Human Leukocyte Antigen-Class II-Positive Human Corneal Epithelial Cells Activate Allogeneic T Cells

Mitsuhiro Iwata,*† Atsuhito Yagihashi,‡ Melvin I. Roat,* Adrian Zeevi,‡ Yuichi Iwaki,‡ and Richard A. Thoft†

Purpose. To achieve a better understanding of the mechanism of corneal immune diseases, including corneal allograft rejection, the authors examined the potential of human corneal epithelial (HCE) cells to activate allogeneic T lymphocytes.

Methods. The mixed lymphocyte–HCE cell reaction (MLCER) was performed as follows: HCE cells from primary cultures, with or without treatment with interferon-γ (IFN-γ), were treated with mitomycin C and then mixed with peripheral blood lymphocytes (PBL) from normal volunteers. Triplicate cultures were incubated for 7 days. Interleukin-1α (IL-1α) was added to some cultures to examine its effect on MLCER. The lymphocyte responses were measured by 3H-thymidine uptake for the last 18 hours of incubation in MLCER.

Results. IFN-γ-treated, HLA-class-II-bearing HCE cells stimulated allogenic lymphocytes, whereas IFN-γ nontreated, class-II-negative HCE cells did not. The stimulation by IFN-γ-treated HCE cells was blocked by anti-HLA class II monoclonal antibody. In addition, exogenous IL-1α reduced the lymphocyte response in MLCER. This effort was inhibited by indomethacin.

Conclusions. This study demonstrates that HLA-class-II-bearing HCE cells can activate allogeneic PBL by a major histocompatibility complex class II-dependent mechanism. In addition, HCE cells may regulate immune reactions, probably through prostaglandin production caused by IL-1.

T lymphocytes recognize antigenic peptides in the context of major histocompatibility complex (MHC) gene products.1 The initiation of T-cell responses requires an antigen-presenting cell (APC). The MHC class II antigens expressed on the surface of APC play a pivotal role in the primary T-cell response.2 Originally, the MHC class II antigens were thought to be expressed only on the surface of the cells of the immune system. However, with inflammation such as that found with autoimmune diseases or allograft rejection, class II antigen expression has been detected on many cell types that do not normally express class II antigens.3-6 Aberrant class II antigen expression on the cell surface may be critical to the pathogenesis of such diseases.3-7 Cytokines such as interferon-γ (IFN-γ) can induce class II antigen expression on various kinds of cells in vitro.8-11 The function of IFN-γ-induced, class II-bearing cells to present antigen to T cells has been studied in such cells as renal tubular cells,12 vascular endothelial cells,13 liver sinusoidal lining cells,14 epidermal keratinocytes,15,16 and fibroblasts.17,18 According to the results of those studies, the ability of these cells to function as APCs varies and depends on cell type origin. For example, IFN-γ-treated, class II-bearing fibroblasts are an effective APC for allografted CD4-positive T cells. This is in contrast to their inability to stimulate freshly isolated CD4-positive T cells.17 It is suggested that the initiation of the primary T-cell responses requires another interaction with APC, in addition to the interaction through antigen peptide–MHC class II complex of
APC and T-cell receptor–CD3 molecules. Recently, adhesion molecules, such as ICAM-1, ICAM-2, or LFA-3 expressed on APC and LFA-1 or LFA-2 expressed on T cells, have been shown to play a role in the APC–T-cell interaction. Adhesion molecules such as LFA-1 and LFA-2 produce important signals inside T cells and may be especially important to the primary T-cell response.

In the cornea, it is thought that Langerhans cells, which exist in limbal and peripheral cornea, can be potent APCs. It has been shown that rabbit corneal endothelium, expressing class II antigen after treatment with IFN-γ, could stimulate fresh allogeneic lymphocytes. On the other hand, human corneal fibroblasts, treated with IFN-γ and expressed class II antigen, were not capable of inducing the primary response of allogeneic lymphocytes. The ability of human corneal epithelial cells to function as APCs and to initiate T cell-responses has not been studied.

It has been demonstrated that there is no class II antigen expression on cultured human corneal epithelial (HCE) cells and that the expression of the human leukocyte antigen (HLA) class II could be induced on HCE cells by IFN-γ.

To investigate the potential of human corneal epithelial cells to function as APCs, we examined the ability of cultured HCE cells to stimulate allogeneic lymphocytes in the primary mixed lymphocyte–HCE cell reaction (MLCER).

