The induced expression of HLA antigens on a variety of tissues involved in autoimmune diseases has been reported by several groups. In this study, we have examined the expression of HLA antigens in iris biopsy specimens from patients with anterior uveitis and compared them to patients with non-inflammatory senile cataracts. In addition, we measured the levels of gamma interferon in the aqueous humor of the same subjects. Our results show that there was induced expression of class I and class II HLA antigens in iris biopsies from patients with anterior uveitis and that the level of induced expression correlated with the concentration of gamma interferon found in the aqueous humor. These results suggest that induced expression of HLA antigens on iris cells may play a role in the pathogenesis of anterior uveitis and that gamma interferon may be one of the mediators for this induced expression.

The cellular expression of HLA antigens is central to immune responsiveness and has thus been studied in a variety of tissues involved in autoimmune disorders. Thyroid epithelial cells, for example, which do not normally express class II HLA antigens, express these antigens in thyroid specimens from patients with Hashimoto's thyroiditis. Other examples include the induced expression of class II antigens on pancreatic islet beta cells in patients with insulin dependent diabetes mellitus, on retinal pigment epithelial cells in patients with sympathetic ophthalmia and on salivary gland epithelial cells in patients with Sjögren's syndrome. Induced expression of class I HLA antigens has been reported in skeletal muscle cells from patients with Duchenne muscular dystrophy. These cells do not normally express class I HLA antigens. It has been hypothesized that induced expression of class II, and probably also class I antigens in some tissues, contributes to the immunopathogenesis of certain autoimmune diseases involving these tissues.

We have previously reported that class I and class II HLA antigens are not normally expressed on human uveal cells, with the exception of vascular endothelium. We have shown that these antigens were inducible in vitro by a variety of lymphokines, especially gamma interferon (submitted for publication).

Based on these results, we speculated that autoimmune responses may not be possible in the eye without the prior induction of HLA antigens on ocular cells. To further address this issue, we have examined the expression of HLA antigens on iris biopsies obtained from patients with anterior uveitis (AU) and from patients with uncomplicated, senile cataracts. Additionally, we obtained samples of aqueous humor from these patients and quantitated their concentration of gamma interferon.

Iris biopsies from patients with AU expressed class I and class II HLA antigens, although those from patients with senile cataracts did not. Furthermore, the level of expression of HLA antigens correlated with the concentration of gamma interferon in the aqueous humor of the patients with AU.

Materials and Methods

Iris Biopsies

After obtaining informed consent to examine iris biopsies and samples of aqueous humor in these experiments, ten iris biopsies were obtained at lens replacement surgery. Five (two females) were from patients with chronic anterior uveitis (age; 59.4 ± 10.5 years, range 45–70 years), the other five (two females), from patients with noninflammatory senile cataracts (age; 64.8 ± 10.3 years, range 48–73 years). Patients with AU were receiving topical steroid therapy at the time of surgery, while the other patients were not on any medication. Iris biopsies were placed in embedding fluid and transported without delay to the laboratory (maximum time of delay was 2 hr). They were then snap-frozen in liquid nitrogen and
sectioned on a cryotome, following which they were fixed in acetone and stored at −20°C until stained.

**Aqueous Humor**

Samples of aqueous humor (100–200 µl) were obtained from the same subjects donating iris biopsies. These were transported to the laboratory without delay and diluted to 600 µl in sterile phosphate-buffered saline. Aqueous humor samples were stored frozen at −70°C until assayed for gamma interferon concentration.

**Staining of Iris Biopsies**

All biopsies were stained within 1 month of sectioning by an indirect immunoperoxidase technique, as previously described. Briefly, the first layer was a monoclonal antibody, followed by a peroxidase-conjugated antiserum. Color development was produced using the Harker-Yates reagent. To differentiate the brown pigment of uveal cell melanin from the brown color reaction product of peroxidase, the sections were counterstained with Azure A. This reagent stains melanin green and imparts a light blue color to nuclei. The monoclonal antibodies used were: ASH 620 (class I monomorphic, H. Vaughan, personal communication), W6/32 (class I monomorphic), ASH 622 (class II monomorphic), OKM2, OKB1 and OKT11 (monocyte, B cell and T cell specific monoclonal antibodies, respectively, purchased from Orthoclone, Raritan, NJ). Sections with the first, second or both antibodies omitted were included in all staining experiments as background controls.

Two independent observers examined sections from all iris biopsies of patients with AU and ranked the intensity of staining in the following manner. Sections were labeled as negative when no staining was seen except for blood vessels and/or lymphoid cells. In sections where iris stromal cells were positively stained for HLA antigens, they were ranked as low, moderate or high intensity staining. This ranking was relative to the staining intensity of other biopsy specimens.

**Gamma Interferon Assay**

Samples of aqueous humor were assayed in duplicate using a commercially available radioimmunoassay specific for biologically active gamma interferon (Centocor Corp., Malvern, PA). The results of duplicate samples were always within 10% of each other and the mean of the duplicates was used in all calculations. In patients with chronic AU, a mean level of 11.5 ± 7.7 U/ml (mean ± SD) with a range of 4.0–21.6 U/ml was found in the aqueous humor. A mean level of gamma interferon of 1.1 ± 0.4 U/ml (mean ± SD) with a range of 0.6–1.6 U/ml was found in aqueous humor samples from patients with senile cataracts. The difference between these groups was statistically significant by the nonparametric Mann-Whitney U-test (U = 0, P = 0.002).

