Function and Cell Surface Phenotype of Dendritic Cells From Rat Cornea

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Purpose. To isolate dendritic cells (DC) from rat corneas and to examine their functions and surface markers in vitro.

Methods. Cells were isolated enzymatically from dissected rat corneas and cultured for various intervals of time. Dendritic cells were enriched immunomagnetically from corneal cell preparations using monoclonal antibodies against DC surface antigens and tested for functional activity in lymphocyte stimulation assays. In vivo migration of DC was induced by traumatizing the corneal epithelium. Whole mounts of epithelial sheets were stained immunofluorescently with anti-DC antibodies and examined by confocal microscopy. Dendritic cells isolated from traumatized corneas were tested for functional activity.

Results. Corneal DC exhibited the properties of other members of the DC family, i.e., low buoyant density, lymphoid DC-specific markers, and lymphostimulatory function. In fresh unfractionated cell preparations of normal cornea, no functional activity was detected. However, DC immunomagnetically purified from fresh preparations were functionally active. Injury to the corneal epithelium induced the migration of DC from the periphery to the central cornea; DC measured in this situation showed significantly increased functional activity. Finally, IL-1β and GM-CSF enhanced the functional activity of corneal DC.

Conclusions. Corneal DC have lymphostimulatory capacity in situ, but they may be maintained in a state of latency by the suppressive influence exerted by neighboring cells. Injury to the corneal epithelium results in functional activation of the corneal DC, which may be caused by cytokines such as IL-1β or GM-CSF. Thus, corneal DC may be important in the immune regulation of the anterior segment.

Dendritic cells (DC) are a family of bone marrow-derived leukocytes whose properties make them extremely effective as antigen-presenting cells and stimulators of T-cell responses. These cells form an interdigitating network in the limbus and normally are absent from the central cornea. However, various stimuli can induce the migration of DC from the periphery to the central cornea. The presence of DC in the central cornea has a profound effect on the immunologic properties of the cornea, manifested as increased rejection of central corneal grafts and keratitis, iritis, and encephalitis after intranasal herpesvirus infection. More remarkably, antigens injected into the anterior chamber fail to evoke the anterior chamber-associated autoimmune deviation phenomenon if these eyes contain DC in the central cornea. Coinjection of DC along with the antigen into the anterior chamber-associated also fails to elicit anterior chamber autoimmune deviation. Thus, corneal DC appear to have a profound influence on the immune regulation of the cornea and of the anterior chamber. However, there are no previous studies examining the properties of these cells as an isolated population. Consequently, there is little information on the function of corneal DC or the factors that regulate their function. The current study was, therefore, undertaken to isolate and culture DC from rat corneas and to study their functional and phenotypic properties in vitro. We have compared as well the activity of normal DC and DC that migrate to the central cornea after trauma to the corneal epithelium.
METHODS

Inbred Lewis rats (250 to 300 g) were purchased from commercial vendors (Charles River, Raleigh, NC or Harlan Inc., Indianapolis, IN) and were housed in the Animal Resource Facility at the School of Medicine. Inbred SHR rats were a kind gift from Dr. James Buggy (Department of Physiology, School of Medicine, University of South Carolina). All procedures were approved by the Institutional Animal Care and Use Committee and conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Hanks’ balanced salt solution (HBSS) was purchased commercially (Gibco BRL, Grand Island, NY) as a X10 liquid and diluted with sterile distilled water. In some procedures, 2% heat-inactivated horse serum (HS) was added to HBSS (HBSS + HS). Horse serum was purchased commercially (JRH Biosciences, Lenexa, KS) and was heat inactivated by immersion in a water bath at 55°C for 30 minutes. RPMI-1640 was purchased as a sterile liquid (JRH Biosciences) and supplemented with 1% penicillin-streptomycin solution (Gibco BRL) and 1% glutamine solution (Gibco BRL). Cells were cultured in RPMI + 10% horse serum unless otherwise indicated. Dispase was purchased as a sterile liquid (Boehringer Mannheim, Indianapolis, IN), 2.4 IU/ml. DNase was purchased commercially as a lyophilized powder (2 X 10^6 Kunitz U/mg protein; Calbiochem, La Jolla, CA). A 0.5% stock solution was made in HBSS and sterilized by filtration through a 0.22-μm filter (Gelman Sciences, Ann Arbor, MI).

