Effect of Intraocular Gamma-Interferon on Immunoregulatory Properties of Iris and Ciliary Body Cells

J. Wayne Streilein,*† Scott Cousins,*† and Debbie Bradley*

Resistance of the anterior chamber (AC) of mouse eyes to expression of cell-mediated immunity can be overcome by pre-treating the eye with a dose of recombinant rat gamma-interferon (γIFN) that is of itself noninflammatory. To study the mechanism of this form of intraocular inflammation, cells of the tissues surrounding the AC (iris, ciliary body, cornea) were studied in vivo for alterations in phenotype and in vitro regarding their effects on antigen-driven T cell activation. The results indicate that γIFN: (1) induced class II major histocompatibility complex (MHC) expression on resident bone marrow-derived cells of iris and ciliary body (I/CB), but not the cornea; (2) led to recruitment of bone marrow-derived cells into the I/CB stroma; and (3) failed to induce class II MHC expression on ocular epithelial cells. Cell suspensions prepared from γIFN-treated I/CB superficially resembled normal I/CB cells in that neither were able to activate allogeneic T cells and both were able to suppress antigen-driven T cell activation in vitro. However, unlike cells from normal eyes, I/CB cells from γIFN-treated eyes suppressed T cell activation primarily through the secretion of prostaglandins. These results indicate that the ability of γIFN-treated eyes to display immunogenic inflammation probably does not result merely from the restoration of conventional antigen presenting cells to this environment, but appears to correlate with a critical change in the molecular mediators of immunosuppression. The findings are discussed in terms of the possibility that the eye may be able to respond to abrogation of its primary immunosuppressive microenvironment by erecting a secondary microenvironment that also is capable of suppressing immunogenic inflammation with a different set of antiinflammatory mediators. Invest Ophthal Vis Sci 33:2304-2315, 1992

The anterior chamber (AC) of the eye has proven to be a rather inhospitable environment for the expression of cell-mediated immunity of the delayed hypersensitivity type. This has been especially well demonstrated in mice, where injection of antigen (prepared from Mycobacterium tuberculosis) into the AC of tuberculin-primed mice failed to elicit a delayed-in-time intraocular inflammatory response. Similarly, parental strain lymphocytes injected into the AC of suitable F1 hybrid mice failed to induce a local graft-versus-host response. At least two unusual features of the anterior segment of the eye are thought to be involved in limiting or preventing immunogenic intraocular inflammation. First, histologic surveys of the tissues that surround the AC (iris, ciliary body, lens, and corneal endothelium) have revealed a paucity of potential antigen presenting cells, ie, bone marrow-derived cells expressing class II major histocompatibility complex (MHC) antigens. In fact, attempts to use class II-positive cells prepared from iris and ciliary body as antigen presenting cells in vitro have failed.

Second, the aqueous humor (the clear fluid that fills the AC) contains factors that inhibit antigen-driven T cell activation in vitro. One prominent immunosuppressive factor has been identified as transforming growth factor-beta (TGFβ), although other inhibitory substances are present and remain to be identified. The aqueous humor is secreted by epithelial cells of the ciliary body, and it has been demonstrated recently that cells contained within the iris and ciliary body (I/CB) can secrete immunosuppressive factors, such as TGFβ, that similarly inhibit T cell responses to antigens. The nature of the inhibitory factors produced by these cells remains ill defined, although prostaglandins are not thought to be included.

Recently, Cousin et al have attempted to alter the murine anterior chamber experimentally in a way...
that would permit the display of cell-mediated immune responses. Because TGFβ appears to be the primary immunosuppressant in normal aqueous humor, and because this cytokine has been shown by others to be antagonized by gamma-interferon (γIFN),10 these investigators injected a subinflammatory dose of γIFN (100 U rat recombinant γIFN) intracamerally. Although injection of this dose of γIFN into the AC of normal mouse eyes elicited no clinically detectable inflammation, injection of mycobacterial antigen into γIFN-treated eyes of tuberculin-sensitized mice evoked a delayed-in-time intraocular inflammatory response that was made up chiefly of polymorphonuclear neutrophilic and mononuclear leukocytes and T cells.11

Because γIFN was able to alter the immunoregulatory properties of the anterior chamber under these in vivo conditions, we wished to determine whether cells of the iris and ciliary body (I/CB) were altered by in vivo treatment with this cytokine. Specifically, we wanted to know whether I/CB cells from γIFN treated eyes (1) could activate allogeneic T cells; and (2) whether the cells ceased secreting immunosuppressive factors. The results of our experiments that form the basis of this report indicate that although intraocular γIFN increases the number of class II-bearing bone marrow derived cells in the I/CB, cells from these tissues remain unable to activate T cells, partially because they continue to secrete inhibitory factors. Of particular interest is the finding that the major inhibitory mediators secreted by γIFN-treated I/CB cells are prostaglandins.

Materials and Methods

Experimental Animals

BALB/c and C3H/HeN female mice were bred in our domestic colony and used for experiments at the ages of 6 to 8 wk. These strains differ at the major histocompatibility complex as well as at numerous minor histocompatibility loci. All experimental procedures conformed to the ARVO Resolution on the Use of Animals in Research.