It has been shown that IL-1 is necessary and increases lymphocyte proliferation in the primary mixed lymphocyte reaction (MLR). IL-1 can be produced by corneal epithelial cells. Therefore, we examined IL-1 production by cultured HCE cells, and we also examined the effect of the addition of exogenous recombinant human IL-1 on MLCER.

**MATERIALS AND METHODS**

**Human Corneal Epithelial Cell Culture**

Human donor eyes were provided by the Medical Eye Bank of Western Pennsylvania (Pittsburgh, PA).

Primary cultures of HCE cells were prepared as described. Briefly, limbal explants without endothelium were incubated with modified SHEM, which consists of Ham’s F12 and Dulbecco’s modified Eagle’s medium (1:1), supplemented with mouse epidermal growth factor (10 ng/ml), bovine insulin (5 µg/ml) (Collaborative Research, Bedford, MA), cholera toxin (0.1 µg/ml) (Sigma, St. Louis, MO), L-glutamine (1 µg/ml), dimethylsulfoxide (0.5%), gentamicin (40 µg/ml), and 10% fetal calf serum (Irvine Scientific, Santa Ana, CA) in 35-mm tissue culture dishes (Falcon 3001, Becton Dickinson, Franklin Lakes, N.J). The cultures were incubated at 37°C in 5% CO₂, and the medium was changed twice a week. The explants were removed after confluence was reached.

**Induction of the HLA Class II Antigens on HCE Cells**

HCE cell cultures were treated with human recombinant human IFN-γ (500 U/ml) (Genzyme, Boston, MA) for the last 4 days of each incubation period.

**Flow Cytometric Analysis**

Primary HCE cell cultures were treated with 0.25% trypsin and 0.5% ethylenediaminetetraacetic acid (EDTA, Sigma) and were converted to single-cell suspension. After the cells were settled in 10% fetal calf serum at room temperature for 1 to 2 hours for cellular recovery, the cells were stained by the following indirect immunofluorescence technique. The cells were incubated with anti-HLA-DR monoclonal antibody (Becton Dickinson, Mountain View, CA) or anti-HLA class I monoclonal antibody (DAKO, Carpinteria, CA) or control mouse IgG (Organ Teknika Cappel, Westchester, PA) and then with fluorescein isothiocyanate-conjugated goat anti-mouse IgG second antibody (Organ Teknika Cappel) for 30 minutes at 4°C. The cells were analyzed by flow cytometry using FACStar (Becton Dickinson, San Jose, CA).

**Keratin Expression in Primary HCE Cell Cultures**

Primary cultures from limbal explants grown in Lab-Tec chamber slides (Nunc, Naperville, IL) were prepared as described by Kiritoshi et al. Also frozen OCT sections from scraped primary cultures were prepared as described. The cells and OCT sections were examined by immunoperoxidase staining with AE1 and AE3 (gifts of Tung-Tien Sun, PhD), using a previously described procedure.

**Isolation of Lymphocytes**

Peripheral blood was collected from normal, healthy volunteers and then heparinized, and lymphocytes were isolated by density step gradient centrifugation with lymphocyte separation medium (LSM, Organ Teknika, Durham, NC). Peripheral blood lymphocytes (PBL) were washed and suspended in RPMI 1640 (Gibco, Grand Island, NY) with 10% heat-inactivated (at 56°C, for 60 minutes) human AB serum (NABI, Miami, FL).

**Mixed Lymphocyte–HCE Cell Reaction**

We examined the function of HCE cells from five different donors to stimulate responder lymphocytes, which were isolated from two different individuals. HCE cells from primary cultures, with or without treat-
Allo Mixed Lymphocyte Reaction

Allo mixed lymphocyte reaction (allo MLR) was established with mitomycin C-treated (20 μg/ml, for 30 minutes) stimulator allogenic lymphocytes (1 × 10^5) and an equal number of responder lymphocytes in 96-well microtiter plates. Triplicate cultures were incubated in RPMI 1640 with 10% heat-inactivated human AB serum at 37°C in 5% CO_2. The lymphocyte proliferation was assessed in the same fashion as with the MLCER.

