often accompanies decreased foveal vision was responsible for incomplete loss of hyperopia in these children.

Arguing against the role of accommodation in the refractive changes we observed in rhesus monkeys is the failure of atropine to result in hyperopic anisometropia in two rhesus monkeys raised with chronic unilateral atropine cycloplégia (although atropinization was not begun in these animals until 25 and 28 days of age). Also, atropine has been ineffective in preventing lid-suture myopia in rhesus monkeys, even though it has prevented lid-suture myopia in other monkey species. 11

Whether or not accommodation is the primary cause of the normal loss of hyperopia in infant monkeys, the technique of dark rearing should prove useful in providing a laboratory model of hyperopia and in understanding the mechanisms of refractive change and emmetropization in early life.

Key words: dark-rearing, emmetropization, hyperopia, accommodation, monkey

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References

Expression of la Antigen by Ocular Tissues of Mice Treated with Interferon Gamma

Mihoko Kusuda,* Anthony A. Gaspari,† Chi-Chao Chan,* Igal Gery,* and Stephen I. Katz†

Intraperitoneal injections of interferon gamma (IFN-γ) induced or enhanced the expression of la antigen by selected murine ocular tissues. In contrast, topical application of this lymphokine had no effect. Ia antigen expression was noted in most cellular components of the conjunctiva, iris, ciliary body, choroid and sclera of treated mice. Intense staining was also found in stromal cells of the cornea, but no la reactivity was detected in the corneal epithelium. The lack of staining in the corneal epithelium is in contrast to the strong reactivity in the adjacent conjunctival epithelium, as well as in the skin epithelium. There was no increase in the number of Langerhans cells in the peripheral cornea and limbus, or in their intensity of staining with the anti la antibodies. The pattern of la expression in ocular tissues of mice treated with IFN-γ is similar to that of rats with experimental autoimmune uveoretinitis (EAU) except that la is expressed more regularly and with higher intensity by the retinal vascular endothelium of rats with EAU than by that of IFN-γ-treated mice. The findings in the current study thus support the notion that IFN-γ may be involved in immune-mediated inflammation in the eye. Invest Ophthalmol Vis Sci 30:764–768, 1989

The pivotal role of antigen-presenting cells (APC) in antigen recognition by helper/inducer T-lymphocytes has been well established. In order to be effective in this process, APC must express on their sur-
face class II (la) molecules of the major histocompatibility complex (MHC). Normally, the expression of class II molecules is limited to lymphoid cells such as mononuclear phagocytes, dendritic cells or B-lymphocytes. In certain circumstances, however, non-lymphoid cells may also express class II molecules: a variety of resident tissue cells (endothelia, epithelia or fibroblasts) were found to express class II molecules in individuals affected by inflammatory as well as noninflammatory diseases. Moreover, some non-lymphoid cells which express class II molecules were found capable of presenting antigens to T-lymphocytes in vitro. It has been suggested, therefore, that nonlymphoid cells which express class II antigens may actually function as APC in vivo as well.

Expression of class II molecules on nonlymphoid cells of the eye has been reported to occur in various pathological conditions in both humans and experimental animals. This phenomenon was recorded mainly in eyes affected by inflammatory conditions, but class II MHC expression has also been observed on ocular cells in noninflammatory diseases.

There is ample evidence to indicate that class II antigen expression is induced on both lymphoid and nonlymphoid cells by interferon gamma (IFN-γ). Incubation with IFN-γ brings about class II antigen expression on a variety of cells in culture, including endothelial and stromal cells of the cornea. Furthermore, studies in vivo have shown that injections of IFN-γ induce class II antigen on many nonlymphoid cells in various tissues of experimental animals. In a recent study we have described the expression of la antigens on epidermal cells of mice injected with IFN-γ. The current study was performed to determine the effect of both systemically and topically administered IFN-γ on various ocular tissues in vivo. In addition, a comparison between the eye and the skin is of particular interest since two ocular tissues, the cornea and conjunctiva, contain epithelium and subepithelial tissue which closely resemble the epidermis and dermis of the skin. Moreover, similar to the skin, the peripheral cornea (mainly at the limbus) and the conjunctiva contain dendritic Langerhans cells which constitutively express class II antigens.

Materials and Methods. Animals: Female BALB/c (H-2d) mice, 6-12 weeks old, were purchased from Charles River Breeding Laboratory (Wilmington, MA). Treatment of animals in this study was in compliance with the ARVO Resolution on the Use of Animals in Research.

Interferon-γ: Recombinant murine IFN-γ was a generous gift from Dr. H. M. Shepard, Genentech (San Francisco, CA).