Ocular Injections

Mice were anesthetized with an intraperitoneal injection of sodium pentobarbital. AC inoculations were performed with a blunted 30 G needle attached via plastic tubing to an automated Hamilton syringe. For each injection, 2 µl of air followed by 2 µl of cytokine or phosphate-buffered saline (PBS) were drawn into the tubing. While the eye was viewed under a dissecting microscope, an oblique transcorneal paracentesis tunnel was formed with a sharp 30 G needle, carefully avoiding iris or lens injury. A small amount of aqueous humor exuded from the opening and was blotted with filter paper. The blunt needle then was inserted into this track, followed by inoculation of 2 µl of cytokine and 2 µl of air, sealing the injection tract.

Clinical Examination of Injected Eyes

A previously described clinical grading scale1 was used to evaluate ocular inflammation, using a slit-lamp or dissecting microscope. Points were assigned according to a weighted scoring system for various clinical findings, including limbal hyperemia, AC fibrin, AC cells, iris nodules, and hypopyon. The maximal possible score was 28.5. All clinical examinations were performed in a masked fashion without knowledge of treatment of the animal. All clinical data are presented as the percent of maximal score (raw score/maximal score).

Preparation of Excised I/CB and Cornea

Mice were killed by cervical dislocation; their eyes were removed and microdissected to obtain the anterior half of each. The I/CB were carefully teased away from their attachment site and placed in wash medium—RPMI-1640 (Gibco Laboratories, Grand Island, NY) containing 5% heat-inactivated fetal calf serum (FCS; Hyclone Laboratories, Logan, UT), L-glutamine (2 mmol/l; Gibco), penicillin/streptomycin (100 U + 100 µg/ml; Gibco), and HEPES buffer (5 mmol/l; Gibco). The cornea was placed in a 20 mmol/l solution of EDTA (Sigma, St. Louis, MO) and incubated for 30 min at 37°C. The corneal epithelium was carefully separated from the stroma and endothelium while viewed with a dissecting microscope. The corneal epithelium then was placed in wash medium. The I/CB and corneal epithelia were stained with monoclonal antibodies and examined by fluorescent microscopy or prepared as single cell suspensions for use in mixed lymphocyte responses and T cell hybridoma activation assays.

Monoclonal Antibody Staining of I/CB and Corneal Epithelium

After washing, the whole I/CB and corneal epithelia were fixed in 95% ethyl alcohol for 30 min at room temperature. The specimens were rehydrated by
washed in two changes of PBS for 10 min each on a rocker platform. The specimens were incubated for 1 hr at 37°C in the primary monoclonal antibody I-A<sup>4</sup> (used at 1/20; Becton Dickinson, Los Angeles CA) or T200 (used at 1/100; Boehringer Mannheim, Indianapolis IN). T200 is a marker found on the surface of all bone marrow-derived cells. The specimens then were washed twice in PBS and placed in the secondary fluoresceinated antibody for 30 min at 37°C. Fluorescein isothiocyanate conjugated F(ab')<sub>2</sub> goat antimouse Ig (GAM-FITC; used at 1/100; Cappel, Malvern, PA) was used for the I-A<sup>4</sup> samples. The secondary antibody used for the T200 samples was F(ab')<sub>2</sub> goat antirat FITC (GAR-FITC; used at 1/100; Cappel). The specimens were again washed twice in PBS and carefully mounted on a microscope slide in glycerol/PBS (9:1). The positively stained cells were viewed with a fluorescent microscope. The cell counts were made from 10 random 400X fields per iris and the mean numbers calculated.

### Dispersion of Iris and Ciliary Body Cells

The excised I/CB to be used in assays requiring cell suspensions were placed in collagenase/dispace (Boehringer Mannheim) at 1 mg/ml in wash medium and incubated at 37°C for 1 hr. After incubation, single cell suspensions were obtained by trituration with a 21 G needle, followed by a 23 G needle attached to a syringe. The cells were washed twice in wash medium and resuspended in standard culture medium (SCM): RPMI-1640 containing 10% FCS, 2 X 10<sup>5</sup> M 2-mercaptoethanol, MEM nonessential amino acids (0.1 mmol/l; Gibco), MEM sodium pyruvate (1 mmol/l; Gibco), L-glutamine (2 mmol/l), penicillin/streptomycin (100 U + 100 µg/ml), and HEPES (5 mmol/l).

### Preparation of Spleen Cell Suspensions

Mice were killed by cervical dislocation and their spleens were removed aseptically. Single cell suspensions were obtained by pressing the minced tissue through a sterile 250 µm nylon mesh (Tetko, Elmsford, NY). The cells were washed twice in wash medium and resuspended in SCM. The aliquots to be used as stimulator cells were irradiated with 2000 rads. The spleen cells were used at a concentration of 2 X 10<sup>5</sup> cells/well.