Blocking of MLCER and Allo MLR With Monoclonal Antibodies

Blocking assays with monoclonal antibodies (mAb) were performed as follows: Anti-HLA-DR mAb (clone L243, Becton Dickinson, Mountain View, CA) (0.5 μg/ml), anti-HLA class I mAb (clone W6/32, DAKO) (0.5 μg/ml), or control mouse IgG (Organ Teknica Cappel, Westchester, PA) (1.0 μg/ml) were added to the wells at the initiation of MLCER and allo MLR and remained throughout the culture period.

Sandwich Enzyme Immunoassays for Human Interleukin-1

The enzyme-linked immunosorbent assay for human interleukin-1 (hIL-1-α and hIL-1-β) were performed with hIL-1 ELISA kit (Otsuka Pharmaceutical, Tokushima, Japan) as follows: Mouse anti-hIL-1-α or -β mAb was immobilized on 96-well microplates. Recombinant hIL-1-α or -β, or culture supernatants of HCE cells, were added to the 96-well plate to a total volume of 200 μl and then incubated overnight at 4°C. After washing the plates, rabbit anti-IL-1-α or -β antisera was added and incubated for 2 hours at room temperature. The plates were incubated with anti-rabbit IgG-peroxidase conjugate for 2 hours at room temperature. After washing the plates, the substrate o-phenylene-diamine was added and incubated for 30 minutes. The enzyme reactions were stopped with 1 N H_2SO_4.

Effects of the Addition of Recombinant hIL-1 and Indomethacin

Recombinant human interleukin 1 (rIL-1) (Genzyme, Boston, MA) or indomethacin (INDO, Sigma), or the combination of rIL-1 and indomethacin, were added in some cultures of MLCER and allo MLR for the whole incubation period.

RESULTS

Characterization of Cell Cultures

The cell shape of the primary cultures of HCE cells consisted of small polygonal cells. All the HCE cells showed strong reactivity with AE1-AE3 by immunohistochemical staining of the cells cultured in Lab-Tec chamber slides (Nunc) and frozen sections of scraped HCE cell cultures (data not shown).

Expression of the HLA Antigens

By flow cytometric analysis, as shown in Figure 1a, there was no positive staining with anti-HLA-DR monoclonal antibody on HCE cells without IFN-γ treatment. This finding supports our previous report. Approximately 50% of HCE cells treated with human recombinant IFN-γ 500 U/ml for 4 days expressed HLA-DR antigen after 0.25% trypsin and 0.5% EDTA treatment to detach the cells from culture dishes (Fig. 1b). HLA class I expression on HCE cells was detected without IFN-γ treatment. IFN-γ (500 U/ml, for 4 days) enhanced HLA class I expression on HCE cells (Fig. 1c). As we previously demonstrated, HLA class II induction is nearly maximized by incubation with IFN-γ (500 U/ml) for 4 days.

We examined the effect of mitomycin C treatment on HLA expression by HCE cells. We compared HLA class I or IFN-γ-induced class II antigen expressed on HCE cells treated with mitomycin C (20 μg/ml) for 30 minutes to the expression on HCE cells without mitomycin C treatment. We could not find any major difference between the HLA antigen expression on HCE cells before and after mitomycin C treatment (data not shown).

Ability of HCE Cells to Stimulate Allogenic Lymphocytes in MLCER

After stimulation by HCE cells, nearly maximum lymphocyte responses were obtained at day 7 of incubation (Fig. 2). A high level of response was obtained when 0.6 × 10^4 to 2.5 × 10^4 HCE cells, as stimulators, were mixed with responder lymphocytes (Table 1). The response of lymphocytes declined when the num-
Figure 1. Flow cytometric analysis of the HLA-antigen expression on cultured HCE cells, treated or not treated with IFN-γ (500 U/ml). (a) HCE cells were stained with control IgG. (b) HCE cells were stained with anti-HLA-DR mAb. (c) HCE cells were stained with anti-HLA class I mAb. HLA = Human leukocyte antigen; HCE = human corneal epithelial; IFN = interferon; IgG = immunoglobulin G; mAb = monoclonal antibody.

As shown in Table 3, the stimulation by IFN-γ-treated HCE cells could be blocked by anti-class II (HLA-DR) mAb to the level of stimulation of HCE cells without IFN-γ treatment in MLCER (P < 0.005, Student's t-test), whereas anti-class I mAb did not significantly (P < 0.2, Student's t-test) block stimulation by IFN-γ-treated HCE cells. Ninety percent of the proliferation response of lymphocytes to allogenic PBL in allo MLR could be blocked by the same concentration of the anti-class II mAb. In donor 4 (see Table 2), HCE cells without IFN-γ treatment stimulated lymphocytes but much less than did IFN-γ-treated HCE cells. The stimulation by HCE cells without IFN-γ treatment could not be blocked either by anti-class II or anti-class I mAb (data not shown).