Isolation of Corneal Cells

Rats were killed by ether inhalation. Enucleation was performed by severing the conjunctiva, orbital muscles, and optic nerve with curved scissors and transferring the globes to a petri dish containing HBSS. The individual globes were then placed on sterile paper towels and stripped of adherent muscle and conjunctival tissue. With a scalpel, a single transverse cut was made at the equator so that the eye was dissected into a segment anterior to the lens and a segment posterior to it. The lens and the iris-ciliary body, teased away from the anterior segments, were discarded. The pooled anterior segments, which consisted of the cornea and limbus, were immersed in 10 ml of 50% dispase in HBSS. The tissues were incubated for 90 minutes with occasional, gentle agitation. They were then carefully overlayered with 1 ml of “light” BSA solution and centrifuged at 10,000g for 30 minutes. The low-density fraction at the interface of the two solutions and the high-density cell pellet were collected separately, washed three times with HBSS + HS before use.

Immunomagnetic Separation of Corneal Cells

Immunomagnetic separation of corneal cells was carried out by using a modification of a procedure described by Gee et al.15 Monoclonal antibodies reactive to dendritic cell surface antigens, listed in Table 1, were used to enrich corneal DC immunomagnetically. Purified mouse immunoglobulin G (IgG; all isotypes) used as a control in immunomagnetic and immunofluorescence experiments was purchased commercially (Sigma, St. Louis, MO). Corneal cells were treated with the ascites or affinity-purified antibodies at a concentration of 1 to 2 μg/10^6 cells for 45 minutes at 4°C with occasional, gentle agitation. They were then washed three times with HBSS + HS and incubated for 45 minutes with magnetic beads coated with anti-mouse IgG (Dynal, Great Neck, NY) at a bead:cell ratio of 1:5. After exposure of the cells to a magnetic field, the nonrosetted cells and rosetted cells were collected separately, washed, and assayed for accessory activity.

### TABLE 1. Monoclonal Antibodies Reactive to Rat Lymphoid Dendritic Cell Surface Antigens

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Molecule Detected</th>
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<tbody>
<tr>
<td>G489</td>
<td>Major histocompatibility complex class II (Ia)</td>
</tr>
<tr>
<td>B6G</td>
<td>Lymphoid dendritic cell surface antigen (70 kDa)</td>
</tr>
<tr>
<td>A3C</td>
<td>Lymphoid dendritic cell surface antigen (70 kDa)</td>
</tr>
<tr>
<td>C11B</td>
<td>Lymphoid dendritic cell surface antigen (70 kDa)</td>
</tr>
<tr>
<td>F280</td>
<td>Rat Thy-1 (expressed on lymphoid dendritic cells)</td>
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Ia = immune region associated; ICAM = intercellular adhesion molecule.
FIGURE 1. Accessory activity of corneal cells after various intervals of culture. Cells isolated from normal rat corneas were cultured for various time intervals. Various numbers of recovered nonadherent cells were tested for accessory activity in the oxidative mitogenesis assay. Incorporation of \(^{3}\)H-thymidine was measured during the last 4 hours of a 48-hour incubation. Values represent the mean ± standard deviation of triplicate cultures. • = Freshly isolated cells. • = Cells cultured for 24 hours. • = Cells cultured for 72 hours.

**Oxidative Mitogenesis and Mixed Leukocyte Response Assays**

Oxidative mitogenesis assays were performed according to a previously described procedure.\(^1\) Lymph node cells were fractionated on a discontinuous BSA gradient, and the high-density cells, which have negligible accessory activity, were treated with sodium periodate. Periodate-treated cells (2 \(\times\) 10\(^5\)) were added to serial dilutions of cell preparations in round-bottomed, 96-well plates (Corning Glass Works, Corning, NY). Lymphocyte proliferation was measured during the last 4 hours of a 48-hour incubation by incorporation of \(^{3}\)H-thymidine (6.7 Ci/mM, ICN Radiochemicals, Irvine, CA) added under saturating conditions as described previously.\(^1\) Cells were harvested with a cell harvester (Skatron, Sterling, VA), and \(^{3}\)H label incorporated into DNA was measured using a scintillation counter (Pharmacia LKB, Piscataway, NJ). In all experiments, background \(^{3}\)H-thymidine incorporation by periodate-treated or allogeneic lymphocytes alone was less than 1000 cpm, was not subtracted from test values, and is represented on the ordinate corresponding to X = 0.