### Mixed Lymphocyte Reaction

Responder spleen cells (2 X 10<sup>5</sup> cells/well) were cultured with irradiated stimulator cells (2 X 10<sup>5</sup> syngeneic or allogeneic spleen cells/well) in round bottomed 96-well plates. Regulators (I/CB cells, 5 X 10<sup>4</sup>/well) were added to the cultures so that a total volume of 200 µl/well was maintained. The cultures were incubated at 37°C and, on day 4, pulsed with tritiated thymidine (Amersham Corp., Arlington Heights, IL) at a concentration of 0.5 µCi/well. The cells were harvested on day 5 and the thymidine incorporation was measured. All in vitro experiments were repeated at least two times, with essentially identical results.

### Assay of T Cell Hybridoma Activation (DG11 Assay)

DG11 is a T cell hybridoma that secretes IL-2 in the presence of stimulator cells bearing the I-A<sup>4</sup> alloantigen. A20.1-11 (ATCC, Rockville, MD) is a BALB/c-derived B cell lymphoma that expresses I-A<sup>4</sup> on its cell surface. DG11 and A20 cells were washed once with wash medium and resuspended in SCM. A20 cells were irradiated with 2000 rads (X-A20). DG11 cells (2 X 10<sup>5</sup>/well) were cultured with medium alone (spontaneous IL-2 production control) or with X-A20 (2 X 10<sup>3</sup>/well) in 96-well round bottom plates. I/CB cells (5 X 10<sup>5</sup>/well) were added to the DG11/X-A20 cultures so that a total volume of 200 µl was maintained. Triplicate wells were plated for each experimental situation and incubated for 24 hr at 37°C. The supernatants from these cultures were harvested and assayed for the presence of IL-2 using the CTLL assay.

### CTLL Assay for IL-2

Serial two-fold dilutions of each supernatant with SCM (final volume = 100 µl/well) were placed in 96-well flat bottomed plates. IL-2 was assayed as previously described. Briefly, 100 µl of CTLL cells (1 X 10<sup>4</sup>) with the blocking antibody (anti-IL-4) was added to each well. Sextuplet cultures of CTLL cells and medium alone were used as negative controls. Positive controls of CTLL cells with serial dilutions of mouse recombinant IL-2 (Genzyme, Cambridge, MA) were included in all experiments. The CTLL cultures were incubated for 20 hr at 37°C, after which tritiated thymidine was added to the cultures at a concentration of 0.5 µCi/well. The cultures were incubated for an additional 4 hr before the cells were harvested and the thymidine incorporation was measured.

### Evaluation of Prostaglandin-Dependent Suppression

To determine whether suppression associated with I/CB cells was mediated by prostaglandins, neutralizing antiprostaglandin E<sub>2</sub> (PGE<sub>2</sub>) antibodies (Sigma, St. Louis, MO) were added to some mixed lymphocyte reaction (MLR) containing I/CB cells, as described previously. The antibodies were diluted in PBS to 0.1 µg/ml before they were added to the culture wells. In other experiments, indomethacin
(Sigma) was added (5 μg/ml) to some MLR containing I/CB cells, as described previously.11

Results

Clinical and Histologic Evaluation of Anterior Segment After Injection of γIFN Into the Anterior Chamber

We recently reported that immunogenic inflammation can be induced in the anterior chamber of mouse eyes if the AC has been first perturbed with a sub-inflammatory dose of γIFN.1 To document that this dose of γIFN did not induce inflammation, panels of normal BALB/c mice received rat recombinant γIFN (100 U) or PBS into the AC of one eye. The injected eyes then were examined clinically at 24, 48, and 72 hr and at 7 d. These examinations, the results of which are summarized in Table 1, revealed that eyes that received 100 U of rat γIFN displayed only minimal inflammation. Most eyes displayed only limbal hyperemia, or (infrequently) fibrin deposits at 24 hr. These changes usually resolved by 72 hr post inoculation. The quantitative clinical assessments of γIFN-treated eyes were not different from controls that received PBS alone. Histologic sections of eyes stained with hematoxylin-eosin revealed virtually normal structures at 24 hr, except for a rare neutrophilic leukocyte within the AC (data not shown). No infiltrate was observed after 48 hr. It is interesting that the cells of both layers of the ciliary epithelium of γIFN-treated eyes often appeared distended with increased amounts of eosinophilic cytoplasm. Ciliary epithelial cells from eyes treated with PBS alone did not have this appearance. Thus, 100 U of rat recombinant γIFN induced little if any clinical or histologic evidence of inflammation in the anterior segment of mouse eyes.

Phenotypic Features of Bone Marrow Derived Cells in Iris and Ciliary Body After Anterior Chamber Injection of γIFN

Although the evidence just described implies that γIFN at the dose injected had little visible effect upon the anterior segment of the eye, we examined the tissues surrounding the AC for more subtle effects of this cytokine—up-regulation of expression of class II MHC molecules and increased numbers of bone marrow-derived cells (T200+). Cornea and I/CB were excised from eyes of BALB/c mice that received AC injections of 100 U rat recombinant γIFN 24, 48, and 72 hr and 7 and 14 d previously. Tissues from control eyes, injected with PBS alone, also were examined. Whole mounts of corneal endothelium and of I/CB were stained with fluorescein-tagged monoclonal antibodies directed at I-Aδ and T200 (CD45), a reliable marker of bone marrow derived cells. The density of stained cells was assessed on whole mounts by fluorescent microscopy. The results of four separate experiments are summarized in Table 2. Similar to what we have reported previously, the normal I/CB contained approximately 610 ± 25/mm2 bone marrow-derived cells (T200+). These cells were located within the stroma and assumed a variety of forms, ranging from round (majority) to extensively dendritic (minority). These cells were usually not located adjacent to stromal blood vessels.