IL-1 Effect on MLCER

As shown in Table 4, with the sandwich enzyme immunoassays for IL-1, we detected IL-1-α in the supernatants of HCE cell cultures, which supports the results of other studies. Lipopolysaccharide added to the culture medium enhanced IL-1-α production by HCE cells. We did not detect IL-1-β in the supernatants of HCE cell cultures, either with or without lipopolysaccharide stimulation.

As shown in Table 5, exogenously added recombinant human IL-1-α (rIL-1-α) enhanced the lymphocyte proliferation in allo MLR. However, the addition
Lymphocyte Activation by Human Corneal Epithelial Cells

FIGURE 2. Time course of 3H-thymidine incorporation by responder allogeneic lymphocytes (1.0 x 10^5) cultured with IFN-γ-treated HCE cells (IFN-γ HCE) or HCE cells without IFN-γ treatment (HCE) (2.0 x 10^5). Triplicate cultures were incubated for 5, 7, and 9 days. Experimental counts—background counts (PBL alone + HCE cells alone) are expressed as the mean cpm of triplicate determination. IFN = Interferon; HCE = human corneal epithelial; PBL = peripheral blood lymphocytes; cpm = counts per minute.

of 40 U/ml (400 pg/ml) of rIL-1-α reduced the response significantly in MLER (Table 6).

We examined the effect of indomethacin, an inhibitor of prostaglandin production. One microgram per milliliter of indomethacin was added to the well at the initiation of MLER. The addition of indomethacin blocked the inhibition of rIL-1-α in MLER completely (Table 6, Experiment 2).

DISCUSSION

Langerhans cells are thought to be potent antigen-presenting cells found in the peripheral and limbal cornea, which is also the source of the primary HCE cell cultures. By using immunohistochemical staining, we and others have found HLA-DR positive, but CD1(a Langerhans cells marker)-negative Langerhans cells in fresh normal limbal corneal sections. Also, we examined the cultures from limbal explants after 1 to 4 weeks of incubation with immunohistochemical staining. We did not detect any HLA-DR-positive or CD1-positive cells in the cultures. In addition, using flow cytometric analysis, we did not detect any HLA-DR-positive cells in the HCE cell cultures. These findings indicate that Langerhans cells are not in the cultures. Therefore, we concluded that the lymphocyte proliferation in MLER was the result of contamination by Langerhans cells. In addition, there were no fibroblasts in our culture, because 100% of the cultured cells showed strong reactivity with AE1-AE3 mAb. AE1-AE3 mAb recognizes acidic and basic cytokeratins, characteristic of epithelial cells and not of fibroblasts. In some experiments, we used pretreated HCE cells with 0.02% EDTA for a short time after removal of the explants and before the addition of IFN-γ, a method described by Sun et al to remove fibroblasts selectively from epithelial cell cultures. However, we did not find any difference in the ability of HCE cells pretreated and not pretreated with 0.02% EDTA to stimulate lymphocytes (data not shown).

We demonstrate that IFN-γ-treated, HLA class II-bearing cultured HCE cells stimulated freshly isolated allogeneic lymphocytes in the primary MLER, whereas without IFN-γ treatment, class II-negative HCE cells induced little or no allogeneic lymphocyte proliferation. Blocking assays with mAb showed that anti-class II (HLA-DR) mAb blocked the stimulation by IFN-γ-treated HCE cells to the level of non-IFN-γ-treated HCE cells, whereas neither anti-class I mAb nor control mouse IgG blocked the stimulation by IFN-γ-treated HCE cells. These data demonstrate that IFN-γ, in leading to class II antigen expression on HCE cells, can result in these cells initiating allogeneic lymphocyte responses by an MHC-dependent mechanism. In addition, these findings suggest that class II-bearing HCE cells can participate in corneal allogeneic responses.

