Induction of HLA-DR Expression in Human Lamina Cribrosa Astrocytes by Cytokines and Simulated Ischemia

Junjie Yang, Ping Yang, Gülgün Tezel, Rajkumar V. Patil, M. Rosario Hernandez, and Martin B. Wax

PURPOSE. Recent evidence strongly suggests that activated immunity occurs during the neurodegenerative process of glaucomatous optic neuropathy. Although activation of lamina cribrosa astrocytes has been identified in glaucomatous optic nerve head, their role on the activated immune responses seen in glaucoma patients is unknown. Here, the authors aimed to study the potential role of lamina cribrosa astrocytes as a component of activated immune responses seen in glaucoma patients.

METHODS. Expression of HLA-DR in optic nerve head astrocytes was studied using immunohistochemistry in postmortem eyes of patients with glaucoma and normal donors. Serum cytokine levels of patients with glaucoma and control subjects were measured using enzyme-linked, immunosorbent assay. In addition, in vitro experiments were performed using astrocyte cultures derived from human optic nerve head or fetal human brain. The cultured astrocytes were incubated under selected stress conditions such as exposure to cytokines, IFN-γ and IL-10, or simulated ischemia for up to 48 hours. The expression of HLA-DR was studied in these cells using flow cytometry and immunocytochemistry.

RESULTS. Immunohistochemistry demonstrated an upregulation of the HLA-DR expression in the optic nerve head astrocytes in glaucoma. In addition, serum levels of IL-10 was higher in the patients with normal pressure glaucoma compared to age-matched control subjects (P = 0.001). Regarding in vitro experiments, unlike brain astrocytes, the percentage of cells expressing HLA-DR was approximately 3 times higher in the cultures of optic nerve head astrocytes exposed to simulated ischemia compared to cultures incubated under normal conditions (P = 0.09). Incubation with IFN-γ induced HLA-DR expression in brain and lamina cribrosa astrocytes, up to 25-fold, (P < 0.001) either in the absence or presence of simulated ischemia. Induction of HLA-DR expression by IFN-10 was approximately 6 times higher in lamina cribrosa astrocytes incubated under simulated ischemia compared to that incubated under normal condition (P = 0.004) and was not prominent in brain astrocytes.

CONCLUSIONS. These findings suggest that optic nerve head astrocytes function as antigen-presenting cells and that their immunogenic capacity is more sensitive to ischemia than brain astrocytes. Taken together, these findings provide novel evidence that regulation of immunogenic capacity of optic nerve head astrocytes by cytokines or ischemic stress may have a role during the neurodegeneration process in patients with glaucoma. (Invest Ophthalmol Vis Sci. 2001;42:365–371)

Glaucma is the second leading cause of permanent vision loss in the world.1 However, the pathogenic mechanism(s) underlying glaucomatous optic neuropathy remains unknown. Recently, compelling observations have provided insight as to potential roles for autoimmune-mediated mechanisms in the development and/or progression of the glaucomatous optic neuropathy. Cartwright et al.2 found that a cohort of glaucoma patients with normal intraocular pressure had an increased prevalence of systemic autoimmune diseases. An increased prevalence of monoclonal gammapathy,3 retinal immunoglobulin deposition,4 and elevated serum autoantibody titters to retinal antigens including heat shock proteins have been reported in some patients with glaucoma.5–8 Additional evidence indicates that alterations in the cellular immune system such as increased percentage of CD8 T lymphocytes and altered serum levels of soluble IL-2 cytokine receptors occur in patients with glaucoma.9,10 These findings suggest that changes in systemic immunity likely accompany optic neuropathy in some patients with glaucoma.

Although elevated intraocular pressure is typically observed in most patients with glaucoma,11 other factors have long been thought to be important for the pathogenesis of optic neuropathy in glaucoma. These include vascular abnormalities, such as ischemia, vasospasm, and systemic hypotension, which may result in tissue hypoxia and reduced retinal perfusion.12-14 In addition, other proposed pathogenic factors include neurotrophin withdrawal secondary to blockade of retrograde axonal transport,15-17 excitotoxicity,18 and increased peroxynitrite production.19