After γIFN injection into the AC, the density of T200+ cells remained unchanged after 24 hr, but rose to 826 ± 10/mm2 at 48 hr (30% increase over control, P < 0.05). The content of T200+ cells remained elevated throughout the 14 d observation period. The vast majority of labeled cells displayed a rounded configuration. At 48 hr, many of the stained cells were associated with blood vessels (Fig. 1), a finding compatible with the suggestion that these cells were recently recruited from the blood. By contrast, no significant change in density of T200+ cells was observed in I/CB removed from PBS-injected eyes. Thus, γIFN appears to induce the immigration of hematogenous cells into the I/CB in a slightly delayed pattern—between 24 and 48 hr after injection. Many of these recruited cells appeared to remain at the site for at least 14 d.

The density of Ia+ cells in the stroma of normal I/CB was found to be approximately 225 ± 25/mm2. The configuration of these cells ranged from dendritic

<table>
<thead>
<tr>
<th>Injection†</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>γIFN</td>
<td>2.6 ± 0.7 (13)</td>
<td>1.3 ± 0.7 (12)</td>
<td>0.9 ± 0.3 (12)</td>
<td>0.3 ± 0.3 (6)</td>
</tr>
<tr>
<td>PBS</td>
<td>2.3 ± 1.2 (3)</td>
<td>0.6 ± 0.6 (3)</td>
<td>0.6 ± 0.6 (3)</td>
<td></td>
</tr>
</tbody>
</table>

* Clinical scores were determined by evaluating ocular inflammation, as described in Materials and Methods. Scores were based on limbal hyperemia, AC fibrin, AC cells, iris nodules, and hypopyon. Maximal score possible is 28.5. Scores presented as percent of maximum score ± standard error of mean.
† γIFN (100 U rat recombinant gamma interferon) or PBS was injected (2 μl) into the anterior chamber of eyes of normal BALB/c mice.

Table 1. Clinical scoring of ocular inflammation resulting from anterior chamber injections
within the stroma of the I/CB. Subsequently, new cells on resident bone marrow derived cells located among bone marrow-derived cells within the I/CB. The evidence suggests that the earliest effect of γIFN is to induce the expression within 24 hr of la molecule on resident bone marrow derived cells located within the stroma of the I/CB. Subsequently, new T200+ cells are recruited into the stroma, presumably from the blood, and, in the aggregate, the density of la-bearing cells within the I/CB after γIFN is significantly enhanced. These changes may be relevant to the capacity of γIFN-treated eyes to sustain the expression of immunogenic inflammation.

**Allostimulatory Properties of Iris and Ciliary Body Cells From Eyes Treated With γIFN**

We previously reported that single cell suspensions prepared from normal I/CB, which contain 1–2% la+ cells, are incapable of activating allogeneic T cells, whereas comparable cell suspensions prepared from the limbal area of the corneal epithelium (containing similar numbers of la+ cells) can vigorously stimulate allogeneic T cell proliferation. Because the experiments above revealed that γIFN induced significant phenotypic alterations among I/CB bone marrow-derived cells, we examined the capacity of cells prepared from I/CB of γIFN-treated eyes to activate allogeneic T cells in vitro. Single cell suspensions were prepared from eyes of C3H/HeN mice that received AC injections of γIFN or PBS 48 hr earlier. The proportion of la-bearing cells in these two cell suspensions was similar but not identical. Among cells prepared from PBS-treated I/CB (n = 10), 1.1 ± 0.6% were la+, whereas among cells prepared from γIFN-treated I/CB (n = 10), 2.8 ± 0.4% were la+. These cell suspensions were plated in tissue culture wells (5 × 10⁴/well) as “stimulators.” Cell suspensions also were prepared from corneal limbal epithelium of untreated eyes and used as positive control “stimulators.” Allogeneic (BALB/c) or syngeneic spleen cell suspensions were added to these wells (2 × 10⁵) as “responders.” After 4 d, ³H-thymidine was added, and 24 hr later the cultures were harvested and assayed for radioactivity content.