The function of class II-bearing nonlymphoid cells as APC is variable and depends on cell type. Keratinocytes, renal tubular cells, and fibroblasts from several kinds of tissue do not stimulate allogenic lymphocytes in the primary mixed lymphocyte reaction, even when HLA class II antigen expression has been induced by IFN-γ. On the other hand, IFN-γ-treated, class II-bearing vascular endothelial cells are capable of inducing the primary allogeneic lymphocyte re-

<table>
<thead>
<tr>
<th>Number of Stimulator Cells</th>
<th>Lymphocyte Proliferation (mean CPM ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCE</td>
</tr>
<tr>
<td>0.6 x 10⁵</td>
<td>950 ± 920</td>
</tr>
<tr>
<td>1.2 x 10⁵</td>
<td>1250 ± 180</td>
</tr>
<tr>
<td>2.5 x 10⁵</td>
<td>660 ± 250</td>
</tr>
<tr>
<td>5.0 x 10⁵</td>
<td>390 ± 80</td>
</tr>
</tbody>
</table>

Lymphocyte proliferation represented by 3H-thymidine incorporation by responder allogeneic lymphocytes (1.0 x 10⁵) cultured with a variable number of HCE cells without IFN-γ treatment (HCE) or IFN-γ-treated HCE cells (IFN-γ HCE). Triplicate cultures were incubated for 7 days.

* Stimulator cells were pretreated with mitomycin C.
CPM = Counts per minute; SD = standard deviation.

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Table 2. Ability of HCE Cells to Stimulate Allogeneic Lymphocytes in MLCER

<table>
<thead>
<tr>
<th>Donor</th>
<th>Responder</th>
<th>HCE stimulator only</th>
<th>Responder (A, B) only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>A</td>
<td>1260 ± 440</td>
<td>4850 ± 270</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>850 ± 150</td>
<td>3160 ± 790</td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>1070 ± 280</td>
<td>2500 ± 130</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>1530 ± 200</td>
<td>4120 ± 700</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>1090 ± 210</td>
<td>2470 ± 250</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>2350 ± 640</td>
<td>5050 ± 530</td>
</tr>
<tr>
<td>HCE stimulator only</td>
<td>&lt;200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responder (A, B) only</td>
<td>&lt;1000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Lymphocyte Proliferation (mean CPM ± SD)**

<table>
<thead>
<tr>
<th>Stimulator Cells*</th>
<th>HCE</th>
<th>IFN-γ HCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responder (A, B)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Lymphocyte proliferation represented by ³H-thymidine incorporation by responder allogeneic lymphocytes (1.0 x 10⁵), isolated from individuals A or B, cultured with HCE cells without IFN-γ treatment (HCE) or IFN-γ-treated HCE cells (IFN-γ HCE) (1.2 x 10⁶ to 2.0 x 10⁶). Triple cultures were incubated for 7 days. * Stimulator cells were pretreated with mitomycin C.

CPM = Counts per minute; SD = standard deviation.

Table 3. Effect of Anti-Class I or Class II Monoclonal Antibodies on Lymphocyte Proliferative Responses in MLCER

<table>
<thead>
<tr>
<th>Monoclonal Ab</th>
<th>HCE</th>
<th>IFN-γ HCE</th>
<th>Stimulator Cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2140 ± 660</td>
<td>7350 ± 1030</td>
<td>HCE</td>
</tr>
<tr>
<td>Control IgG</td>
<td>2890 ± 300</td>
<td>7270 ± 1120</td>
<td>Responder (A, B)</td>
</tr>
<tr>
<td>Anti-class I</td>
<td>2810 ± 230</td>
<td>6170 ± 680†</td>
<td>&lt;200</td>
</tr>
<tr>
<td>Anti-class II</td>
<td>1740 ± 110</td>
<td>1690 ± 50†</td>
<td>&lt;1000</td>
</tr>
</tbody>
</table>

*Lymphocyte proliferation represented by ³H-thymidine incorporation by responder allogeneic lymphocytes (1.0 x 10⁵), isolated from individuals A or B, cultured with HCE cells without IFN-γ treatment (HCE) or IFN-γ-treated HCE cells (IFN-γ HCE) (1.2 x 10⁶ to 2.0 x 10⁶). Allo MLR was established with stimulator lymphocytes (1.0 x 10⁶) and responder allogeneic lymphocytes (1.0 x 10⁵). Anti-class II (HLA-DR) MAb (0.5 μg/ml) or anti-HLA class I MAb (0.5 μg/ml), or mouse IgG control (1.0 μg/ml) were added at the initiation of the culture and remained through the incubation period. Triplicate cultures were incubated for 7 days. * Stimulator cells were pretreated with mitomycin C.

