The Distribution and Ontogeny of MHC Antigens in Murine Ocular Tissue

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The distribution of H-2 (Class I) and Ia (Class II) antigens in the mouse eye was determined with the use of monoclonal antibodies and found to be different. H-2 antigen was observed in the corneal epithelium, conjunctival epithelium, choroid, and inner and outer nuclear layer of the retina—in order of decreased intensity of staining. In contrast, Ia was detected most strongly, in a patchy distribution, in the choroid, limbus epithelium, and peripheral corneal stroma, followed by the iris and ciliary body. Thus, distinctively different patterns of distribution of H-2 and Ia were observed in the normal mouse eye. Surprisingly, adult eyes stained more intensely for both H-2 and Ia antigens than neonatal eyes, implying that the expression of MHC antigens varies with ontogeny. Since unique and important immunologic functions have been ascribed to class I and II antigens, their different distribution within the eye may indicate that various ocular structures can play distinctive roles in the immune response.


Materials and Methods

Preparation of Purified Monoclonal Antibodies

Hybridoma cell lines which produce monoclonal antibodies against either H-2 (all haplotypes, ATCC No: TIB 126, Rockville, Maryland)15 or Ia (I-A^d^k, ATCC No: TIB 120) antigens were cultured in Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO) containing 5% fetal bovine serum (FBS, Sterile Systems, Inc., Logan, UT), penicillin-G (100 unit/ml, GIBCO, Grand Island, NY) and streptomycin (100 μg/ml, GIBCO) in a humidified incubator filled with 5% CO₂ and 95% air. The supernatant of the confluent culture was pooled. Antibody from 800 ml of the pooled supernatant was precipitated by the addition of an equal volume of 100% saturated (NH₄)₂SO₄, pH 7.0, for 1 hr at 4°C. The precipitate was collected by centrifugation and concentrated by dialysis overnight against two changes of 2 l 0.04 M phosphate buffer, containing 0.03 M NaCl, pH 8.0. It was cleared of any precipitate by centrifugation and concentrated by dialysis.
Amicon ultrafiltration with a pM-10 membrane (Amicon Co., Danvers, MA) to 20 ml. The concentrated solution was passed through a DEAE-Cellulose (Sigma, St. Louis, MO) column (2.6 × 20 cm). The effluent fraction with highest protein concentration was collected, pooled and concentrated again with Amicon ultrafiltration. A control was obtained by purifying the pooled supernatant from the p3 × 63 cell line as detailed above.

Preparation of Frozen Sections For Immunohistology

Mice were used in accordance with the ARVO Resolution on the Use of Animals in Research. Balb/C mice of three different age groups, ie, adult (>6 weeks old), young (14–18 days) and neonatal (<7 days) were sacrificed with metofane (Pitman-Moore, Inc., NJ). Eyes were enucleated and oriented on a thin square cork board so that the cornea and retina would be obtained on a single section. The eye was covered completely with OCT compound (Miles Scientific, Naperville, IL) and snap-frozen in an isopentane-dry ice bath. Four µ frozen sections were cut at −21°C using a cryostat (Tissue-Tek II, Miles Scientific) and placed on a chrome alum precoated slide. They were then quickly fixed by dipping into cold acetone.

Staining of Eye Sections With Monoclonal Antibodies Against H-2 or Ia Antigens

The vectastain ABC (Avidin-Biotin Complex) immunoperoxidase staining procedure (Vector Lab, Burlingame, CA) was followed with some modification. Four µ frozen sections from mouse eyes were fixed in acetone for 10 min. After washing for 15 min in 0.05 M Tris-saline, pH 7.6, the sections were incubated for 20 min with 1:5 diluted normal rabbit serum (NRS) in Tris-saline containing BGEN (bovine serum albumin [3%], gelatin (0.25%), EDTA [5 mM] and NP-40 [0.025%]). Excess serum was blotted from the sections, and 50 µl of a 1:5 dilution of purified rat monoclonal antibody against either the mouse H-2 or Ia antigen (in Tris-saline-BGEN) was added and incubated overnight at 4°C in a moisture chamber. The sections were washed 3 times for 10 min in Tris-saline, and incubated for 30 min with biotinylated rabbit anti-rat IgG, diluted with a mouse skin extract, which was prepared as follows: 1 ml of 0.5 M Tris, pH 7.6 and 5.7 ml of distilled water were mixed with 3.3 ml of the supernatant of a mouse skin extract obtained from shaking minced mouse skin overnight at 4°C with 10 ml of 0.45 M NaCl solution. The slides were again washed 2 times for 8 min with Tris-saline. They were incubated for 10 min in 3% H2O2 in methanol and washed 2 times for 10 min with Tris-saline. The sections were then incubated for 30 min with vectastains ABC reagent (Avidin biotinylated peroxidase complex, Vector Lab., Burlingame, CA). They were washed for 40 min in Tris-saline, dipped in 0.1 M acetate buffer, pH 5.2 for 1 min, and incubated for 5 to 10 min in AEC (3-amino-9-ethylcarbazole, Sigma) solution, which was made from 3 ml of AEC (4 mg/ml) in N,N-dimethyformamide, 42 ml of acetate buffer and 60 µl of 30% H2O2. They were then washed in tap water for 5 min, counterstained with Mayer's hematoxylin for 4 min, and quickly dipped in 1% ammonia water. After 10 min of washing under tap water they were covered with a glass coverslip, using a drop of warm glycercine jelly, containing 8% gelatin and 50% glycercine.