High-density lymph node cells prepared from SHR rats were used as allogeneic responders in mixed leukocyte reaction assays. Cell numbers and culture conditions were identical to those described above. Cultures were maintained for 4 days, and \(^{3}\)H-thymidine was added for the final 18 hours of culture. \(^{3}\)H-thymidine, prepared as described above, was added for the final 18 hours of a 4-day incubation period before cell harvest and scintillation counting.

**Preparation of Epithelial Sheets From Normal and Traumatized Corneas**

Corneal trauma and removal of epithelial sheets were performed as described by Miller et al\(^1\)\(^5\) with minor modifications. Rats were anesthetized with intraperitoneal injection of sodium pentobarbital (Abbott Labora-

**Antigen Presentation Assays**

Myelin basic protein as well as the synthetic peptide (pMBP) were kind gifts of Dr. W. Hickey (Dartmouth Medical College, Hanover, NH), Dr. D. Mannie (East Carolina University, Greenville, NC) and Dr. C. Heber-Katz (Wistar Institute, Philadelphia, PA). The lyophilized powders were dissolved in RPMI, filter sterilized, and stored at 4°C. Freund's complete adjuvant containing 1 mg/ml heat-killed *Mycobacterium bovis* was purchased commercially (Sigma). Lewis rats were injected in one hindfoot pad with 0.1 ml of a 1:1 emulsion of the antigen in Freund's complete adjuvant. The total antigen delivered into the foot pad was approximately 50 \(\mu\)g. Seven to 8 days later, the rats were killed by ether inhalation, and the popliteal and inguinal lymph nodes were removed. Cells were isolated from the lymph nodes and fractionated on a BSA gradient as described above. High-density lymph node cells (2 \(\times\) 10\(^5\)) were cultured with various concentrations of accessory cells and protein or peptide antigen in a procedure identical to that described for the oxidative mitogenesis and mixed leukocyte reaction assays. \(^{3}\)H-thymidine, prepared as described above, was added for the final 18 hours of a 4-day incubation period before cell harvest and scintillation counting.

FIGURE 2. Ability of low-density and high-density corneal cells to stimulate periodate-treated and allogeneic lymphocytes. Corneal cells were fractionated into low-density (○) and high-density (▲) cells and cultured for 2 days. Various numbers of low-density and high-density cells were tested for accessory activity in a standard oxidative mitogenesis assay. Values represent the mean ± standard deviation of triplicate cultures.
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FIGURE 3. Accessory activity of corneal cells fractionated by immunomagnetic separation. Corneal cells were incubated with various monoclonal antibodies or normal mouse IgG (control), washed, and further incubated with immunomagnetic beads coated with anti-mouse IgG. The rosetted and nonrosetted fractions were separated using a magnet and cultured for 48 hours. For each antibody, rosetted cells (2 to 2.5 × 10³ cells/well, cross-hatched bar) and nonrosetted cells (50 × 10³ cells/well, hatched bar) were tested for accessory activity in an oxidative mitogenesis assay. Values represent the mean ± standard deviation of triplicate cultures.

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RESULTS

Lymphostimulatory Activity of Dendritic Cells in Cultures of Corneal Cells

Corneal cells were obtained by enzymatic treatment of dissected rat globes as described in Materials and Methods. In preliminary experiments, trypsin was used and yielded 0.92 ± 0.5 × 10⁶ cells per animal (n = 10) with a viability of 55% ± 9%. The use of dispase to disrupt the tissue yielded 1.4 ± 0.5 × 10⁶ cells per animal, with a mean viability >95% (n = 13). Thus, in all experiments described in this article, dispase was used to disrupt the corneal tissue. This method of enzymatic digestion for isolation of single cells does not affect the accessory function of rat DC isolated from skin and other tissues (unpublished observation, 1993). Cells were cultured for various intervals of time and assayed for their ability to stimulate periodate-treated lymphocytes. Of the initial corneal cell population, 37.2% ± 28.8% was recovered after 24 hours of culture, and 23.3% ± 13% was recovered after 72 hours of culture (n = 4). As seen in Figure 1, freshly isolated corneal cells had negligible accessory activity, which increased significantly after 24 hours of culture. Further culture up to 72 hours did not result in a significant increase in accessory activity.