The major histocompatibility complex (MHC) Class II antigens (HLA-DR), which are displayed on the surface of antigen-presenting cells, are highly polymorphic cell surface glycoproteins encoded by specific genes.20,21 Under physiologic conditions, in vivo, MHC Class II expression is relatively limited to cells of the immune system. If this restriction is violated as occurs in organ-specific pathologic states, autoimmunity or immunopathology is likely to be involved in disease pathogenesis.22 The significance of MHC Class II display in organs undergoing autoimmune attack is supported by the correlation between tissue destruction and expression of MHC Class II by resident cells of the involved organ.22

In view of the potential importance of MHC Class II expression in immune-related disease, we sought to examine HLA-DR expression in optic nerve head sections from postmortem eyes with glaucoma in comparison to that from age-matched normal donors. Immunohistochemistry revealed an upregulation of HLA-DR expression in the optic nerve head astrocytes in glaucoma. In addition, we examined whether there is a difference
in serum cytokine levels between patients with glaucoma and normal subjects that might affect the HLA-DR expression and thereby the immunogenic capacity of optic nerve head astrocytes in glaucomatous eyes. Using enzyme-linked, immunosorbent assay, serum titers of the cytokine IL-10 were found elevated in glaucoma patients compared to age-matched normal subjects. To examine the feasibility of these observations, we then performed in vitro experiments using cultured lamina cribrosa astrocytes derived from human optic nerve head. We specifically studied responses of cultured optic nerve head astrocytes to induction by cytokines (IFN-γ and IL-10) under normal or ischemic conditions. Our in vitro observations using flow cytometry and immunocytochemistry revealed that the expression of HLA-DR and its induction by selective cytokines increase in optic nerve head astrocytes exposed to simulated ischemia. Findings from in vitro studies and glaucoma patients, in which elevated serum cytokines and ischemia have been identified, suggest that cytokines and/or ischemia may induce HLA-DR expression in optic nerve head astrocytes. Therefore, optic nerve head astrocytes may be a key component of immunoregulatory events that participate in the pathogenesis of neurodegeneration in patients with glaucoma.

**Materials and Methods**

**Immunohistochemistry**

To examine HLA-DR expression and its colocalization to astroglial cells, we performed double immunofluorescence labeling in optic nerve head sections from postmortem human eyes. For this purpose, 6 postmortem eyes with a diagnosis of glaucoma and 6 eyes from age-matched normal donors were obtained from the Glaucoma Research Foundation (San Francisco, CA), The National Disease Research Interchange (NDRI, Philadelphia, PA), and the Mid-America Eye Bank (St. Louis, MO). Clinical findings of the patients were well documented including intraocular pressure readings, optic disc assessments, and visual field tests (Table 1). Normal donors had no history of eye disease or diabetes. There was no infection or sepsis in any of the donors. The cause of death for all of the donors used in this study was myocardial infarction or cardiopulmonary failure.

The eyes were enucleated within 2 to 4 hours after death and processed and fixed within following 6 to 12 hours in either 10% buffered formaldehyde or 4% paraformaldehyde. The posterior poles were dissected free of surrounding tissues and were washed extensively in 0.2% glycine in phosphate-buffered saline solution at pH 7.4, embedded in paraffin, and oriented sagittally for 6 μm sections. Serial sections from the glaucomatous eyes and normal donor eyes were stained simultaneously to control for variations in the immunostaining.

For double immunofluorescence labeling, sections were incubated with a mixture of mouse antibody against HLA-DR and rabbit antibody against glial fibrillary acidic protein (GFAP), a marker for astrocytes, at 1:100 dilution for 30 minutes (Sigma, St. Louis, MO). The sections were then incubated with a mixture of Rhodamine-Red and Oregon-Green-labeled secondary antibodies at 1:200 dilution (Molecular Probes, Eugene, OR) for another 30 minutes. Negative controls were performed by replacing the primary antibody with non-immune serum or by incubating sections with the each primary antibody followed by the inappropriate secondary antibody to determine that each secondary antibody was specific to the species it was made against. Slides were examined and documented using the fluorescence microscope.