The results of a typical experiment are summarized in Figure 2. As expected, I/CB cells from eyes treated with PBS alone failed to induce proliferation among allogeneic T cells. Surprisingly, I/CB cells from C3H eyes treated with γIFN also utterly failed to activate BALB/c spleen cells. For comparison’s sake, this experiment included cultures in which BALB/c responder cells were stimulated with cells harvested from corneal limbus epithelium obtained from normal eyes of C3H mice. These suspensions contain approximately 2% la+ cells (predominantly Langerhans cells). As the data presented in Figure 2 reveal, C3H corneal limbal cells were readily able to induce proliferation on the part of BALB/c spleen cells. In the aggregate, the results displayed in this figure indicate that γIFN treatment of the AC, which permits the intracellular expression of immunogenic inflammation and which alters the phenotypes of I/CB bone marrow-derived cells, does not restore the capacity of these cells to activate allogeneic T cells. In contrast, corneal lim-

### Table 2. Effect of gamma-interferon on expression of Ia and T200 on cells of iris and ciliary body

<table>
<thead>
<tr>
<th>Injection</th>
<th>Time post injection</th>
<th>(n)</th>
<th>Ia⁺</th>
<th>% Inc</th>
<th>T200</th>
<th>% Inc</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>(18)</td>
<td>223 ± 22</td>
<td>—</td>
<td>614 ± 22</td>
<td>—</td>
</tr>
<tr>
<td>γIFN</td>
<td>24 hr</td>
<td>(6)</td>
<td>366 ± 10</td>
<td>55*</td>
<td>627 ± 8</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>48 hr</td>
<td>(6)</td>
<td>447 ± 9</td>
<td>89†</td>
<td>825 ± 10</td>
<td>30†</td>
</tr>
<tr>
<td></td>
<td>72 hr</td>
<td>(6)</td>
<td>453 ± 8</td>
<td>92†</td>
<td>835 ± 11</td>
<td>32†</td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>(2)</td>
<td>401 ± 19</td>
<td>73*</td>
<td>801 ± 18</td>
<td>26*</td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td>(2)</td>
<td>363 ± 5</td>
<td>54*</td>
<td>763 ± 28</td>
<td>20</td>
</tr>
<tr>
<td>PBS</td>
<td>24 hr</td>
<td>(4)</td>
<td>242 ± 17</td>
<td>8</td>
<td>633 ± 25</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>48 hr</td>
<td>(4)</td>
<td>223 ± 25</td>
<td>0</td>
<td>652 ± 17</td>
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<td>72 hr</td>
<td>(4)</td>
<td>223 ± 17</td>
<td>0</td>
<td>633 ± 17</td>
<td>4</td>
</tr>
</tbody>
</table>

Gamma interferon (100 U rat recombinant) or PBS was injected (2 µl) into AC of eyes of normal BALB/c mice. Iris and ciliary body were removed periodically thereafter and examined by immunofluorescent microscopy with anti Ia⁺ and T200 antibodies for density of positive cells. Values reported as mean positive cells/mm² ± standard error of mean.

* Significantly increased compared to un.injected control, P < 0.05.
† Significantly increased compared to un injected control, P < 0.02.
bus cells (from normal C3H eyes), which contain approximately the same number of Ia+ positive cells as do I/CB cell suspensions, are vigorous activators of allogeneic T cells.

Immunosuppressive Properties of I/CB Cells From γIFN-Treated Eyes

Cells prepared from normal I/CB body have been reported to possess potent and constitutive immunosuppressive properties that are revealed when (1) the cells are cocultured with responders and allogeneic stimulators, and (2) supernatants from cultured I/CB cells are added to conventional T cell activation assays. We next examined whether I/CB cells harvested from γIFN-treated eyes still retained immunosuppressive properties. For these experiments, panels of BALB/c and C3H/HeN mice received intracameral injections of γIFN or PBS 48 hr prior to harvest of I/CB. Single cell suspensions were prepared and
Effect of Gamma-Interferon on Capacity of Iris and Ciliary Body Cells to Stimulate Allogeneic T cells

Fig. 2. BALB/c spleen cells (2 × 10^5 cells/well) were exposed to various stimulator cells in vitro for 5 days. ^{3}H-thymidine (0.5 μCi/well) was added for the terminal 24 hr, and amount of radioactivity incorporated is expressed as counts per minute (± standard error of mean), and as stimulation index (S.I.) Stimulators included (1) X-C3H-SC (2000 R irradiated C3H spleen cells, 2 × 10^5 well), (2) X-BALB/c SC (2000 R irradiated BALB/c spleen cells, 2 × 10^5 well), (3) C3H-I/CB, PBS (iris and ciliary body cells harvested 72 hr after injection of PBS into AC of C3H mice, 5 × 10^4/well), (4) C3H-I/CB, γIFN (iris and ciliary body cells harvested 72 hr after injection of γIFN (100 U) into AC of C3H mice, 5 × 10^4/well), and (5) C3H-CL (cells harvested from epithelial layer of corneal limbus of normal C3H mice, 2 × 10^4/well).

Fig. 3. BALB/c spleen cells (responders, 2 × 10^5/well) were stimulated with X-irradiated syngeneic (X-BALB/c) or allogeneic (C3H) spleen cells (SC, 2 × 10^5/well) without, or with various regulators (5 × 10^4/well): BALB/c-I/CB-iris and ciliary body cells harvested 72 hr after injection of PBS or γIFN into AC of BALB/c mice; C3H-I/CB-iris and ciliary body cells harvested 72 hr after injection of PBS or γIFN into AC of C3H mice. Cultures were conducted for 5 days; during terminal 24 hr ^{3}H-thymidine was added. Results presented as described in legend to Figure 2.