Corneal cells were fractionated on a discontinuous BSA gradient; 44.7% ± 17.0% of the corneal cells were recovered in the low-density fraction, and 51.9% ± 22.2% were recovered in the high-density fraction (n = 4). When tested for accessory activity in an oxidative mitogenesis assay, virtually all the accessory activity was associated with the low-density fraction (Fig. 2). Accessory activity for periodate-treated lymphocytes and a low buoyant density are characteristic properties of dendritic cells.

Functional Activity of Immunomagnetically Enriched Corneal Dendritic Cells

To isolate corneal DC for functional studies with minimum influence of contaminating cells, freshly prepared corneal cells were subjected to immunomagnetic separation. Approximately 1% of the total corneal cells were recovered in the rosetted fraction, and >95% of the cells were partitioned into the nonrosetted fraction. The addition of 2 to 2.5 × 10³ immunomagnetically enriched cells to periodate-treated lymphocytes resulted in a 35- to 60-fold increase over...
FIGURE 4. Lymphostimulatory activity of freshly isolated and cultured corneal dendritic cells, immunomagnetically enriched with anti-major histocompatibility complex class II antibody G489. Various numbers of rosetted corneal dendritic cells that were freshly isolated (•) or cultured for 24 (A) and 72 hours (•) were tested for immunostimulatory activity with periodate-treated (A) or allogeneic (B) high-density lymph node cells. Values represent the mean ± standard deviation of triplicate cultures.

Baseline incorporation with the antibodies G489, A3C, and C11B; the increase noted with B6G was of a lower magnitude (3- to 4-fold)(Fig. 3). The antibody F280 failed to enrich corneal DC selectively. None of the nonrosetted fractions displayed accessory activity except the cells rosetted with control mouse IgG.

In subsequent functional assays, DC were immunomagnetically enriched from corneal cells using the anti-major histocompatibility complex class II antibody, G489. The recovery of Ia-rosetted cells was 1.1% ± 0.6%, and that of nonrosetted cells was 88.5% ± 8.6% (n = 14). Immunomagnetically enriched corneal DC were tested for their ability to stimulate periodate-treated and allogeneic (Fig. 4B) lymphocytes at time 0. The activity of the corneal DC increased slightly after culture for 24 or 72 hours.

The finding that fresh, unfractionated corneal cells lacked accessory activity (Fig. 1) and DC immunomagnetically separated from fresh corneal cells showed robust functional activity (Figs. 3, 4) suggested that corneal cells may be inhibitory. In preliminary experiments, fresh corneal cells and corneal cells cultured for 72 hours that were immunomagnetically depleted of cells expressing class II major histocompatibility complex were able to inhibit lymphoproliferation in a standard oxidative mitogenesis assay with lymph node-derived accessory cells (data not shown). Other studies have reported that corneal epithelial cells and fibroblasts exert an inhibitory effect on lymphocyte activation directly and/or through soluble factors.16-18

Antigen Presentation by Corneal Dendritic Cells

Corneal DC also were examined for their ability to stimulate antigen-primed lymphocytes in standard assays for antigen presentation. Dendritic cells were immunomagnetically enriched from corneal cells and cultured for various times before their use in assays as antigen-presenting cells. Fresh as well as cultured corneal DC were efficient stimulators of myelin basic protein-primed lymphocytes, and no significant difference in the antigen-presenting cell function of fresh and cultured corneal DC was observed (Fig. 5). Similar results were noted when ovalbumin protein or peptides obtained by trypsin digestion were used as antigen (data not shown).

Phenotypic Characteristics of Corneal Dendritic Cells and Their Migration After Injury to the Central Cornea

Previous studies9 have documented the migration of DC from the peripheral cornea toward the center after injury to the corneal epithelium. One of the aims of this study was to compare the properties of corneal DC from normal and traumatized corneas. Migration of corneal DC was induced, and corneal epithelial sheets were stained immunofluorescently with the panel of monoclonal antibodies listed in Table 1. An anti-keratin monoclonal antibody was used as a positive control, and normal mouse IgG was used as a negative control. The results of the immunofluorescence studies are presented in Figure 6. Confocal microscopy of the epithelial sheets from the normal corneas revealed the presence of immunofluorescently stained cells of interdigitating morphology, arranged regularly in circular patterns along the periphery of the cornea (Figs. 6A to 6C). Positive staining was observed with the antibodies G489, A3C, and C11B (Figs.
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The appearance of epithelial sheets from traumatized corneas differed significantly from that of normal eyes. Fluorescent cells of interdigitating morphology were observed along the periphery in traumatized epithelia; however, the concentric arrangement of these cells noted in the normal peripheral corneas was disrupted (Fig. 7A to 7C). The fluorescent cells were not contained in a well-defined region at the periphery. Instead, the cells were scattered throughout the entire area of the epithelium with decreasing density from the periphery to the center. In contrast to normal eyes, the central cornea in traumatized eyes contained many dendritic cell clusters or isolated cells (Figs. 7a to 7c). The phenotype of the DC in traumatized corneas was identical to that of DC in normal eyes because they stained strongly with the antibodies G489 (Fig. 7Aa), A3C (7Bb), and C11B (7Cc), weakly with B6G (not shown) and did not stain with F280 (not shown). The observed phenotype of corneal DC correlated with the findings of the immunomagnetic studies.