Results

Immunohistochemical staining of adult mouse eyes demonstrated that H-2 (Figs. 1a and 3a) and Ia (Figs. 2a and 4a) antigens were distributed differently. Strong staining for H-2 antigen was observed in the conjunctival epithelium, corneal epithelium and choroid; moderate staining in the ciliary body and iris; weak staining in the outer nuclear layer, inner nuclear layer and ganglion cell layer of the retina; and no staining in the corneal endothelium and stroma, the retinal pigment epithelium and photoreceptor layer (Figs. 1, 3, 5). In contrast, Ia antigen was detected most strongly, but in a patchy distribution, in the choroid, conjunctival epithelium and peripheral...
Cornea, Limbus and Conjunctiva

The corneal epithelium stained intensely with the monoclonal antibody to H-2 (Figs. 1, 5a). Within the corneal epithelium the most superficial layer stained lightest and the epithelial cell plasma membrane was most intensively stained. Since the inside of the cell remained clear, the contour of the cells was easily observed. The junctions between cells, as well as that between the cell plasma membranes and Bowman's membrane stained prominently. No difference was noted between the central and peripheral portions of the corneal epithelium. No staining was noted in the corneal stroma or endothelium.

In contrast to anti-H-2, the monoclonal antibody to la produced a very weak and even pattern of staining in the corneal epithelium (Fig. 2a); no staining of the central corneal stroma or endothelium was observed. In the peripheral corneal stroma, as well as the limbus, a spot-like staining pattern was frequently noted (Fig. 6c). This was most prominent in the anterior rather than posterior stroma. It was frequently associated with a few spots of heavy staining for la at the basal layer of the epithelium, near the limbus.

Iris and Ciliary Body

As demonstrated in Figures 5d and e, patchy staining for H-2 was observed in the iris and ciliary body. It appeared most intense in the inner rather than the outer cell layers of the ciliary body. The pattern of la staining was somewhat different. Patchy, but rather strong staining was observed in both the ciliary body and iris (Fig. 6e, d). Unfortunately, the poor resolution of histologic details in our frozen sections did not allow us to more precisely identify the cells being stained.

Choroid

As shown in Figure 5c, H-2 antigen was abundantly present in the choroid, especially in the vicinity of Bruch's membrane. Frequently, intense staining was noted in the endothelium of the blood vessels. A strong patchy pattern of staining was observed with the monoclonal antibody to la (Fig. 6a). Most of the staining appeared in the plasma membrane of cells scattered throughout the choroid, and not near large blood vessels.

Retina

A weak uniform pattern of staining for H-2 was observed in the outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer and retina ganglion cell layer (not shown). In general, the nuclear layers stained less intensely than the plexiform layers. The photoreceptor layer did not stain at
Comparison of MHC Expression by the Corneal Epithelium and Choroid of Different Age Groups

Corneal epithelium: The staining characteristics of the corneal epithelium was studied in three different age groups—the adult (>6 weeks), the young mouse (14–18 days) and the neonate (<7 days). As seen in Figure 1a, b and c, the corneal epithelium stained in each of these age groups. However, it was noted that the intensity of staining increased with the age of the mouse and paralleled the increase in thickness of the corneal epithelium. There were only two cell layers in the neonatal corneal epithelium, whereas the young and adult mouse had many more. The intensity of H-2 staining was weak, but even, in the neonate; moderate in the outer layer, and weak in the basal layer, of the young; strong throughout all layers of the adult.

Only in the adult corneal epithelium was weakly positive staining for Ia observed (Fig. 2a). In neither the neonate nor young mouse was Ia detected (Fig. 2b).

Choroid: A strong belt-like staining pattern was observed in the choroid of the adult mouse with the monoclonal antibody to the H-2 antigen (Fig. 3a). This was not observed in either the neonate or young mouse. Instead, a patchy pattern of distribution was observed in the young mouse (Fig. 3b) and no staining in the neonate (Fig. 3c). In contrast, although a strong, patchy pattern of staining for Ia was noted throughout the choroid in the adult mouse (Fig. 4a), no detectable Ia was present in either the young mouse or the neonate (Fig. 4b and c).

