Monoclonal Antibodies to Human Amnion Recognize Different Components of the Rabbit Eye

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The epithelium of the eye originates from embryonic ectoderm, whereas the amnion is an extra-embryonic membrane that bears close relationship with many ectodermal tissues. Shared antigens have been identified between human amnion and cornea using rabbit antisera to human amnion. Three monoclonal antibodies to human amnion, GB4, GB9, and GB11 were studied by immunofluorescence on the anterior segment of the rabbit eye. GB4 recognized the epithelium of the conjunctiva and the subcapsular epithelium of the lens. GB9 reacted only with the central four fifths of the corneal epithelium; the peripheral epithelium near the limbus was not reactive. GB11 detected the pigmented epithelial cells in ciliary processes.

Human amnion is an extra-embryonic ectoderm that lines the amniotic cavity, wraps around the umbilical cord, and continues with the fetal skin. Many antigenic similarities have been demonstrated between amnion and various ectoderm-derived tissues. The eye is essentially an ectodermal structure; only the stromas of the cornea, iris, and ciliary body are of mesoderm and neural crest ectoderm origin. Several groups of amnion antigens that have been characterized by rabbit antisera to amnion showed cross-reactivity with human cornea.

Recently, eight monoclonal antibodies to human amnion have been developed. Three of these antibodies, GB4, GB9, and GB11, showed interesting reactivities to the rabbit cornea, conjunctiva, ciliary processes, and lens. GB4 recognized cytoplasmic structures in the amniotic epithelium; GB9 reacted with amniotic epithelium and sub-epithelial connective tissue; and GB11 detected the supra-basal epithelium of the skin. Each of these antibodies recognized defined structures in the anterior segment of the rabbit eye.

Materials and Methods. Tissues: Eye specimens were obtained from five New Zealand Albino and three Fauve de Bourgogne rabbits. After enucleation, a circumlimbal incision was made (2 mm beyond the limbus, along the conjunctiva), the whole lens was extracted with the anterior segment of the eye. The aqueous humor and vitreous body were removed by a filter paper. The eye specimens were then placed between two slices of rabbit liver that were used as supporting tissues. Rabbit skin, lung, trachea, kidney, stomach, esophagus, intestine, eye lid, and lip were also collected. The specimens were frozen in liquid nitrogen and stored at −20°C. Studies using these animals were performed in conformance with the ARVO Resolution on the Use of Animals in Research.

Antibodies: Three mouse monoclonal antibodies to human amnion GB4, GB9, and GB11 were used for this study. The production and their reactivities to human amnion and other extra-embryonic tissues were detailed by Hsi and Yeh. Undiluted supernatant fluids of the hybridoma cell lines were used. The culture medium of P3-NS1-Ag4-1 (NS-1) cell line was used as a control. Fluorescein isothiocyanate (FITC) conjugated rabbit anti-mouse immunoglobulin (Ig) were purchased from Dakopatts (Copenhagen, Denmark).

Immunofluorescence: Frozen sections (5 μm) were prepared by using a cryostat (Bright Instrument; Huntingdon, England) and air-dried at room temperature. Sections were pre-washed for 10 min in 0.15 M phosphate buffered saline (PBS), pH 7.2, then incubated with 50 μl of the supernatant fluid for 2 hr. After being washed in PBS for 5 min, sections were reacted with a 1:40 dilution of 50 μl FITC conjugated rabbit anti-mouse Ig. Some of the sections were counterstained with propidium iodide. After the last incubation, the sections were washed in PBS for 5 min, then mounted in AF1 mounting media (Citifluor Ltd., London). Sections were studied by epi-illumination with the use of a Zeiss Universal Microscope (Carl Zeiss, Inc.; Oberkochen, West Germany) fitted with HBO 50 mercury arc lamp, epifluorescence condenser III RS and filter sets for FITC and propidium iodide studies.

Results. GB4: GB4 reacted with the conjunctival epithelium. The reactivity began at the limbus where the stratified squamous epithelium of the cornea changed to the simple columnar epithelium of the conjunctiva (Fig. 1), continued to the inner surface of the eye lid, and disappeared where the epithelium changed to the stratified squamous epithelium of the skin. GB4 also reacted with the subcapsular epithelium of the lens (Fig. 2); the epithelial cells at the equatorial zone of the lens were nonreactive. Among the other rabbit tissues examined, the reactivities could be identified on the basal epithelium of the hair follicles and some of the sweat glands in skin, the epithelium of trachea, bronchi, and epithelium lining the calyces of the kidney. In some instances, the reactivity could also be identified on a few stromal cells in the sub-epithelial connective tissues of these specimens.
Fig. 1. The reactivity of GB4 on the rabbit conjunctiva (×150). The reactivity of GB4 can be identified in the epithelium of the conjunctiva. Note that the corneal epithelium (C) and the stromal tissues (S) were nonreactive.