Comparison of Dendritic Cell Activity From Normal and Traumatized Corneas

The immunofluorescence studies demonstrated the migration of DC from the periphery to the center of the cornea after injury to the epithelium. The phenotype of DC in normal and injured corneas was identical. For a comparison of functional activity, DC were immunomagnetically enriched from normal and traumatized corneas using the antibody G489. Freshly isolated DC then were used to stimulate periodate-treated and allogeneic lymphocytes in standard lymphoproliferative assays. Comparison of functional activity on a per cell basis revealed that DC from traumatized corneas were 2.5 to 4 times more potent stimulators of periodate-treated or allogeneic lymphocytes (Table 2). These experiments, however, did not provide data on the activity of those DC that had migrated to the central cornea compared to those that remained in the limbus after trauma. To address this question, central corneal buttons were removed from normal and traumatized corneas using a trephine with a diameter of 1.7 mm. The average diameter of excised rat corneas measured approximately 3 to 5 mm. Corneal cells were obtained enzymatically from the corneal buttons as well as from peripheral rings and were immunomagnetically rosetted with the antibody G489. No significant difference in total cell yield, or immunomagnetically separated populations, was observed between normal and traumatized corneas, central buttons, and limbic rings. The rosetted cells were tested for accessory activity in an oxidative mitogenesis assay. As shown in Table 2, the activity of cells from central and peripheral corneas of traumatized eyes was greater than that of counterparts from normal eyes. Rosetted cells from the central cornea of normal

6A, 6B, and 6C, respectively). No fluorescent cells were observed with the control IgG (negative control) (Fig. 6D). The antibody B6G stained these cells very weakly, and staining with the antibody F280 was comparable to the negative control (not shown). The region along the circumference that contained the fluorescent cells was well demarcated at the periphery, and no positively stained cells were observed in the central or paracentral cornea. Figure 6E shows the central corneal epithelium stained with the antibody G489 and demonstrates the absence of positively stained cells. Staining with the anti-keratin antibody revealed the cobblestone appearance of the epithelial cells in a continuous sheet (Fig. 6F).
FIGURE 6. Immunofluorescent staining of epithelial sheets from normal cornea with antidendritic cell monoclonal antibodies. Epithelial sheets were removed from dissected corneas using ethylenediaminetetraacetic acid as described in Materials and Methods. The sheets were adhered to poly-lysine-coated slides, fixed with paraformaldehyde, and incubated with anti-dendritic cell monoclonal antibodies. They were then washed and further treated with goat anti-mouse IgG conjugated with Texas Red. Fluorescent cells were visualized using a confocal microscope. (A to C) Regions in the periphery of the cornea (limbus): A, G489; B, A3C; C, C11B; D, negative control; E, staining of the central corneal epithelium with G489; F, staining of the central corneal epithelium with anti-keratin antibody.
FIGURE 7. Immunofluorescent staining of epithelial sheets from traumatized corneas with anti-dendritic cell monoclonal antibodies. The corneal epithelium of rat eyes was abraded as described in Materials and Methods. Five days later, the corneas were dissected, and the epithelial sheets were stained immunofluorescently as described for Figure 7 and visualized by confocal microscopy. Parts A to C depict regions in the periphery of the cornea (limbus), and subparts a to c depict regions of the central cornea. Aa = G489; Bb = A3C; Cc = C11B.
TABLE 2. Immunostimulatory Activity of Dendritic Cell Immunomagnetically Enriched From Normal and Traumatized Corneas