As reported previously, I/CB cells from normal mouse eyes and added directly to the culture inhibit IL-2 secretion by X-A20-stimulated DG11 cells. In the present experiments, I/CB cells were obtained from γIFN- or PBS-treated eyes of BALB/c mice and added as “regulators” to cultures containing DG11 cells (2 × 10^5/well) and X-A20 cells (2 × 10^5 cells/well). The results of one such experiment, presented in Figure 4, indicate that IL-2 secretion by DG11 cells was markedly curtailed in cultures to which I/CB cells were added, irrespective of whether the “regulators” were obtained from PBS- or γIFN-treated eyes. The extent of reduction of IL-2 secretion was comparable when I/CB cells from PBS-treated (72% reduction) or γIFN-treated (69% reduction) eyes were used. Thus, by two different assays of alloantigen-driven T cell activation, I/CB cells from γIFN-treated eyes displayed immuno-suppressive properties quantitatively similar to those not mediated via the T cell receptor for antigen and molecules encoded by class I or class II genes of the MHC.
displayed by I/CB cells prepared from normal (or PBS-treated) eyes.

**Role of Prostaglandins in Suppressive Properties From I/CB Cells From γIFN-Treated Eyes**

The immunosuppressive properties of I/CB cells from normal eyes are a result of, at least in part, the capacity of these cells to secrete soluble mediators such as TGF-β. However, virtually none of the suppression arising from normal I/CB cells is mediated by prostaglandins. Because γIFN may be able to activate prostaglandin synthetase, we determined whether the suppression mediated by γIFN-treated I/CB cells could be ascribed to prostaglandins. Two types of experiments were performed. In the first, mixed lymphocyte cultures were established with BALB/c spleen cells as “responders” and X-irradiated C3H spleen cells as “stimulators.” “Regulator” cells then were prepared as above from I/CB harvested from γIFN- or PBS-treated eyes, and added to the cultures. In preliminary experiments, it was determined that exogenous PGE2, in doses ranging from 50 μg to 180 pg/ml, was able to completely suppress MLR made up of BALB/c responders and C3H stimulators. Moreover, neutralizing anti-PGE2 antibodies were able to reverse the inhibition mediated by 180 pg/ml PGE2 completely and to partially reverse inhibition that resulted from 370 pg to 1.5 μg/ml PGE2. Therefore, the neutralizing anti-PGE2 antibody was added to some of the cultures to reverse suppression ascribed to I/CB cells. The results of a representative experiment are presented in Figure 5. Although, as reported previously, anti-PGE2 antibodies had no effect on suppression mediated by normal (PBS-treated) I/CB cells, anti-PGE2 antibodies almost completely reversed the suppression caused by I/CB cells harvested from γIFN-treated eyes.

In separate experiments, indomethacin (5 μg/ml) was added to MLR in which I/CB cells from γIFN- or PBS-treated eyes were used as “regulators.” As displayed in Figure 6, indomethacin partially relieved the suppression mediated by I/CB cells obtained from γIFN-treated eyes (stimulation index [SI] of 13, compared to SI of positive control of 21), but this drug failed to alter the inhibition caused by I/CB cells harvested from PBS-treated eyes (SI of 2.2). Moreover, in an experiment covering a broad range of indomethacin concentrations, the suppressive activities of γIFN-treated I/CB cells were progressively eliminated with increasing doses of indomethacin (See Table 3). At 10 μg/ml indomethacin, γIFN-treated I/CB cells displayed only marginal capacity to suppress T cell activation. Based on these results, we conclude that prostaglandin synthesis is induced among I/CB cells harvested from γIFN-treated eyes, and at least a portion
Ability of Indomethacin to Reverse Suppression Mediated by Gamma-Interferon Exposed Iris and Ciliary Body cells

![Graph](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933392/ on 11/22/2018)

Table 3. Dose response of effects of indomethacin on suppression mediated by γIFN-exposed iris and ciliary body cells

<table>
<thead>
<tr>
<th>Stimulators</th>
<th>Regulators</th>
<th>Indomethacin (μg/ml)</th>
<th>C.P.M.</th>
<th>Stimulation index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syngeneic</td>
<td>—</td>
<td>—</td>
<td>681 ± 139</td>
<td>—</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>—</td>
<td>—</td>
<td>16,573 ± 1528</td>
<td>24.0</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>I/CB—Normal</td>
<td>—</td>
<td>1630 ± 260</td>
<td>2.4</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>I/CB—γIFN</td>
<td>—</td>
<td>1675 ± 201</td>
<td>2.4</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>I/CB—γIFN</td>
<td>1</td>
<td>3893 ± 219</td>
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<tr>
<td>Allogeneic</td>
<td>I/CB—γIFN</td>
<td>10</td>
<td>12,258 ± 233</td>
<td>18.0</td>
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BALB/c spleen cells (responders, 2 × 10⁵/well) were stimulated with x-irradiated syngeneic (BALB/c) or allogeneic (C3H) spleen cells (2 × 10⁵/well) with regulators (BALB/c I/CB cells harvested 72 hr after injection of γIFN into AC). 3H-thymidine (0.5 μCi/well) was added after 4 days, and radioactivity incorporation assessed 24 hr later. Results expressed as counts per minute (C.P.M.) and stimulation index.

of the immunosuppressive properties of these cells can be ascribed to the production of PGE₂.