### Table 1. la expression by ocular tissues following systemic treatment with IFN-γ

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Cornea epithelium</td>
<td>0</td>
</tr>
<tr>
<td>stroma</td>
<td>±</td>
</tr>
<tr>
<td>Conjunctiva epithelium</td>
<td>±</td>
</tr>
<tr>
<td>substancia propria</td>
<td>±</td>
</tr>
<tr>
<td>Ciliary body epithelium</td>
<td>0</td>
</tr>
<tr>
<td>stroma</td>
<td>±</td>
</tr>
<tr>
<td>Iris epithelium</td>
<td>0</td>
</tr>
<tr>
<td>stroma</td>
<td>±</td>
</tr>
<tr>
<td>Retina</td>
<td>0</td>
</tr>
<tr>
<td>Retinal pigment epithelium</td>
<td>0</td>
</tr>
<tr>
<td>Choroid</td>
<td>+</td>
</tr>
<tr>
<td>Sclera</td>
<td>±</td>
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</tbody>
</table>

* Observations made in mice treated for 7 or 14 days; the two groups responded very similarly.
† la expression was found on Langerhans cells in the epithelium as well as on many cells in the stroma.
‡ Positivity in untreated mice was exclusively due to la expressed by Langerhans cells. Following treatment, however, cells other than Langerhans cells became intensely la-positive.

Systemic and topical use of IFN-γ: Systemic treatment was carried out as described in detail elsewhere. Using this treatment protocol made it possible to compare the response in the mouse eyes to that in the skin or other tissues. In brief, mice were injected intraperitoneally with 50,000 U IFN-γ in a volume of 1 ml daily, for 7 or 14 days. Control mice were injected with phosphate-buffered saline (PBS). Topical application was carried out by dropping the IFN-γ solution, at 50,000 U/ml, on the right eye, four times a day, for 3 consecutive days. The left eyes of these mice were used as controls. Five mice were tested following systemic treatment with IFN-γ for 7 days, three were examined after 14 days while five were PBS-treated controls. Five mice were topically treated with IFN-γ.

Immunohistochemical staining: Frozen sections of the eyes were cut at 4 μm and placed on gelatin-coated slides and stained for la, using a rat hybridoma-derived monoclonal antibody, M5/114 (Hybritech Incorporated, San Diego, CA), which is specific for murine la antigens I-Aβ, I-Aβ, I-Eα and I-Eβ. Control "irrelevant" antibodies included nonspecific mouse ascites and antibodies against Lyt2. The staining procedure was the avidin-biotin-peroxidase complex (ABC), as described in detail by Hsu et al. In brief, the sections were fixed with acetone, washed with Tris-buffered saline, 0.05 M, pH 7.6 and incubated sequentially with the following reagents: 10%...
Fig. 1. Effects of treatment with IFN-γ on la expression in the anterior segment of the mouse eye. (A, B), a control mouse, injected with PBS (14 daily injections); (C, D) a mouse treated with IFN-γ (14 daily injections of 50,000 units). All sections were similarly stained with antibodies against la antigen and counterstained with methyl green as described in Materials and Methods. Sections are shown at the magnification of X160 (A, C) or X80 (B, D). A and C are sections from the central cornea (CO). No la is expressed by cells of the control cornea (A), while intense staining is seen in the stromal cells (st) of the cornea from the IFN-treated mouse (C). In contrast to stromal cells, no la is expressed by the corneal epithelium (ep) of the IFN-treated mouse. B and D are sections of the peripheral cornea (right of the arrows), limbus, conjunctiva and ciliary body of eyes of a control and an IFN-treated mice. The only cells to stain strongly for la in this segment of the control eye (B) are the Langerhans cells, mainly in the limbus. In contrast, intense staining of cells in the conjunctiva and ciliary body (CB) is seen in the eye of the treated mouse (D). Intense la expression is also found in iris cells (IR) of the IFN-treated mouse. Please note the lack of la expression by the corneal epithelium of the IFN-treated mouse (right of the arrow), in contrast to the intense staining of the conjunctival epithelium of the same eye.

normal goat serum, M5/114 diluted 1:25 (8 μg/ml), biotinylated goat anti-rat IgG (H and L chains, American Qualex Inc., La Mirada, CA, diluted 1:100), avidin-biotin-peroxidase complex (Vector Lab, Burlingame, CA, diluted 1:100) and the substrate, diaminobenzide-Ni-H2O2 solution. The slides were counterstained with methyl green, dehydrated and examined by a light microscope.