<table>
<thead>
<tr>
<th>Source/Number of Rosetted Cells</th>
<th>Normal (cpm ± SD)</th>
<th>Traumatized (cpm ± SD)</th>
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<tbody>
<tr>
<td>Background</td>
<td>220 ± 69</td>
<td></td>
</tr>
<tr>
<td>Whole corneas (oxidative mitogenesis)</td>
<td>1000</td>
<td>1293 ± 262</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>3293 ± 609</td>
</tr>
<tr>
<td>Whole corneas (mixed leulocyte response)</td>
<td>1000</td>
<td>1404 ± 224</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>2492 ± 234</td>
</tr>
<tr>
<td>Corneal buttons (oxidative mitogenesis)</td>
<td>1000</td>
<td>254 ± 91</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>781 ± 186</td>
</tr>
<tr>
<td>Limbic rings (oxidative mitogenesis)</td>
<td>1000</td>
<td>578 ± 245</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>1799 ± 207</td>
</tr>
</tbody>
</table>

SD = standard deviation.

eyes did not display accessory activity over background, which correlates with the observation that no immunofluorescent DC were observed in this region (Fig. 6E). Thus, nonspecific binding most likely accounts for the small population (<1%) of cells from the central corneas of normal eyes recovered in the rosetted fraction. Taken together, the findings of these experiments indicate that the signal for functional activation delivered to corneal DC after injury is not restricted to those DC that have migrated into the central cornea; rather, it extends throughout the corneal epithelium.

Effect of Cytokines and Metabolic Agents on the Function of Corneal Dendritic Cells

Cytokines such as IL-1β, TGFβ, TNFα, and several others are known to be present within the normal ocular environment or to be secreted during inflammatory responses. These cytokines have potent immunomodulatory effects. Additionally, IL-1β has been demonstrated to induce the migration of corneal DC from the periphery to the central cornea. The effect of these cytokines and other metabolic agents on the function of corneal DC was, therefore, examined in this study. Immunomagnetically enriched corneal DC were cultured for 2 days in serum-free HL-1 medium in the presence of various cytokines and metabolic agents at concentrations listed in Table 3. They were then washed and used as accessory cells in an oxidative mitogenesis assay. Among the various agents tested, IL-1β and GM-CSF increased the accessory activity of corneal DC by 3.5-fold and 14-fold, respectively. TNFα also caused a slight increase in activity (approximately 1.5-fold), although the increase was not as great as that caused by IL-1β and GM-CSF. TGFβ caused a 50% inhibition of DC function. None of the other agents had a significant effect on the accessory function of corneal DC (Fig. 8). Cycloheximide and staurosporine displayed little effect on the function of corneal DC; however, cycloheximide abrogated the increase in accessory activity mediated by IL-1β and GM-CSF (Fig. 9), which suggests that these cytokines exert their effect through a mechanism that required de novo protein synthesis.

DISCUSSION

In this study, we have outlined an approach to enrich corneal DC on the basis of physical and phenotypic characteristics. Rat corneal DC have a low buoyant density similar to that of dendritic cells from lymphoid tissues. These cells also expressed the lymphoid DC antigens detected by the antibodies G489, C11B, and
FIGURE 8. Effect of cytokines and metabolic agents on the accessory activity of corneal dendritic cells. Corneal dendritic cells, immunomagnetically purified with the anti-major histocompatibility complex class II antibody G489, were cultured for 2 days with various cytokines or metabolic inhibitors in serum-free HL-1 medium, washed, and used as accessory cells to stimulate periodate-treated lymphocytes. The source of the cytokines and the concentrations used are given in Table 3. Approximately 1.5 to 2 × 10⁶ rosetted cells treated with the various agents were added per well as accessory cells. Values represent the mean ± standard deviation of triplicate cultures.

A3C, although the antigen detected by the antibody B6G was very weakly expressed. The Thy-1 antigen of rat detected by the antibody F280 was not expressed on corneal DC, although it is known to be expressed on lymphoid DC. Conical and lymphoid DC also were similar functionally in their ability to stimulate periodate-treated allogeneic lymphocytes as well as to process and present antigen. Approximately equal numbers of corneal DC were detected in situ (data not shown) or were enriched immunomagnetically from corneal cells when the antibodies G489, C11B, and A3C were used. Moreover, the DC isolated with each of the three antibodies had comparable functional activity, suggesting that the same cell population expressed the three markers. Corneal DC exposed to cytokines, such as IL-1β or GM-CSF, in vitro or recovered from corneas after injury to the epithelia in situ exhibited an increase in functional activity, but they retained the phenotype of DC isolated from normal corneas. The relationship between functional properties and phenotypic characteristic of DC remains to be elucidated.