**Measurement of Serum Cytokine Levels in Patients with Glaucoma**

Blood samples obtained from 20 patients with primary open angle glaucoma, 35 patients with normal pressure glaucoma and 20 healthy age-matched controls were studied. All of the experiments were performed in accordance with the Declaration of Helsinki. The inclusion and exclusion criteria for these groups were described previously. Briefly, normal pressure glaucoma consisted of the presence of open irido-corneal angles, no evidence of intraocular pressure greater than 23 mm Hg, glaucomatous changes in visual fields and optic disc cupping and the absence of alternate causes of optic neuropathy. The diagnostic criteria for the primary open angle glaucoma were similar to those of normal pressure glaucoma except their untreated intraocular pressure levels were greater than 23 mm Hg. Visual field loss of patients was evaluated with the Humphrey Field Analyzer, 30-2 program (Humphrey Instruments, San Leandro, CA). Our criteria for visual field abnormalities included a corrected pattern SD with a P value < 0.05 or a glaucoma hemifield test outside normal limits obtained with at least two reliable and reproducible visual field examinations. The subjects in the control group had no evidence of ocular or systemic disease.

Serum was collected after centrifugation and stored at -80°C until further use. Cytokine levels in the collected serum samples were assessed by enzyme-linked, immunosorbent assay (ELISA) using a commercial kit (R&D Systems, Minneapolis, MN). The sensitivities were as follows: IFN-γ (sensitivity < 3 pg/ml; intra-assay precision < 5.3%), IL-10 (sensitivity < 24 pg/ml; intra-assay precision < 6.1%).

**Cell Cultures**

Human lamina cribrosa astrocytes were cultured from adult normal optic nerve heads in DMEM/F-12 supplemented with 10% fetal bovine serum, 5 ng/ml of human basic fibroblast growth factor (Biomedical Technologies, Stoughton, MA), 5 ng/ml of human platelet-derived growth factor-A chain (Sigma) and PSFM (10,000 U/ml penicillin, 10,000 μg/ml streptomycin, and 25 μg/ml amphotericin B) as described previously. To suppress fibroblasts, which divide faster than astrocytes, cytosine was added every 2 days. The medium was exchanged every 2 days. All tissue culture reagents were purchased from Gibco (Grand Island, NY). Primary cultures of optic nerve astrocytes were characterized by staining with GFAP. In addition, cultured fetal human brain astrocytes (Clonetics, Walkersville, MD) served as a control, which were incubated under identical conditions to that for optic nerve head astrocytes as described next.

**Study Design**

Lamina cribrosa and brain astrocytes plated in 6-well plates (Costar, Cambridge, MA) at a density of 5 × 10⁶ cells/well and grown to 75% confluence were then incubated under normal or simulated ischemia conditions. During incubations, astrocyte-defined, serum-free medium containing DMEM, 1.3% bovine albumin fraction V, (Sigma), 2 μ/l IL-1 culture supplement (Collaborative Biomedical Products, Bedford, MA), and 1 μ/l antibiotic and antimycotic mixture (Gibco) was used. In addition, incubations were performed in the presence or absence of cytokines such as recombinant human IFN-γ (100 μg/ml) and IL-10 (10 ng/ml; R&D Systems). For simulated ischemia, astrocytes were exposed to reduced oxygen tensions in medium lacking glucose. Hypoxia was maintained by placing the cultures in a dedicated culture incubator with a controlled flow of 95% N₂/5% CO₂ and 0% O₂ for up to 48 hours. This incubation was purged overnight with a mixture of CO₂/N₂ to achieve a final O₂ concentration of 2% at the beginning of the experiment. Control cells from identical passage of the cultured cells were simultaneously incubated in a tissue culture incubator at 95% air/5% CO₂, at 37°C. At the end of the incubation period, the cells were immediately subjected to experiments described next, including flow cytometry and immunocytochemistry.

**Table 1. Clinical Data of Postmortem Glaucomatous Eyes**

<table>
<thead>
<tr>
<th>Number</th>
<th>Age (years)</th>
<th>Gender</th>
<th>C/D</th>
<th>VF Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58</td>
<td>M</td>
<td>0.9</td>
<td>Advanced</td>
</tr>
<tr>
<td>2</td>
<td>74</td>
<td>F</td>
<td>0.9</td>
<td>Advanced</td>
</tr>
<tr>
<td>3</td>
<td>67</td>
<td>F</td>
<td>0.5</td>
<td>Moderate</td>
</tr>
<tr>
<td>4</td>
<td>93</td>
<td>F</td>
<td>0.7</td>
<td>Moderate</td>
</tr>
<tr>
<td>5</td>
<td>56</td>
<td>F</td>
<td>0.9</td>
<td>Advanced</td>
</tr>
<tr>
<td>6</td>
<td>67</td>
<td>F</td>
<td>0.4</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