**Aqueous Humor Gamma Interferon Levels and Intensity of HLA Staining in Corresponding Iris Biopsy**

Table 1 combines the results of staining for HLA antigens in the group of five patients with AU, with their respective aqueous humor gamma interferon levels. There was excellent correlation between the intensity of staining for class I and class II HLA antigens and the level of aqueous humor gamma interferon.

**Discussion**

We have previously shown that normal postmortem human iris cells, with the exception of vascular endothelium, do not express class I or class II HLA antigens. These results were further confirmed in this study using iris biopsy specimens, taken at surgery, from patients with senile cataracts. We have also previously reported that class I HLA antigens with senile cataract. As can be seen, the blood vessel endothelium stained positively for class I antigens, but iris stromal cells did not. Staining for class II antigens was consistently negative both in the stromal cells and the vascular endothelium (not shown).

Figure 2 shows the staining results for class I antigens of an iris biopsy taken from a patient with chronic AU. As can be seen, the vascular endothelium, as well as the iris stromal cells, stained positively for class I HLA antigens. Similarly, class II positive iris cells can be seen in Figure 3. Overall, all five iris biopsies taken from patients with AU showed class I-positive iris stromal cells. Additionally, three of the five biopsies from patients with AU showed class II positive iris stromal cells (Table 1). In all iris sections from patients with AU, there was a large number of monocytes and a small number of T lymphocytes. These cells were always positive for class I antigens and were easily distinguished from iris cells morphologically (see Fig. 2).
can be induced in vitro on cultured uveal cells by a variety of lymphokines, especially gamma interferon. Additionally, gamma interferon was the only lymphokine capable of inducing the expression of class II antigens on cultured uveal cells (submitted for publication). These results suggested that HLA antigens may be induced on ocular cells during the development of immune or autoimmune responses in the eye.

In the current study we found that class I and class II HLA antigens were expressed on iris cells from patients with AU. These findings confirm that HLA antigens are induced in the course of development of autoimmune responses in the eye. Additionally, aqueous humor gamma interferon levels were significantly elevated in patients with AU, and paralleled the level of induction of class I and class II HLA antigens in the respective iris biopsies.
The possible involvement of gamma interferon in AU was suggested by our previous observation that the sera of patients with active AU had higher levels of neopterin than those of patients with inactive disease or control subjects. Neopterin is a pteridine derivative whose synthesis is induced by gamma interferon. In localized immune responses, neopterin can be found to be elevated in serum, without evidence of elevated gamma interferon levels. This is thought to be due to the longer half-life of neopterin and its ability to better penetrate tissue barriers. This latter finding thus suggested a localized elevated level of gamma interferon leading to elevation of neopterin levels systemically. The results of this study indicate that the anterior chamber is the probable site of gamma interferon elevation in subjects with AU.

The significance of our finding of induced HLA antigen expression on iris cells in patients with AU is not entirely clear. One of the most important clues to the pathogenesis of AU is its link to HLA B27. Clearly, expression of this antigen, or other class I or class II HLA antigens on iris cells may be important in the pathogenesis of an autoimmune response in the eye. Our observations do not indicate whether HLA antigen expression in the eye precedes an autoimmune inflammatory response, or is consequent upon it. It is not possible to answer this question in the human eye, although similar observations in other organs suggest that the induced expression of HLA antigens may precede the inflammatory response. In the pancreas of subjects with insulin-dependent diabetes mellitus, for example, class II HLA antigen expression is found to be induced exclusively on beta cells in islets not yet involved in the inflammatory process. This may indicate that class II expression may be related causally to such an autoimmune process. In the rat model of generalized uveitis, induced by retinal S-antigen, class II MHC antigens were found to be induced on the retinal pigment epithelial cells 4 days prior to the development of macroscopic or microscopic signs of uveitis.

These observations suggest that the induced expression of HLA antigens in the iris may precede the development of the inflammatory response in AU and that this response may be related to the presence of lymphokines, such as gamma interferon, in the anterior chamber.

Table 1. Intensity of HLA staining and corresponding aqueous humor gamma interferon levels

<table>
<thead>
<tr>
<th>Iris donor</th>
<th>Aqueous humor gamma interferon</th>
<th>Staining intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Class I</td>
</tr>
<tr>
<td>1</td>
<td>21.6</td>
<td>High</td>
</tr>
<tr>
<td>2</td>
<td>17.0</td>
<td>Moderate</td>
</tr>
<tr>
<td>3</td>
<td>10.0</td>
<td>Moderate</td>
</tr>
<tr>
<td>4</td>
<td>4.8</td>
<td>Low</td>
</tr>
<tr>
<td>5</td>
<td>4.0</td>
<td>Low</td>
</tr>
</tbody>
</table>

The concentration of gamma interferon (U/ml) found in the aqueous humor of the five patients with AU is shown in column 2. Columns 3 and 4 show the intensity of staining for class I and class II HLA antigens in the corresponding iris biopsy of these patients. A correlation between staining intensity and aqueous humor gamma interferon levels can be seen.
Key words: HLA antigens, anterior uveitis, autoimmunity, gamma interferon, senile cataract

References