One of the functions of the cornea is to serve as a physicochemical and mechanical barrier that protects the integrity of the eye from potential pathogens and ocular trauma. Our study provides evidence that DC in the corneal epithelium can function as accessory cells, which suggests that they may play a critical role in immune surveillance against pathogenic invasion. Based on the unique distribution of corneal DC and the ability of other corneal cells to regulate DC function, it is possible that local environmental factors prevent DC migration from the limbus and control functional activation. Corneal epithelial cells and fibroblasts have been reported to exert an inhibitory effect on lymphocyte activation directly and through soluble factors. Immune privilege of the anterior segment could thus be sustained until the stimulatory signal(s) is delivered after infection or injury to the central corneal epithelium.

How corneal DC are regulated in situ is unknown. Studies on human corneal cells by Shams et al have demonstrated that γ-interferon, Staphylococcus aureus, or lipopolysaccharide can stimulate the secretion of IL-1β. We have shown that cytokines such as IL-1β and granulocyte-macrophage colony-stimulating factor upregulate DC activity, whereas cytokines such as TGFβ have a suppressive effect. In addition to the stimulatory effect of IL-1β noted in the current study, a previous article demonstrated that it induces the migration of DC into the central cornea. With regard to inhibitory factors, corneal epithelial cells are known to produce inhibitory prostaglandins and other undefined, membrane-associated factors capable of suppressing lymphocyte responses. Other regulatory peptides may be transported across the corneal stroma from the aqueous humor of the anterior chamber, which contains an assortment of inhibitory factors such as TGFβ, α-melanocyte stimulating hormone, calcitonin gene-related protein, and vasoactive intestinal peptide. Similarly, regulatory factors present in lacrimal secretions may play a role in regulating the function of corneal DC.

The purpose of the migration of DC from the periphery to the central cornea after trauma remains unclear. It has been suggested that the migration may be driven along a chemotactic gradient extending from the central corneal injury to the
Values represent the mean ± standard deviation of triplicate invading pathogens. The migration of Langerhans
These DC may then exit through efferent lymphatics which mediate adhesion to extracellular matrix pro-
cation of chemical irritants to the surface of the skin or the blood vessels during subsequent neovasculari-
migration of the cornea to the lymphoid tissue, where uptake of antigen at the site of injury or infection.
They may initiate an effective response against the invading pathogens. The migration of Langerhans cells from the epidermis to lymph nodes after application of chemical irritants to the surface of the skin is a well-documented phenomenon. The movement of corneal DC after epithelial injury may, therefore, be parallel to the process that occurs in the skin during contact sensitization. Little is known about the molecules on the cell surface that may play a role in the process of migration. Our immunofluorescence data did not reveal any significant differences in the phenotype of resident and migrant corneal DC. However, it is likely that changes in other surface molecules occur. Integrins that mediate attachment to extracellular matrices would be likely candidates. Human epidermal Langerhans cells are known to express α5β1 and α6β1 integrins, which mediate adhesion to extracellular matrix proteins such as laminin, fibronectin, and type IV collagen. Modulation of surface adhesion molecules of corneal DC may be involved in their mobilization after injury.

Our findings suggest an involvement of DC in the regulation of immune responses in the cornea and the anterior chamber. Development of methods specifically to deplete DC in corneal grafts in vivo would be of use in improving the outcome of corneal transplantation. Moreover, modulation of the in vivo function of DC may provide approaches for the therapy of inflammatory diseases of the anterior segment. Exposure of corneal allografts to ultraviolet radiation before transplantation not only reduces the immunogenicity of the graft but renders them tolerogenic. In addition to phototherapy, antibodies against surface molecules of DC may modulate DC activity. Antibodies to cell adhesion molecules, such as LFA-1 and ICAM-1, have been demonstrated to increase the survival of murine corneal allografts. These molecules are expressed abundantly on the surface of DC, and antibodies against them may affect the progress of the disease condition by modulating DC activity. Thus, future directions in therapy for immunopathologic diseases may involve the specific modulation of DC rather than empirical treatment with drugs such as corticosteroids, which have a nonspecific mechanism of action and many side effects. The understanding of DC regulation and differentiation is crucial for the development of these approaches.

Key Words
corneal epithelium, cytokines, dendritic cells, immunoregulation, T-cell responses

References
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