† P < 0.05, for different from control IgG; Student’s t-test.

Table 4. Interleukin 1 (IL-1) Production by Cultured HCE Cells

<table>
<thead>
<tr>
<th>Experiment</th>
<th>IL-1-α (pg/ml)</th>
<th>IL-1-β (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>120</td>
<td>ND (&lt;7.8)</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>ND (&lt;7.8)</td>
</tr>
<tr>
<td>+LPS</td>
<td>190</td>
<td>ND (&lt;7.8)</td>
</tr>
<tr>
<td>3</td>
<td>85</td>
<td>ND (&lt;7.8)</td>
</tr>
<tr>
<td>+LPS</td>
<td>135</td>
<td>ND (&lt;7.8)</td>
</tr>
</tbody>
</table>

Interleukin 1 (IL-1) in culture supernatants produced by primary cultures of HCE cells (2 x 10⁶ per 35-mm culture dish) incubated in 10% FCS with or without LPS (20 μg/ml). ND = Not detected.
TABLE 5. Effect of Exogenous Interleukin 1 (IL-1) on Allo MLR (Allogeneic PBL as Stimulator Cells)

<table>
<thead>
<tr>
<th>Addition</th>
<th>Lymphocyte Proliferation (mean CPM ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>22590 ± 3560</td>
</tr>
<tr>
<td>rIL-1 (10 U/ml)</td>
<td>30560 ± 4220*</td>
</tr>
<tr>
<td>rIL-1 (40 U/ml)</td>
<td>40600 ± 5530†</td>
</tr>
</tbody>
</table>

Lymphocyte proliferation represented by "H-thymidine incorporation by responder lymphocytes (1.0 X 10^5) mixed with mitomycin C treated (20 µg/ml, 30 minutes) allogeneic stimulator lymphocytes (1.0 X 10^5) and incubated with recombinant human IL-1-alpha (10 U/ml [100 pg/ml] or 40 U/ml [400 pg/ml]). Triplicate cultures were incubated for 7 days.

* P < 0.02, for different from addition: none, Student's t-test.
† P < 0.01, for different from addition: none, Student's t-test.
CPM = Counts per minute; SD = standard deviation.

In some experiments, HCE cells without IFN-γ treatment stimulated allogeneic lymphocytes, but always less than IFN-γ-treated HCE cells did. The stimulation by non-IFN-γ-treated HCE cells was observed when large numbers of stimulator HCE cells (2 X 10^4/well) were employed. Regardless of the treatment with IFN-γ, lymphocytes cultured with an excess of 2.5 X 10^4 stimulator HCE cells did not proliferate in MLR. This may be due to either the excessive consumption of nutrition because of the high metabolic rate of epithelial cells or the prevention of sufficient interaction between lymphocytes and HCE cells by the aggregation of large numbers of HCE cells in the well.

The stimulation of HCE cells without IFN-γ treatment could not be blocked by anti-class I or anti-class II (HLA-DR) mAb. Therefore, it is unlikely that, within the wells, T cells that responded to donor HLA class I antigen would produce IFN-γ. Similarly, it is unlikely that, during the incubation period, IFN-γ would induce HLA class II antigen expression on class II-negative HCE cells. It is possible that small numbers of lymphocytes in the G1 phase might respond to IL-1, which could be produced by HCE cells.

Interleukin 1 is a powerful inflammatory cytokine. It can be produced not only by leukocytes but by various cell types of nonhematopoietic lineage. IL-1 produces its effect by binding to an IL-1 receptor. Two different isoforms of IL-1 have been identified, IL-1-α and IL-1-β. Both isoforms bind the same receptor, and their function is nearly the same. It has been shown that IL-1 is necessary and increases lymphocyte proliferation in the primary MLR by means of induction of IL-2 production and IL-2 receptor expression. Large amounts of IL-1 have been detected in the synovial fluid of patients with rheumatoid arthritis. In addition, it has been shown that rheumatoid arthritis synovial mononuclear cells and dendritic cells produce IL-1 activity in vitro. IL-1, produced abnormally, may be critical in the progressive destruction of joints of patients with rheumatoid arthritis. IL-1 can be produced by HCE cells and fibroblasts. Therefore, in corneal immune responses, it is possible that IL-1 may be produced by corneal cells at the inflammatory sites as well as by infiltrating leukocytes.