Fig. 2. The reactivity of GB4 on the rabbit lens (×150). The reactivity of GB4 can be detected in the subcapsular epithelium of the lens (L). Some very weak reactivities can also be observed in the iris (I). The lens epithelium at the equatorial zone did not react with GB4 (not shown).

Fig. 3. The reactivity of GB9 on the rabbit cornea (×150). GB9 recognized the upper layers of corneal epithelium (C). The corneal epithelium at the limbus (Lb) did not react with this antibody.

Fig. 4. The reactivity of GB11 on the rabbit ciliary processes (×150). The epithelial cells situated on the basement membrane of the ciliary processes reacted with GB11. The upper nonpigmented epithelium was nonreactive. Some reactivities can also be found in a few cells in the stromal tissues.

GB9: GB9 reacted with the central four fifths of the corneal epithelium; its reactivity stopped abruptly near the limbus region (Fig. 3). No morphological difference could be discerned between the GB9-positive and GB9-negative corneal epithelium. In the middle of the cornea, the basal layer of the corneal epithelium reacted more weakly than the upper layers. None of the other rabbit tissues examined reacted with GB9.

GB11: GB11 reacted with the pigmented epithelial cells of ciliary processes (Fig. 4). The epithelial cells of the iris were nonreactive. The reactivity was more obviously observed in the albino rabbit eyes. Some cells in the stromal tissues of ciliary processes and iris also weakly reacted. No other rabbit tissues examined reacted with this antibody, except the skin; the reactivity of GB11 could be seen in the cytoplasm of the suprabasal epithelium of the skin which was similar to the pattern found in human epidermis.

Discussion. The three monoclonal antibodies described in this communication provided extremely specific epithelial markers for the anterior segment of the rabbit eye. GB4 reacted with the conjunctival epithelium and the subcapsular epithelium of the lens; GB9 recognized the corneal epithelium; and GB11 detected the pigmented epithelium of the ciliary processes. In clinical ophthalmology, inflammation and lesions...
of the anterior segment of the eye, such as acid and alkaline burns to the epithelium and stroma of the cornea, are frequent pathological cases. These conditions can be easily produced in animals, particularly in rabbits for experimental studies. These three monoclonal antibodies should provide valid means to follow the migration, proliferation, and differentiation of the epithelial cells during wound healing. In extra-capsular surgery of cataract, lens is removed with its epithelium. However, secondary cataract often occurs when the remaining epithelial cells at equatorial zone of the lens proliferate and eventually cover the posterior capsule.

The results of this study demonstrate that GB4 reacted with the subcapsular epithelium of the lens, but not with the cells located at the equatorial zone. Thus, GB4 will be particularly useful in studying the posterior capsule epithelialization during the formation of secondary cataract.

Although the biochemical nature and the function of the antigens recognized by GB4, GB9, and GB11 await further studies, these antibodies offer valuable tools for ophthalmologists to investigate the epithelial differentiation in the anterior segment of the rabbit eye.

**Key words:** monoclonal antibodies, conjunctiva, cornea, ciliary process, lens

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**References**


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**Tear Immunoglobulins Measured by ELISA**

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The authors applied an ELISA to measure IgG, IgA, and IgM concentration in tears from 20 normal subjects. This assay was more sensitive than any other previously reported technique to quantitate tear immunoglobulin. Only 2 μl of tears were required and concentrations as small as 1 ng/ml could be detected. IgM was present in all samples at a geometric mean level of 5.6 μg/ml. Mean IgA level was 186 μg/ml and mean IgG was 67 μg/ml. No correlation was found between tear and serum levels, suggesting that local synthesis was responsible for most of the tear immunoglobulin. This ELISA offers a sensitive and reliable method to analyze very small volumes of tears. It can be modified to test for many different antigens and antibodies. Invest Ophthalmol Vis Sci 27:622–625, 1986

Previous investigators have applied a variety of tear collection methods and immune assays to measure tear immunoglobulins. In addition to inherent variation between individual subjects, these studies have sometimes produced a wide range of immunoglobulin concentration. In part because of the lack of standardization, relative insensitivity of the assays, and differences in assay and collection techniques. The relatively large volumes of tears which most assays require has made research in this area difficult. We applied a modified ELISA immunoassay to study the concentration of immunoglobulins in normal tears. We found this technique could reliably measure immunoglobulins in smaller volumes of tears with greater sensitivity than has been previously reported using other techniques. The purpose of this study is to present our findings on immunoglobulin concentration in normal tears using a modified ELISA assay.

**Materials and Methods. Subjects:** Simultaneous tear and serum samples were collected from 20 subjects without eye disease (11 men and 9 women) ranging in age from 21 to 62 yr (mean 35 yr). None was taking any medications that could interfere with tear production. Serum was collected from an additional ten nor-