Recombinant interferon-gamma induces HLA-DR expression on human corneal epithelial and endothelial cells in vitro: a preliminary report

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SUMMARY The effect of interferon-gamma on the expression in situ of major histocompatibility complex (MHC) products in human corneas was studied in vitro. Incubation for four days with 5 or 50 mg/l of *Escherichia coli*-derived recombinant human interferon-gamma resulted in the appearance de novo of MHC class II or HLA-DR antigens on variable numbers of corneal epithelial cells as well as on corneal endothelium, whereas it had no effect on the expression of MHC class I or HLA-ABC antigens. These results may help to explain the mechanism underlying the expression of HLA-DR antigens on corneal and limbal epithelium in various inflammatory eye diseases.

Major histocompatibility complex (MHC) class II products or HLA-DR antigens are highly polymorphic cell surface glycoproteins involved in antigen presentation and initiation of T lymphocyte-dependent immune responses. HLA-DR antigens are predominantly expressed on immunocompetent cells such as B lymphocytes, activated T lymphocytes, macrophages, dendritic cells, and endothelial cells.¹ In addition a variety of epithelial cell types are now known to express HLA-DR antigens constitutively or following induction with lymphokines.²

In the normal human cornea HLA-DR antigens are expressed on occasional dendritic cells in the epithelium and stroma^{3,4} and on the endothelial cells lining the limbal blood vessels.⁵⁻⁷ Normal corneal epithelium and endothelium, as well as the majority of stromal cells, lack HLA-DR antigens.

We recently observed HLA-DR antigen expression on corneal epithelium in herpetic keratitis,⁸ on limbal epithelium in phlyctenular eye disease,⁹ and on conjunctival epithelium in trachomatous conjunc-

tivitis.¹⁰ In all these conditions variable numbers of T cells were found in the epithelial and stromal inflammatory infiltrate, suggesting that lymphokines liberated by these T cells were responsible for the epithelial HLA-DR expression. The lymphokine, interferon-gamma, produced by activated T cells has been shown to induce synthesis and expression of HLA-DR antigens in a variety of epithelial cells in vitro.^{2,11}

In the present study we have investigated whether interferon-gamma can induce HLA-DR expression by the corneal and limbic epithelium in vitro.

Materials and methods

Eyes were obtained at necropsy within a few hours of death. The corneas used in this study were excised with a 3 mm scleral margin and divided into three parts. Each piece of cornea was placed in a separate bottle containing 10 ml of organ culture medium.¹² The first corneal part served as a control. To the culture medium of the second and third bottles human pure recombinant *Escherichia coli*-derived interferon-gamma was added in a dose of 5 and 50

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Fig. 1 Effect of interferon-gamma on HLA-DR expression by corneal endothelium. 1a. No endothelial reactivity is seen in the control specimen. 1b. Four days of incubation with 50 mg/l interferon-gamma reveals HLA-DR expression by corneal endothelium and by variable numbers of stromal cells.

mg/l respectively. Recombinant interferon-gamma was kindly supplied by Dr W Wolf (Bioferon GmbH & Co, Laupheim, West Germany). The three bottles

were incubated at 37°C in 5% CO₂ for four days; subsequently the corneal pieces were snap frozen in isopentane cooled liquid nitrogen.

Fig. 2 Effect of interferon-gamma on HLA-DR expression by limbal epithelium. 2a. Weak reactivity is seen on the endothelium of some limbal blood vessels in the control specimen. 2b. Four days of incubation with 5 mg/l interferon-gamma reveals strong membranous and cytoplasmic HLA-DR expression by many epithelial cells as well as by the endothelium of most limbal blood vessels. (Three-step indirect immunoperoxidase method for TAL1B5, slightly counterstained with Harris's haematoxylin; all figures $\times 260$.)

Serial 5 µm thick cryostat sections were stained with the three-step indirect immunoperoxidase method using monoclonal antibodies directed to MHC products. Monoclonal anti-HLA-ABC (Cappel Laboratories, Cochranville, PA) reacts with the constant part of HLA-ABC antigens. Monoclonal antibody TAL-1B5 was a generous gift from Dr W F Bodmer, Imperial Cancer Research Fund, London, and is directed against the monomorphic part of HLA-DR alpha chains,¹³ and monoclonal antibody L243 (Becton-Dickinson, Sunnyvale, CA), is directed against the monomorphic part of HLA-DR β chains.¹⁴

Results

The control cornea that did not receive interferon-gamma expressed HLA-ABC antigens on epithelial cells, stromal cells, corneal endothelium, and on the endothelial cells lining the limbal blood vessels. HLA-DR expression, identified with monoclonal antibodies L243 and TAL-1B5, was restricted to occasional dendritic cells in the limbal epithelium and corneal stroma as well as to the endothelium of limbal blood vessels (Figs. 1a and 2A).

After four days of incubation with interferon-gamma the expression of HLA-ABC antigens was similar to that of the control cornea. However, monoclonal antibodies L243 and TAL-1B5 showed additional membranous and cytoplasmic HLA-DR expression by the corneal endothelium (Fig. 1b) as well as by variable numbers of epithelial cells, the most intense reactivity being found in the deepest epithelial layers (Fig. 2b). This additional HLA-DR expression was found with both concentrations of interferon-gamma employed.

Discussion

In this study we have shown that MHC class I products or HLA-ABC antigens are expressed on normal human corneal epithelium and stromal cells. These data are in accordance with earlier findings⁵⁻⁷ and indicate that epithelial and stromal cells can serve as target cells involved in allograft rejection.¹⁵ Moreover, the high density of HLA-ABC antigens on corneal epithelium provides an explanation for the decreased rejection rates when the epithelium is removed prior to transplantation,¹⁶ as well as for the prolonged graft survival, observed in MHC class I matched corneal transplants.¹⁷

Normal corneal endothelial cells have previously been found to lack MHC class I products. Our demonstration of HLA-ABC antigens on corneal endothelium can be explained by the short term culture of the tissue fragments, a procedure that has

been shown in other studies to induce MHC class I products on corneal endothelium.¹⁸⁻²⁰

We have also shown that MHC class II products or HLA-DR antigens are normally present on occasional dendritic cells in the limbal epithelium and on endothelial cells lining limbal blood vessels. After incubation with interferon-gamma, however, corneal epithelial and stromal cells were found to express HLA-DR antigens. This finding in vitro confirms and extends previous studies on cultured rabbit²¹ and human epithelial and stromal cells.²²⁻²⁴ Moreover, the effect of low concentrations of interferon-gamma, namely, 5 mg/l, provides good evidence that epithelial HLA-DR expression in various inflammatory conditions in vivo is indeed induced by interferon-gamma, released by activated T cells.

Interferon-gamma also induced expression of HLA-DR antigens on corneal endothelium. This finding confirms previous studies employing cultured endothelial cells, and may indicate a potentiating role in the rejection of corneal allografts.¹⁵

HLA-DR expression by corneal epithelial and endothelial cells may render these cells susceptible to immunological attack by MHC class II restricted cytotoxic T-cells²⁵ or may render them capable of acting as antigen presenting cells for T cells, thereby enhancing the immune response and possibly inducing an autoimmune response.²⁶ Elucidation of the precise functional significance of corneal HLA-DR expression awaits further in-vitro studies.

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