Discussion

We studied the tissue distribution and ontogeny of both H-2 (Class I) and Ia (Class II) antigens in normal mouse eyes immunohistologically, using the ABC immunoperoxidase method. The staining patterns of our antigen-specific monoclonal antibodies were initially confounded by both antigen non-specific absorption of our second antibody and the presence of endogenous peroxidase. We found that biotinylated rabbit anti-rat IgG (the second antibody), and not peroxidase, nonspecifically bound to the mouse corneal stroma and sclera, specifically to the collagenous extracellular matrix. By treating the biotinylated rabbit anti-rat IgG with neutral salt-soluble collagen extract from mouse skin, we were able to eliminate this non-specific staining. Endogenous per-
oxidase was removed from mouse eye sections by treatment with 3% hydrogen peroxide in methanol. This also decolorized the red blood cells in vessels, which simplified interpretation of our sections since the AEC substrate can produce a color similar to red blood cells.

As a control for each monoclonal antibody, we either deleted the primary antibody or replaced it with a purified fraction of the p3 X 63 cell culture supernatant. No staining was observed with either control, indicating that the staining patterns demonstrated in our tissue sections were specific for the monoclonal antibodies employed. In the adult mouse eye the corneal epithelium, conjunctiva and limbus stained most intensely for H-2. The junction between epithelial cells was strongly stained, suggesting that the H-2 antigen is membrane-bound. These results are consistent with previously published studies using indirect immunofluorescence in the human cornea. However, in contrast to the human eye, where it appears that the expression of HLA-ABC is greater in the peripheral than central corneal epithelium, we observed no difference. Staining was also not observed in the corneal stroma or endothelium, implying that under normal conditions these tissues expressed minimal or no H-2 antigen, although in vitro culture of human corneal stromal and endothelial cells can result in the expression of Class I MHC antigens.

Adult mouse corneal epithelium stained lightly, but evenly, with our antibody to la. A previous study of human corneal epithelium did not demonstrate the presence of HLA-DR. Since Class II MHC antigens have been detected in epithelial cells from other organs—salivary gland, gut, lactating mammary gland and renal tubules—it is not unreasonable to expect a small amount of Class II antigen in the corneal epithelium. The positive spot-like staining we noted in the epithelium of the limbus may be associated with Langerhan's cells, which have been documented in this location. However, we observed spot-like deposits of la in the peripheral corneal stroma. These have not been previously reported and we do not know with what cell type it is associated. Although there is no in situ expression of la on corneal endothelial cells it does appear that they have the potential to express la during an inflammatory reaction.

Class I MHC antigens were only weakly expressed in the iris and ciliary body of the mouse eye. However, scattered throughout these structures were full size cells that expressed la intensely. It is not known what types of cells express this Class II MHC antigen or if they play an important part in immune response. Additional immunohistologic and functional studies are in progress to further clarify these cell populations.

Both H-2 and la are expressed strongly in the choroid of the normal adult mouse eye. The patchy distribution of la positive cells, which appeared throughout the choroid, may identify a cell similar to the Langerhan's cell which is involved in antigen processing. Preliminary observations in a model of HSV-induced keratouveitis in the mouse suggests that the patchy expression of la within the choroid increases during an active immune response (Wang and Kaplan, submitted for publication).

In contrast to the choroid, the retina did not demonstrate la and only weakly expressed H-2. This is consistent with previous observations that the central nervous system only expresses MHC antigens weakly, if at all. Nevertheless, central nervous system astrocytes, oligodendrocytes, microglia and other neurons can be induced to express H-2 and la after exposure to gamma interferon. Likewise, the RPE in the eye can be induced to express la in experimental EAU and retinitis pigmentosa. Since it has recently been shown that la positive astrocytes and vas-
cular endothelial cells within the central nervous system can serve as antigen presenting cells, it is not unreasonable to postulate a similar role for these cells within the retina.\textsuperscript{35}

Since the MHC antigens were expressed most strongly in the corneal epithelium and choroid, these tissues were chosen to study the effects of age on MHC antigen expression. In general, as the mouse became more mature the quantity of expressed Class I and II antigens increased. The neonatal mouse eye expressed H-2 very weakly and la not at all. These observations are consistent with other reports that indicate: (1) a defect in antigen presenting cell function in neonatal mice\textsuperscript{39}, (2) an increase in the number of Langerhan's cells in the limbal and conjunctival epithelium of the eye with maturity\textsuperscript{40}, and (3) the detection of HLA-ABC and Dr antigens in the human fetus only after 8 weeks of gestation.\textsuperscript{41}

With the development of better immunohistologic techniques the cell populations stained for Class I and II MHC antigens will be more specifically defined. Furthermore, the variable expression of these antigens during the immune response may signal a possible role for these cells in either the induction or expression of immunity.

**Key words:** MHC antigens, ontogeny, monoclonal antibodies, immunoperoxidase method, murine ocular tissue

### References