C/D, cup-to-disc ratio; VF, visual field.
Flow Cytometry

Cultured lamina cribrosa and brain astrocytes were detached from 6-well plates by 0.25% trypsin-EDTA solution (Sigma) and washed twice with phosphate-buffered saline solution at pH 7.4 containing 0.1% bovine serum albumin and 0.01% sodium azide. For labeling of HLA-DR, the cells were incubated with phycoerythrin–conjugated monoclonal antibody to HLA-DR (Sigma) at 4°C for 60 minutes. For labeling of GFAP, the cells were fixed in 4% paraformaldehyde solution at room temperature for 20 minutes and permeabilized using a solution containing 5% fetal bovine serum, 0.2% Triton X-100, and 0.5% glycine. They were then incubated with rabbit antibody to GFAP (1:100, Sigma) on ice for 60 minutes. After washing three times, the cells were incubated with FITC-conjugated goat anti-rabbit antibody (1:200, Sigma) at 25°C for 30 minutes.

To examine the effects of treatment on the cell cycle, after centrifugation at 20 000g, pelleted astrocytes were fixed in 2 ml cold 70% ethanol at 4°C for 60 minutes. The cells were centrifuged and washed in 1 ml phosphate-buffered saline solution and resuspended in 0.5 ml of RNase solution prepared using phosphate-buffered saline (1 mg/ml, Type I-A, Sigma). Then, 1 ml of 100 µg/ml propidium iodide (Sigma) solution was added and gently mixed. The cells were then incubated in the dark at room temperature for 15 minutes and kept in the dark at 4°C until flow cytometric analysis.

The stained cells were measured using a FACScan flow cytometer/CELLQuest Software system (Becton Dickinson, San Jose, CA) equipped with a single air-cooled, argon-ion laser. Data were collected using logarithmic amplification on 5000 cells, excluding cell debris by a combination of forward and side scatter. Frequency histograms representative of three separate experiments are shown.

Immunocytochemistry

For labeling of HLA-DR in cultures, astrocytes grown on sterile coverslips were fixed using 4% paraformaldehyde solution. After washing, coverslips were incubated with monoclonal antibody to HLA-DR (1:50, Accurate Chemical, Westbury, NY) at 23°C for 30 minutes and then with rhodamine-conjugated goat anti-mouse IgG (1:800) at 25°C for 30 minutes. For intracellular labeling of GFAP, after fixation, the cells were permeabilized using a solution containing 5% fetal bovine serum, 0.2% Triton X-100, and 0.5% glycine. The coverslips were then incubated with monoclonal antibody to GFAP (Sigma) (1:100) at 23°C for 30 minutes. Incubation with second antibody was then performed using rhodamine-conjugated goat anti-mouse IgG (1:400) at 25°C for 30 minutes. For negative controls, the primary antibody was replaced by nonimmune mouse serum. After washing, the coverslips were mounted and slides were examined using a fluorescence microscope (Olympus, Tokyo, Japan) and images were recorded using a digital camera attached to the microscope.

RESULTS

HLA-DR Expression in Astrocytes of Glaucomatous Optic Nerve Head

Expression of HLA-DR was examined in postmortem eyes from patients with glaucoma in comparison to eyes from age-matched normal donors using immunohistochemistry. We observed that expression of HLA-DR in optic nerve head astrocytes is upregulated in glaucoma. The examination of optic nerve head sections from normal donor eyes using double immunolabeling revealed that the expression of HLA-DR is not colocalized with GFAP, a marker of astroglial cells. In normal optic nerve heads, HLA-DR was localized to the perivascular microglia. However, colocalization of HLA-DR and GFAP immunolabeling was detected in sections from postmortem glaucomatous eyes. Some, but not all, of the astrocytes exhibited positive immunolabeling for both HLA-DR and GFAP. Astrocytes expressing HLA-DR and GFAP exhibited the morphology of reactive astrocytes and were located in the nerve bundle area in the glaucomatous optic nerve head (Fig. 1).

Serum Cytokine Levels in Patients with Glaucoma

Serum levels of IFN-γ and IL-10 were measured by ELISA in patients with glaucoma and age-matched control subjects. Serum levels of IFN-γ and IL-10 in control subjects and in the patients with primary open angle glaucoma or normal pressure glaucoma are shown in Table 2. There was no difference in the serum levels of IFN-γ between the normal and control groups. However, serum IL-10 levels were significantly higher in patients with normal pressure glaucoma compared to control serum levels (Mann–Whitney U test, P = 0.001).

