Human stromal and endothelial cultures were established from corneas obtained from the Maryland Eye Bank. Cultures were incubated in the presence of human gamma interferon for 4 days, trypsinized, washed, and then stained with a monoclonal reagent specific for human Class II (HLA-DR) antigens. Under these conditions, 100% of human corneal endothelial cells and 40 to 70% human corneal stromal cells expressed HLA-DR antigens. Invest Ophthalmol Vis Sci 26:571–574, 1985

The donor antigens responsible for initiating allograft rejection are primarily those of the Major Histocompatibility Complex. These antigens can be divided into two groups, Class I antigens, which are coded for by the genomic region termed HLA-A,B,C; and Class II antigens, which are coded by a region termed HLA-D,DR. In renal grafts, Class II antigens have been demonstrated to elicit strong immune responses, and compatibility at the HLA-DR (Class II) locus was found to be of overriding importance for graft survival when compared to compatibility at the HLA-A and B loci. Class II antigens have been consistently demonstrated on Langerhans cells of the cornea that are present in the limbal region of human corneal epithelium, but not apparently on the epithelial, stromal, or endothelial cells themselves. Recent studies of human vascular endothelium indicate that although primary isolates do not express Class II antigen under standard culture conditions, they may be induced to do so by treatment of the cultures with activated T-cells or T-cell products. We have recently demonstrated that some alloantigens found on the corneal endothelium are shared with antigens found on lymphocytes. In this report, we demonstrate that cultured human corneal endothelial and stromal cells share the capacity to express Class II antigens after exposure to a lymphokine.

Materials and Methods. Endothelial cultures: Human corneal endothelial cultures were established from normal corneas obtained from the Maryland Eye Bank using material that was not suitable for transplantation. Primary cultures were established by stripping the endothelium, including Descemet’s membrane, from a central corneal button and placing it into a small petri dish containing Minimum Essential Medium (MEM) (Gibco; Grand Island, NY) and 20% fetal bovine serum (FBS). The tissue was overlayed with a coverslip and incubated at 37°C in 5% CO₂ for 1 week, at which time cells were observed growing out from under the coverslip. The coverslip was removed, the cultures fed and allowed to reach 50% confluency. The culture was then trypsinized and placed into a “Primaria” plate (Falcon; Oxnard, CA). These plates are treated by the manufacturer to prohibit adherence by fibroblasts while allowing cells which normally need a matrix, such as endothelial cells, to attach. After 1 to 2 weeks on Primaria plates, cells again reached 50% confluency. At that time, they were passaged to a 75 cm² flask (Corning; Corning, NY) and maintained in 20% FBS/MEM. Four different cultures were used (donor age ranged from 21 to 52): one at passage three (donor age 52), and one at passage eight (donor age 37).

Stromal cultures: After removal of endothelium and epithelium, primary stromal cultures were established by mincing the stromal layer and placing it in a small petri dish containing Nutrient Mixture F12 (Ham’s) medium (Gibco) with 10% FBS. The tissue was overlayed with a coverslip and incubated at 37°C in 5% CO₂ for 5 days. At that time, the coverslip was removed, the cultures fed, and allowed to reach confluence. Cultures were then trypsinized and placed into a 75 cm² flask (Corning) and maintained in 20% FBS/MEM. Four different cultures were used (donor age ranged from 21 to 52): two during early
Fig. 1. Photomicrograph depicting suspension of cultured human corneal endothelial cells showing fluorescence with anti-HLA-DR after 4 days' incubation (a) in the presence of gamma IF, and (b) in the absence of gamma IF (×400).

passages (p-3 and p-4), and two during later passages (p-8 and p-11).

Induction of Class II antigen expression: Endothelial and stromal cell cultures at 50% confluency were treated by the addition of 500 units/ml human gamma interferon (IF) (Interferon Sciences, Inc.; New Brunswick, NJ) in 20 ml of the appropriate growth medium for each cell type. Duplicate cultures of each cell type were fed at the same time with growth medium alone. All cultures were incubated 4 days at 37°C in 5% CO₂.