Results. Topical application of IFN-γ produced no detectable changes in la expression by ocular cells. In contrast, striking effects were found following the systemic administration of this mediator (Table 1). Similar effects were produced in mice treated with IFN-γ for 7 or 14 days. Of particular interest were the effects on tissues of the anterior segment (Fig. 1). In the cornea, the IFN-γ treatment produced an increase in la expression by stromal cells, while no la could be detected on the corneal epithelial cells (Fig. 1C). In contrast, high levels of la were induced on the conjunctival epithelium of the treated mice (Fig. 1D). Little or no change in la staining was seen in the limbus of mice treated with injections of IFN-γ. Langerhans cells and other la-positive cells (including macrophages) normally reside in this tissue (Fig. 1B).
and the treatment had no significant effect on their density or intensity of Ia expression (Fig. 1D). Very few Langerhans or other Ia-positive cells were seen in the sections of the conjunctival epithelium of untreated mice.

A marked effect of IFN-γ treatment was observed in the ciliary body. In contrast to the low level of staining in untreated control mice (Fig. 1B), almost all cells of this tissue became strongly positive in the treated mice (Fig. 1D). A considerable increase in Ia expression was also observed on iris cells following treatment with IFN-γ (Fig. 1D).

No Ia staining was seen in the retinas of the control mice or the majority of the IFN-γ-treated animals (Fig. 2); low levels of Ia expression were detected on retinal vascular endothelium of two of the mice treated with IFN-γ for 7 days. Conversely, the treatment induced positive staining on retinal pigment epithelial cells and increased the Ia expression on choroid and scleral cells (Fig. 2B).

Discussion. The data reported here show that systemic administration of IFN-γ increases Ia expression on cells of various murine ocular tissues. No effect was produced, on the other hand, by topical application of IFN-γ, perhaps because of the inability of this mediator to penetrate to the cornea (A. G. Palestine, personal communication).

Tissues which show increased Ia expression following systemic treatment with IFN-γ included the cornea, conjunctiva, ciliary body, iris, choroid and sclera. A finding of particular interest is the difference between Ia expression by the cell layers of cornea and conjunctiva of mice injected intraperitoneally with IFN-γ. In the conjunctiva, epithelial cells expressed Ia antigenicity, and fibroblasts and vascular endothelium in the substantia propria also demonstrated increased Ia antigenicity. In the cornea, on the other hand, Ia expression was found only on stromal cells. This difference between the cornea and conjunctiva is not understood, but could be related to the lack of...
vasculature in the cornea. It is noteworthy that the response in the cornea also differs from that seen in the skin, where la antigens were expressed by epidermal cells which do not have this capacity constitutively. It is of note that the eyes of mice treated with IFN-γ resembled their skin in showing no apparent increase in the number of Langerhans cells or in their intensity of staining with the anti-la antibody. Our findings in the skin and the eye are in contrast, however, with those of Skoskiewicz et al, who reported that treatment with IFN-γ increased the number of la-expressing dendritic cells in a variety of nonlymphoid tissues. It is possible that the IFN-γ-induced recruitment of dendritic cells is limited to certain organs.

A striking increase in la expression was seen on most cells of the ciliary body of eyes from IFN-γ-treated mice. A similar reaction was seen in rats developing experimental autoimmune uveoretinitis (EAU) following either active immunization with S-antigen or adoptive transfer of lymphocytes specifically sensitized to this protein. This pattern of staining differs, however, from that seen in rats injected with endotoxin in which la expression was found to localize selectively on epithelial cells.

Mice treated with IFN-γ resembled rats developing EAU also in their increased expression of la on cells of the corneal stroma, choroid and RPE. On the other hand, unlike rats with EAU, minimal or no la expression was seen on retinal vascular endothelial cells of the IFN-γ-treated mice. This difference between the two experimental systems could be attributed to the selective accumulation of activated lymphocytes in the retina of rats with EAU. Local release of IFN-γ by these lymphocytes is assumed to bring about la expression by the vascular endothelial cells of the affected retinas. It is conceivable, therefore, that the levels of IFN-γ in the retinas of treated mice in the current study were lower and insufficient for regular induction of intense la expression on the endothelial cells.

Local expression of class II MHC antigens may be essential for presentation of tissue-specific antigens to the lymphocytes that produce autoimmune diseases. Our finding that treatment with IFN-γ increases la expression on ocular cells thus supports the notion that IFN-γ plays a role in the pathogenesis of ocular immune-mediated diseases such as EAU. More studies are needed to further test the effect of IFN-γ by monitoring EAU development in animals treated with this lymphokine.

Key words: class II MHC (la) antigens, interferon gamma (IFN-γ), antigen presentation, ocular and skin tissues, a comparison, experimental autoimmune uveoretinitis

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