TABLE 6. Effect of Exogenous Interleukin 1 (IL-1) on MLCER

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Addition</th>
<th>HCE</th>
<th>IFN-γ HCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>2350 ± 640</td>
<td>5050 ± 530</td>
</tr>
<tr>
<td></td>
<td>rIL-1 (10 U/ml)</td>
<td>2060 ± 510</td>
<td>4750 ± 390</td>
</tr>
<tr>
<td></td>
<td>rIL-1 (40 U/ml)</td>
<td>1330 ± 110</td>
<td>3140 ± 520†</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>2560 ± 340</td>
<td>6840 ± 1210</td>
</tr>
<tr>
<td></td>
<td>rIL-1 (10 U/ml)</td>
<td>2970 ± 280</td>
<td>5540 ± 790</td>
</tr>
<tr>
<td></td>
<td>rIL-1 (40 U/ml)</td>
<td>1840 ± 490</td>
<td>3950 ± 260†</td>
</tr>
<tr>
<td></td>
<td>+ INDO (1 µg/ml)</td>
<td>2560 ± 50</td>
<td>6340 ± 1510</td>
</tr>
<tr>
<td></td>
<td>INDO (1 µg/ml)</td>
<td>2500 ± 50</td>
<td>5590 ± 430</td>
</tr>
</tbody>
</table>

Lymphocyte proliferation represented by "H-thymidine incorporation by HCE cells without IFN-γ treatment (HCE) or IFN-γ treated HCE cells (IFN-γ HCE) (2.0 X 10^5) cultured with responder allogeneic lymphocytes (1.0 X 10^5) and incubated with recombinant human IL-1-α (10 U/ml [100 pg/ml] or 40 U/ml [400 pg/ml]) or indomethacin (INDO) (1 µg/ml), or combination with IL-1-α (40 U/ml [400 pg/ml]) and INDO (1 µg/ml). Triplicate cultures were incubated for 7 days.

* Stimulator cells were pretreated with mitomycin C.
† P < 0.02, for different from addition: none, Student's t-test.
CPM = Counts per minute; SD = standard deviation.
To investigate the effect of IL-1 in the corneal immune reaction, we examined the effect of exogenous recombinant human IL-1-α (rIL-1-α) in MLCER. Forty units per milliliter (400 pg/ml) of rIL-1-α significantly enhanced the lymphocyte response in the allo MLR. Unexpectedly, the addition of rIL-1-α at the same dose in MLCER reduced lymphocyte proliferation. Indomethacin, an inhibitor of prostaglandin production, completely blocked the inhibitory effect of rIL-1-α to MLCER. These findings suggest that the inhibitory effect of rIL-1-α was caused by prostaglandin E synthesis from HCE cells. Indomethacin had little effect in MLCER without the addition of rIL-1-α or with a low concentration of exogenous rIL-1-α 10 U/ml (100 pg/ml). These data suggest that the immune response in corneal epithelial cells could be regulated by an IL-1-rich environment capable of inducing a sufficient amount of prostaglandin E production from corneal epithelial cells to inhibit lymphocyte proliferation.

For a better understanding of the mechanism of corneal immune diseases, it is important to elucidate the immunologic function of the main components of the cornea, such as the epithelial cells, stromal fibroblasts, and endothelial cells. Langerhans cells have been thought to be the only potent APC in the cornea, because only Langerhans cells express the MHC class II antigen constitutively. However, it has been demonstrated clearly, using animal skin graft models, that the presence of allogenic Langerhans cells within a skin graft is not sufficient to induce or cause rejection of the entire graft.

The corneal endothelium can stimulate allogeneic lymphocytes after IFN-γ treatment. In addition, we demonstrate in this study that the IFN-γ-treated, class II-bearing corneal epithelium can induce allogeneic lymphocyte responses. Previous research has shown that corneal stromal fibroblasts are incapable of inducing the primary T-cell response. However, corneal stromal fibroblasts, like dermal fibroblasts, may be able to produce several cytokines, which may regulate the function of other corneal components.

In conclusion, corneal cells as well as Langerhans cells can participate in corneal immune responses. In addition, human corneal epithelial cells may regulate corneal immune reaction by secreting IL-1 and prostaglandins.

**Key Words**

HLA class II antigens, interferon-γ, corneal epithelial cells, mixed lymphocyte–corneal epithelial cell reaction, interleukin-1

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Lymphocyte Activation by Human Corneal Epithelial Cells


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