Discussion

Immunogenic inflammation, especially that mediated by nonspecific defense mechanisms and that leading to significant "innocent bystander" injury, is difficult to elicit in eyes of normal mice. Inoculation of P815 tumor cells into eyes of BALB/c mice previously sensitized to DBA/2 alloantigens produces an evanescent tumor that is eliminated rapidly with minimal evidence of intraocular injury.¹¹ Similarly, injection of herpes simplex virus-1 (HSV-1; KOS strain) into the AC of HSV-immune mice elicits only minor intraocular inflammation, which subsides without apparent damage to the visual apparatus (personal communication, Dr. Sally Atherton, University of Miami, October 1991). Injections of parental strain lymphoid cells into the AC of suitable F1 hybrid mice evoke little or no evidence of local graft-versus-host reactivity.¹² Finally, injection of antigen into the AC of mice pre-sensitized to the same antigen elicits no clinically detectable intraocular inflammation.¹ Understanding the physiologic bases for the lack of intraocular inflammation under these experimental circumstances may help to explain why, under pathologic conditions, intraocular inflammation occurs and is devastating to the delicate microanatomy of the organ, leading to blindness. Cousins et al¹ have begun to attack this important problem experimentally by injecting agents into the eye that render the organ more susceptible to immunogenic inflammation. Specifically, the introduction of subinflammatory doses of the pleiotropic cytokine γIFN into the AC allows the eye to display cell-mediated immunity to mycobacterial antigens. The studies reported here were designed to explore the effects of γIFN on intraocular cells that might be relevant to local expression of immunogenic inflammation. Although the results bear only tangential witness to the pro-inflammatory effects of γIFN, the findings offer new insights into the mechanisms by which intraocular inflammation may be regulated.

Injection of very small amounts of γIFN (100 U rat recombinant γIFN) into the AC of mouse eyes did not induce Ia⁺ cells to migrate into the central corneal epithelium, nor did the cytokine induce Ia expression on epithelial cells of cornea, iris, or ciliary body. However, this dose of γIFN did induce resident macrophages of I/CB stroma to become Ia⁺ and caused the acute recruitment of modest numbers of additional...
blood borne cells into these intraocular tissues. The latter findings led us to postulate that the ability of γIFN-treated eyes to permit the expression of cell-mediated immunity might be a result of the emergence of conventional antigen presenting cells within I/CB. However, when cells harvested from I/CB of γIFN-treated eyes were tested for antigen presenting function in vitro they failed to activate allogeneic T cells and suppressed alloantigen-driven T cell activation. In these regards, the cells from γIFN-treated eyes closely resemble cells obtained from PBS-injected (and uninjected) eyes. However, because even γIFN-treated I/CB cells continued to secrete immunosuppressive factors into the culture medium, our assays might not have been able to detect subtle differences in antigen presenting capacity between normal and γIFN-treated ocular cells. Thus, based on these results, we are unable to account for the ability of γIFN-treated eyes to display immunogenic inflammation. More to the point, our data suggest that expression of cell-mediated immunity in γIFN-treated eyes is not merely predicated upon the conversion of resident bone marrow-derived cells into “professional” antigen presenting cells.

Recently, Sethna and Lampson have injected various doses of recombinant rat γIFN into the subarachnoid space of rat brains and observed intracerebral changes similar to those we have described in the uveal tract. After intracerebral injection of 100 U of γIFN, these workers detected recruitment of lymphocytes and other inflammatory cells to the injection site and observed relatively widespread upregulation of Class II expression on microglial, ependymal, and perivascular cells through both hemispheres of the brain. Thus, the local effects of small doses of injected γIFN on cells of the uveal tract and the brain are very similar.

Gamma-interferon has been injected intraocularly by other investigators to explore the potential role of this cytokine in ocular inflammation. For example, Hamel et al injected 20,000 U γIFN intravitreally or subretinally and achieved intense Ia expression on a variety of ocular cells localized in the conjunctiva and anterior segment. This regimen induced iritis and retinitis, made up of infiltrating mononuclear cells, some of which expressed MHC class II molecules. Kusuda et al injected 50,000 U γIFN intraperitoneally for 7–14 d into Lewis rats and observed a pattern of intraocular Ia antigen expression similar to that of rats with experimental autoimmune uveoretinitis, except for less regular and less intense expression of Ia on retinal vascular endothelium. In neither of these instances were Langerhans cells observed to migrate into the central corneal epithelium, nor was the corneal epithelium converted to Ia expression. Lee and Peose compared the effects of intraocular γIFN in rats and mice and found remarkably little evidence of inflammation in mouse eyes that received 7.6×10⁴ U murine γIFN, even though there was extensive induction of Class II MHC antigens on ocular cells. Thus, intraocular and systemic administration of γIFN can induce intraocular inflammation, in part by the recruitment of massive numbers of blood borne Ia+ cells. It is important to note that the doses of γIFN used in these studies were several orders of magnitude greater than we have employed.