Identification of Astrocytes in Cultures

After labeling of GFAP, flow cytometry demonstrated that the percentage of astrocytes in both cell lines we used was more than 98% homogeneous (Fig. 2).

![Image](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932898/ on 06/24/2017)
Flow cytometry revealed that the percentage of cells expressing HLA-DR was approximately 3 times greater in lamina cribrosa astrocytes exposed to simulated ischemia (8%) compared to astrocytes incubated under normal conditions (2.5%; Fisher’s exact test, \( P = 0.09 \); see Figs. 3 and 4). However, as seen in Figure 4, there was no prominent difference in the expression of HLA-DR in brain astrocytes incubated under normal or simulated ischemia conditions (Fisher’s exact test, \( P = 0.85 \)).

During incubations under normal or ischemic conditions, we simultaneously used selected cytokines, individually or in combination, to induce HLA-DR expression in astrocytes. We observed that under both normal condition and simulated ischemia, IFN-\( \gamma \) (100 \( \mu \)g/ml) significantly induced HLA-DR expression in both brain (approximately 22-fold) and lamina cribrosa astrocytes (approximately 12-fold; Fig. 4; Fisher’s exact test, \( P < 0.0001 \)). There was no difference between the induction of HLA-DR expression by IFN-\( \gamma \) in these cultures following incubation under simulated ischemia or normal conditions (Fig. 4; Fisher’s exact test, \( P > 0.05 \)). However, induction of HLA-DR expression by IL-10 (10 ng/ml) was approximately 6 times higher in lamina cribrosa astrocytes incubated under simulated ischemia compared to that incubated under normal condition (Fisher’s exact test, \( P = 0.004 \)), yet this difference was less than 1.5-fold in brain astrocytes (Fig. 4; Fisher’s exact test, \( P = 0.66 \)). HLA-DR expression induced by IFN-\( \gamma \) was neither inhibited or enhanced by IL-10 in cells incubated under either normal or simulated ischemia conditions.

### Table 2. Serum Cytokine Levels

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<tr>
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Statistical significance (\( P \), Mann-Whitney \( U \) test)

- Control versus POAG: \( P = 0.50 \), \( 0.29 \)
- Control versus NPG: \( P = 0.25 \), \( 0.001 \)
- POAG versus NPG: \( P = 0.25 \), \( 0.03 \)

POAG, primary open angle glaucoma; NPG, normal pressure glaucoma; Mean ± SD, pg/ml.

**Induced HLA-DR Expression in Cultured Astrocytes Incubated under Simulated Ischemia**

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![Image](https://via.placeholder.com/150)

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Incubations were performed in the absence or presence of cytokines, IFN-\(\gamma\) and/or IL-10, under normal conditions and ischemic stress. Therefore, we propose that the immunogenic capacity of optic nerve head astrocytes may be activated in glaucomatous eyes secondary to elevated serum cytokines and/or ischemia, and optic nerve head astrocytes may contribute to immunoregulatory events participating in the neurodegeneration process in glaucoma.

Macrophages, B lymphocytes, and dendritic cells express MHC Class II molecules and function as “professional” antigen-presenting cells. One property of T lymphocytes, which comprise the fundamental basis of cellular immune recognition, is that they recognize antigens in the form of small peptides that are tightly bound to MHC molecules expressed on the surface of the antigen-presenting cells.29 In doing so, the MHC complex provides evidence that optic nerve head astrocytes are immunogenically activated in glaucoma. We also found that serum levels of IL-10 were higher in patients with glaucoma compared to age-matched normal subjects and wondered whether this might affect the immunogenic capacity of optic nerve head astrocytes in glaucomatous eyes. A hallmark of cytokines is their ability to alter the behavior of cells with immunogenic capacity. It has been demonstrated that cytokines such as IFN-\(\gamma\) can induce HLA-DR expression in brain astrocytes.25–27 Interleukin-10, a multifunctional cytokine produced by B cells, stimulated macrophages, and subsets of CD4+ T cells, has immunostimulatory and immunosuppressive properties depending on the target cell type.28 We therefore sought to determine whether HLA-DR expression of optic nerve head astrocytes could be altered by exposure to cytokines, in vitro. Our studies revealed that the expression of HLA-DR increases in optic nerve head astrocytes exposed to selected cytokines, IFN-\(\gamma\) and IL-10, under normal conditions and ischemic stress. Therefore, we propose that the immunogenic capacity of optic nerve head astrocytes may be activated in glaucomatous eyes secondary to elevated serum cytokines and/or ischemia, and optic nerve head astrocytes may contribute to immunoregulatory events participating in the neurodegeneration process in glaucoma.