Staining and analysis: On day 4, all cultures were washed three times in Ca++ , Mg++-free phosphate buffered saline (PBS) and removed from the flask with 0.25% trypsin (Gibco). Cells from each flask were resuspended in medium, counted, and divided into two aliquots for staining. Each aliquot was washed once in 1% FBS/PBS and all supernatant removed from each tube. Aliquots of 5 × 10⁵ to 1 × 10⁶ cells/tube were resuspended in 10 μl of anti-HLA-DR (mouse monoclonal anti-human DR, Becton-Dickinson; Mountain View, CA). The cells were incubated 30 min on ice, washed once in cold 1% FBS/PBS, and resuspended in 30 μl (40 μg/ml) FITC goat anti-mouse IgG + IgM (Tago, Inc.; Burlingame, CA). The cells were again incubated for 30 min on ice, washed once in 1% FBS/PBS, and examined.

Negative control tubes contained the FITC conjugated reagent only or anti-Leu-M2 (mouse monoclonal anti-human monocyte, Becton-Dickinson) in place of anti-HLA-DR. For quantitation, five randomly chosen fields of approximately 100 cells each were first examined under fluorescence for positively stained
cells in each field. Percentages were calculated as follows: Percent positive = Fluorescent cells/Total cells × 100.

Results. Examination of two different human endothelial cell cultures at passage three or eight that had been exposed to gamma IF for 4 days and then stained with a monoclonal antibody to human DR antigen revealed that 100% of the cells were brightly stained (Fig. 1a). In contrast, cells maintained in the absence of gamma IF all failed to express DR antigens (Fig. 1b). Endothelial cells maintained with gamma interferon and then stained only with the fluorescent reagent (goat anti-mouse Ig) or with an irrelevant monoclonal reagent were not stained (data not shown).

Examination of four different human stromal cell cultures at passage three, four, eight, and 11 provided similar data (Fig. 2a), but in this case only 40-70% of the cells stained brightly after incubation in the presence of gamma IF. Stromal cells maintained in the absence of IF failed to express any DR antigen (Fig. 2b). Stromal cells stained only with the fluorescent reagent or with an irrelevant monoclonal reagent were not stained (data not shown).

Discussion. We have demonstrated that gamma IF, a lymphokine produced by activated T-cells, can induce human corneal endothelial cells and a percentage of human corneal stromal cells to express Class II antigens in vitro. These results are consistent with those obtained for vascular endothelial cells.5 Several other investigators have attempted to dem-
onstrate Class II antigens on corneal cells in frozen sections or in culture without success.\textsuperscript{4,7,9} In those experiments, however, there was no attempt to induce the expression of such antigens.

These results have important implications for corneal transplantation. They demonstrate that the donor cornea, in the absence of donor Langerhan's cells or "passenger leucocytes," may have the ability to incite a graft rejection by virtue of Class II antigen expression.

A major question is what induces grafted corneas to express DR prior to any immunologic exposure. We see two possibilities which we are currently testing. First, a small percentage of corneal cells may constitutively express these antigens which could trigger the initial release of lymphokines by T-cells. We have preliminary evidence that approximately 6\% of normal cultured BALB/c mouse corneal endothelial cells appear to constitutively express low levels of Ia (Class II) antigens (unpublished observations). Alternatively, as a result of the trauma of surgery, an influx of recipient Langerhan's cells and lymphocytes into the donor button may induce corneal cells to express Class II antigens. This theory is supported by a report in which mouse epidermal cells were shown to express large amounts of donor specific Ia antigens when skin grafted. This expression was preceded by the influx of host Langerhan's cells into the graft epidermis.\textsuperscript{10} We have not tested corneal epithelial cells for Class II antigen expression, but predict that these cells, too, will prove to be capable of expressing Class II antigens since it was previously demonstrated that basal epithelial cells obtained from some infant corneas were positive for HLA-DR.\textsuperscript{7}

Our results are not consistent with a recently reported study in which interferon failed to induce Class II antigens on a cloned BALB/c 3T3 fibroblast line.\textsuperscript{11} The discrepancy may lie in changes in the 3T3 cell occurring in culture, or the selection of a "non-la-expressing" clone of the 3T3 line. It is interesting to note that in our study only 40–70\% of cultured stromal cells expressed Class II antigens after exposure to gamma interferon. This may indicate a heterogeneity of the cells located in the stromal layer. We are currently separating DR+ and DR− stromal cells to determine what, if any, differences exist in these populations.

In summary, our results indicate that normal corneal cells can be induced to express Class II histocompatibility antigens by exposure to a lymphokine. Establishing the immunological involvement of this expression in corneal rejection and the mechanisms responsible for induction may help to clarify the existing controversy concerning the distribution of such antigens in the cornea.

**Key words:** corneal histocompatibility antigens, Class II antigens, cultured endothelium

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