Our observation that suppression by I/CB cells from γIFN-treated eyes was mediated largely, if not exclusively, by prostaglandins bears comment. We previously reported that prostaglandins play no role in the suppressive activities of cells harvested from normal I/CB. However, there is not universal agreement on this point. Helbig et al have demonstrated that a portion of the inhibitory properties of cultured, irradiated ciliary epithelial cells from various species is mediated by a soluble factor. Using an assay system of antigen-driven T helper cell proliferation, indomethacin reversed a minor portion of inhibition caused by ciliary body cells, suggesting that prostaglandins probably contribute to the inhibition observed. More recently, Knisely et al have shown that supernatants of cultured murine iris and ciliary body cells inhibit proliferation of the TGFβ-sensitive Mv1 Lu epithelial cell line and of thymocytes exposed to phytohemagglutinin. Indomethacin failed to reverse the effects of I/CB supernatant on the Mv1 Lu cells, but did permit thymocyte proliferation, suggesting that prostaglandins are produced by cultured I/CB cells, Streilein and Bradley, Helbig et al, and Knisely each have used different in vitro systems to assay immune suppression by I/CB cells and their supernatants. This may account for why Streilein and Bradley failed to detect a prostaglandin influence emanating from normal I/CB cells, whereas the other investigators did. However, whatever the endogenous prostaglandin production rate of normal I/CB cells, the results presented in the present report clearly indicate that prostaglandin synthesis and release is significantly increased among I/CB cells harvested from eyes exposed to small amounts of γIFN in vivo. We believe that the immunosuppressive microenvironment of the anterior chamber remains inhibitory of lymphocyte activation after γIFN injection and that the major burden of the suppressive activity shifts from cytokines to prostaglandins.

Our experiments do not address the precise cellular source of immunoinhibitory factors released from I/CB cells removed from γIFN-treated eyes. Helbig et al used cultures of virtually pure epithelial cells, and therefore could conclude that the source of immuno-
inhibitory factors in their experiments was the epithelial cells. However, our I/CB preparations, as well as those used by Kniseley et al18 contain both parenchymal cells and bone marrow-derived cells. To this point, Hooper et al20 recently reported that immunoinhibitory factors are produced by both bone marrow-derived (T200+) and parenchymal (T200−) cells of the uveal tract. Thus, the novel immunosuppressive environment created within the AC by γIFN injection may be created by secretory products of parenchymal and passenger cells of the eye.

Treatment of nonlymphoretic cells with γIFN produces major changes in the genetic program of the target cells. Usually (except for neurons and trophoblastic cells), cells that are typically negative convert to a positive after exposure to γIFN. This appears to be the case with many cells from the eye.14,15 However, the ability of γIFN-treated cells to act as antigen presenting cells for T cells is not so uniform. On the one hand, murine keratinocytes,21 human endothelial cells,22 and thyroid epithelial cells23 respond to γIFN treatment by up-regulating class II MHC expression and by displaying the capacity to present antigens to (at least) certain types of T lymphocytes. On the other hand, there is controversy exists about whether γIFN can induce dermal fibroblasts to function as antigen presenting cells.22,24 Our evidence, combined with the results of Helbig et al17 and of Caspi et al,25 clearly indicate that parenchymal ocular cells do not acquire conventional antigen presenting properties when exposed to γIFN. In the case of cells from the iris and ciliary body, which normally suppress T cell activation, suppression of T cell activation is retained after γIFN treatment, although the mediator(s) of the suppression is clearly different.

Our findings have created a paradox. On the one hand, injection of low dose γIFN into the AC allows the eye to display a clinically detectable and flow cytometrically verifiable cell-mediated immune response.1,12 On the other hand, the cells of the γIFN-treated eye neither acquire conventional antigen presenting capabilities nor do they lose the capacity to suppress T cell activation. What changes is the molecular mechanism of suppression detected in vitro. This paradox has caused us to reconsider the meaning of the finding that AC injection of γIFN makes it possible for the AC to express cell-mediated immunity.1 We have reported that injection of mycobacterial antigen into the γIFN-treated AC of Mycobacteria-immune mice resulted in infiltration of leukocytes into the AC within 24 hr.1 We are now entertaining the possibilities that inflammation elicited by antigen in γIFN-treated eyes may be prematurely quenched and that prostaglandins produced locally in response to γIFN are responsible for the quenching. Experiments are currently underway to determine whether immunogenic intraocular inflammation in γIFN-treated eyes can be amplified and extended in duration by systemic inhibition of prostaglandin synthesis and whether this may permit ocular phthisis to develop. Such an outcome would suggest that when the primary immunosuppressive microenvironment of the AC is abrogated (eg, by γIFN), the eye is able to retaliate by creating a secondary immunosuppressive microenvironment that also serves to limit immunogenic inflammation. Possibly, immunogenic inflammation leading to disruption of the visual axis and blindness may occur only after several such ocular barriers to its expression have been eliminated.

Key words: anterior chamber, iris and ciliary body, gamma interferon, prostaglandins, immunosuppression, transforming growth factor-beta

References