Immunocytochemistry similarly revealed that HLA-DR expression was undetectable in lamina cribrosa astrocytes under normal condition (Fig. 5A). However, simulated ischemia induced the expression of HLA-DR in these cells (Fig. 5B). Although there was an increase in the expression of HLA-DR by IL-10 in astrocytes incubated under ischemia compared to normal condition (Fig. 5C and D), this was not as prominent as that detected after treatment with IFN-\(\gamma\). In the presence of IFN-\(\gamma\), the percentage of HLA-DR expressing cells as well as the level of HLA-DR expression increased prominently in lamina cribrosa astrocytes incubated either under control or simulated ischemia conditions (Fig. 5E through 5H).

To determine whether the induction of HLA-DR expression by cytokines was dependent on cell cycle, we examined the effect of IFN-\(\gamma\) and IL-10 on cell cycle of astrocytes by flow cytometry. However, there was no effect of IFN-\(\gamma\) or IL-10 on cell cycle of astrocytes incubated either under normal or ischemic conditions (Table 3). This result suggests that the increased induction of HLA-DR expression in astrocytes we observed was independent from the cell cycle.

**DISCUSSION**

Using immunohistochemistry, we observed an upregulation of the HLA-DR expression in optic nerve head astrocytes in postmortem glaucomatous eyes. While the expression of HLA-DR was not colocalized to astroglial cells in normal eyes, some astrocytes exhibited positive immunolabeling for both HLA-DR and GFAP in the glaucomatous optic nerve head. This observation provides evidence that optic nerve head astrocytes are immunogenically activated in glaucoma. We also found that serum levels of IL-10 were higher in patients with glaucoma compared to age-matched normal subjects and wondered whether this might affect the immunogenic capacity of optic nerve head astrocytes in glaucomatous eyes. A hallmark of cytokines is their ability to alter the behavior of cells with immunogenic capacity. It has been demonstrated that cytokines such as IFN-\(\gamma\) can induce HLA-DR expression in brain astrocytes.25–27 Interleukin-10, a multifunctional cytokine produced by B cells, stimulated macrophages, and subsets of CD4+ T cells, has immunostimulatory and immunosuppressive properties depending on the target cell type.28 We therefore sought to determine whether HLA-DR expression of optic nerve head astrocytes could be altered by exposure to cytokines, in vitro. Our studies revealed that the expression of HLA-DR increases in optic nerve head astrocytes exposed to selected cytokines, IFN-\(\gamma\) and IL-10, under normal conditions and ischemic stress. Therefore, we propose that the immunogenic capacity of optic nerve head astrocytes may be activated in glaucomatous eyes secondary to elevated serum cytokines and/or ischemia, and optic nerve head astrocytes may contribute to immunoregulatory events participating in the neurodegeneration process in glaucoma.

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Using immunohistochemistry, we observed an upregulation of the HLA-DR expression in optic nerve head astrocytes in postmortem glaucomatous eyes. While the expression of HLA-DR was not colocalized to astroglial cells in normal eyes, some astrocytes exhibited positive immunolabeling for both HLA-DR and GFAP in the glaucomatous optic nerve head. This observation provides evidence that optic nerve head astrocytes are immunogenically activated in glaucoma. We also found that serum levels of IL-10 were higher in patients with glaucoma compared to age-matched normal subjects and wondered whether this might affect the immunogenic capacity of optic nerve head astrocytes in glaucomatous eyes. A hallmark of cytokines is their ability to alter the behavior of cells with immunogenic capacity. It has been demonstrated that cytokines such as IFN-\(\gamma\) can induce HLA-DR expression in brain astrocytes.25–27 Interleukin-10, a multifunctional cytokine produced by B cells, stimulated macrophages, and subsets of CD4+ T cells, has immunostimulatory and immunosuppressive properties depending on the target cell type.28 We therefore sought to determine whether HLA-DR expression of optic nerve head astrocytes could be altered by exposure to cytokines, in vitro. Our studies revealed that the expression of HLA-DR increases in optic nerve head astrocytes exposed to selected cytokines, IFN-\(\gamma\) and IL-10, under normal conditions and ischemic stress. Therefore, we propose that the immunogenic capacity of optic nerve head astrocytes may be activated in glaucomatous eyes secondary to elevated serum cytokines and/or ischemia, and optic nerve head astrocytes may contribute to immunoregulatory events participating in the neurodegeneration process in glaucoma.
The lamina cribrosa of the optic nerve head is a specialized connective tissue structure, which provides mechanical and functional support for axons of the retinal ganglion cells as they exit the eye. Damage to nerve bundles in this region satisfactorily accounts for the clinical pattern of the glaucomatous visual field loss. Therefore, the optic nerve head is traditionally considered the sight of glaucomatous injury. However, pathogenic mechanisms that demonstrate convincingly the precise mechanism whereby nerve fiber bundles meet their demise at this location are lacking. There is ample evidence that other immunoregulatory events occur simultaneously in the glaucomatous optic nerve head. We propose that regulation of the immunogenic ability of optic nerve head astrocytes to express HLA-DR in response to cytokines and/or ischemia may play a key role in neuronal damage in glaucoma.

