
</tr>
<tr>
<td>OKIA</td>
<td>1/80</td>
<td>Anti-human DR B-lymphocytes, activated T-lymphocytes, monocytes</td>
<td>Identification of Ia-bearing macrophages</td>
<td></td>
</tr>
<tr>
<td>OKBAI</td>
<td>1/10</td>
<td>B-lymphocytes, monocytes, pre-B-lymphocytes, weakly peripheral blood granulocytes</td>
<td>Identification of B-lymphocytes</td>
<td>Hybriteck (La Golla, CA)</td>
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<td>Anti-Leu-7</td>
<td>1/10</td>
<td>20 ± 7% of adult peripheral blood mononuclear cells</td>
<td>Human lymphocyte antigen: a population that lacks conventional T-cell markers and possesses NK/K function</td>
<td>Beckton (Dickinson, CA)</td>
</tr>
<tr>
<td>Anti-Leu-3A, Anti-Leu-M2, Anti HLA-ABC</td>
<td>1/10</td>
<td>Human monocytes</td>
<td>Helper inducer T-subset, antigen presentation to T-cells</td>
<td>Cappel Laboratories (Cochranville, PA)</td>
</tr>
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Results

Routine Histology

Histologic examination of the hematoxylin-eosin stained sections of all specimens showed a normal appearance of the cornea and the corneoscleral junction or limbus. The cornea was composed of a multilayered epithelium, Bowman’s membrane, stroma, Descemet’s membrane and endothelium. In the connective tissue of the stroma, occasional lymphocytes were observed. The vascularized limbal area contained more lymphocytes, mainly localized around small vessels.

Immunohistochemistry

The cornea and limbus contained both B (BA1 positive) and T-(OKT11 positive) lymphocytes. The vast majority of the cells were detected at the limbus, where moderate numbers of BA1 positive cells were present around the small vessels in the stroma and in the superficial layers of the limbal conjunctival epithelium. The cornea contained only a few BA1 positive lymphocytes in the superficial epithelial layers and in the upper third of the fibrous stroma. No BA1 positive cells were observed in the deeper part of the stroma, in Descemet’s membrane and near or in the endothelium. OKT11 positive cells were found at the limbus as well as in the cornea. At the limbus OKT11 positive, OKT4 positive lymphocytes were concentrated around the vessels.

In the cornea OKT11 positive, OKT4 positive cells were seen in the epithelium as well as in the upper part of the fibrous stroma (Fig. 1). Staining with anti-Leu 3A confirmed the findings observed with the monoclonal antibody OKT4. OKT8 positive cytotoxic/suppressor cells were present in the epithelium and in the fibrous stroma of the cornea and in the epithelium and the connective tissue of the limbus (Fig. 2). In Descemet’s membrane and in the endothelium neither OKT4 positive, nor OKT8 positive cells were found. The cornea and the limbus did not contain any Leu-7 positive cells. Occasional irregularly shaped dendritic OKT8 positive, OKIA positive cells were found in the epithelium of the cornea and in the upper third of the corneal stroma. These cells...
Fig. 1. OKT₄ positive cells are present in the superficial epithelium of the human cornea (immunoperoxidase X125).

Fig. 2. Human cornea: OKT₄ positive cells are found in the stroma and in the epithelium at the limbus (immunoperoxidase X125).
Fig. 3. An irregular OK	extsubscript{II}A positive cell (arrow) is present in the epithelium of the center of the human cornea (immunoperoxidase X50).

were more numerous in the limbic epithelium and stroma (Fig. 3). OK	extsubscript{II}A positive mononuclear cells were seen in the stroma of the limbus. The endothelial cells of the limbic blood vessels showed a positive reaction for OK	extsubscript{II}A (Fig. 4). Epithelial cells of the cornea as well as of the limbus were negative for OK	extsubscript{II}A. Neither Descemet's membrane, nor the underlying endothelium showed any OK	extsubscript{II}A positive staining. HLA-ABC antigens were expressed strongly on the epithelial cells of the cornea and the limbus, as well as on the endothelial cells of the limbic blood vessels. No positive staining was observed on the other layers of cornea and limbus.

Discussion

The availability of monoclonal antibodies allows a more precise and elaborate study of lymphocytes, monocytes, and histocompatibility antigens. It generally is accepted that these components have an important role in various immunologic and inflammatory reactions of known and unknown origin occurring in various tissues. Such diseases also may occur in the eye. The immunopathology of the eye is much less clearly delineated than that of the kidney, the testis, the thyroid gland, or other organs. In order to evaluate the function and the behavior of the various components of the immune system in pathologic conditions in the eye, it is necessary to first study the distribution of these elements in normal conditions. It has been shown that the cornea contains HLA-A and B-antigens. The present observations confirm these data. In contrast, HLA-DR antigens have not been described yet on epithelial cells of the cornea. The studies of M. M. Rodrigues indicate that HLA-DR antigens occur on Langerhans cells in the normal conjunctiva and peripheral cornea. The present observations indicate that similar Ia-like or HLA-DR positive Langerhans cells are occasionally found in the central as well as in the peripheral part of the cornea. Langerhans cells are irregular dendritic cells that have been described in the epidermis, the buccal mucosa, the esophagus, and in general in most squamous epithelia. They express the Ia-like or HLA-DR antigens on their cellular surface and may function as antigen presenting cells and stimulating cells to allogenic lymphocytes. Their phagocytic activity is much less pronounced than that of ordinary macrophages.
Antigen presenting cells are essential for the development of cellular and humoral immunocompetence. A close interaction between these cells and lymphocytes has been described in various organs. From our findings it is clear that antigen-presenting cells and B-lymphocytes as well as T-lymphocytes are present in the normal human cornea albeit in small numbers. Both B- and T-lymphocytes occur mainly in the well-vascularized limbus region but also can be seen in the center of the cornea. The small number of lymphocytes in the central part of the cornea correlates well with the small number of la-like positive cells in this area. The low concentration of these elements in the cornea may be responsible for the low incidence of immunologic reactions in the cornea, in transplantation pathology as well as in other diseases.

It may not be concluded however, that the cornea is immunologically totally incompetent. It contains the various subpopulations of T-lymphocytes and B-lymphocytes. Using monoclonal antibodies, the T-lymphocytes are generally divided into OKT4 positive helper/inducer cells, and OKT8 positive cytotoxic/suppressor cells. These two subpopulations are not necessarily present in the same anatomic localization in the various tissues studied. For instance in the gut, the interepithelial lymphocytes are mainly OKT8 positive cells, whereas the lamina propria lymphocytes are mainly OKT4 positive cells. The present observations indicate that the cornea contains both OKT4 positive and OKT8 positive interepithelial lymphocytes, and that these cells are concentrated densely in the limbus and scarce in the center of the cornea. The OKT4 positive cells are more numerous in the connective tissue of the limbus. Leu7 positive, natural killer cells did not occur in the normal human cornea.

The presence of HLA-ABC antigens, of la-like positive cells and of B- and T-lymphocytes in the normal cornea suggests that the cornea contains an appropriate immunologic system. The distribution of this system, however, is heterogeneous, with a high concentration of elements in the periphery and a low concentration in the center of the cornea.

**Key words:** cornea, B- and T-lymphocytes, monoclonal antibodies, HLA-DR antigen, Langerhans cells
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