We used IFN-γ and IL-10 to induce HLA-DR expression in optic nerve head astrocytes with or without exposure to simulated ischemia. We observed that IFN-γ induced strong HLA-DR expression in lamina cribrosa astrocytes under either normal conditions or ischemic stress. In addition, we observed that simulated ischemia induced an approximate twofold increase in the expression of HLA-DR in lamina cribrosa astrocytes that was further heightened (approximately fivefold) in the presence of IL-10. In contrast, simulated ischemia alone, or IL-10 in the presence of either control conditions or ischemic stress, failed to induce HLA-DR expression in fetal brain astrocytes. These findings indicate that although the expression of HLA-DR is relatively limited to cells of the immune system, stress created by exposure to cytokines or simulated ischemia activate the immunogenic capacity of the lamina cribrosa astrocytes, leading to their function as antigen-presenting cells. We found that serum titers of IL-10 in glaucoma patients were highest in the patients with normal pressure glaucoma. These findings suggest that activation of optic nerve head astrocytes and induction of HLA-DR expression can occur independently from elevated intraocular pressure, which is the most common risk factor associated with glaucoma.

Despite the immune privileged status of the central nervous system, immune responses can occur in this compartment and are implicated in several autoimmune diseases in which endogenous cells of the central nervous system, including glial cells, are thought to initiate, regulate, and sustain an immune response. Here, we demonstrate that astroglial cells of the optic nerve head have the potential to modulate immune responses as evidenced by their increased surface expression of HLA-DR, which is evident in eyes with glaucomatous optic neuropathy. Our findings suggest that activation of lamina cribrosa astrocytes (and perhaps of the retinal glial cells) may initiate an immunogenic cascade characterized by the production of cytokines, antigen presentation, and activation of neuronal cytotoxicity. We conclude that the immunoregulation of astrocytes in the glaucomatous optic nerve head, in addition to increased expression, antigenity or exposure of retinal proteins in response to tissue stress and/or damage, may stimulate

### TABLE 3. Examination of Cell Cycle in Lamina Cribrosa Astrocytes

<table>
<thead>
<tr>
<th>Conditions</th>
<th>G1</th>
<th>S</th>
<th>G2/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>87.19±5.0</td>
<td>1.65±0.5</td>
<td>5.14±0.5</td>
</tr>
<tr>
<td>Simulated ischemia</td>
<td>86.42±2.0</td>
<td>1.46±0.5</td>
<td>6.15±1.3</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>86.05±4.5</td>
<td>1.76±0.9</td>
<td>5.50±0.9</td>
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<tr>
<td>IL-10</td>
<td>86.76±3.8</td>
<td>1.24±0.4</td>
<td>4.31±0.4</td>
</tr>
<tr>
<td>Simulated ischemia + IFN-γ</td>
<td>86.24±4.6</td>
<td>1.14±0.2</td>
<td>5.62±0.9</td>
</tr>
<tr>
<td>Simulated ischemia + IL-10</td>
<td>87.44±3.0</td>
<td>1.05±0.2</td>
<td>5.78±1.8</td>
</tr>
</tbody>
</table>
an immune-mediated process and thereby contribute to the initiation and/or progression of glaucomatous neurodegeneration.

References