Uveal and other ocular tissue reactions to heterologous antiglens capsule antibodies

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The immune reactivities of heterologous antilens substance, and lens capsule antibodies to the host uveal and other ocular tissues were compared by means of the fluorescein conjugated antibody technique. Fluorescein conjugated antibovine lens capsule antibody reacted specifically with the lens capsule and the walls of the vascular tree in iris, ciliary body, retina, choroid, and optic nerve. Fluorescein conjugated antihomologous lens antibody showed specific staining in both the lens capsule and lens substance. The walls of the vascular tree were also stained specifically, but less than the staining obtained by the antilens capsule antibody. Fluorescein conjugated antibovine lens substance antibody reacted with the lens substance but not with the capsule or other eye structures. Eyes sections treated with fluorescein conjugated normal rabbit gamma globulin did not exhibit any specific stainings. The significance of the antigenicity of the lens capsule, its relationship to basement membrane structures, and its possible source as a stimulus to lens-induced uveitis are discussed.

Endophthalmitis phacoanaphylactica has been regarded to occur as a result of autoimmune origin.1-4 The disease is characterized by an allergic uveal inflammation following extracapsular cataract surgery. It has been postulated that lens protein (such as a-crystallin) might be the etiological factor (antigen) of the disease. In some experimental studies, a pathologic picture similar to endophthalmitis phacoanaphylactica has been produced in lens-sensitized animals. These experiments required immunization of the animals with lens substance followed by mechanical needling of the lens capsule to leak out lens substance into the anterior chamber.5-9 Another form of lens-induced uveal inflammation has been described in which the uveitis occurred in the unoperated second eye of patients who had previously undergone extracapsular cataract extraction in the other eye. This type of lens-induced uveitis has some similarity with sympathetic ophthalmia.5,6,7 An increased permeability of the lens capsule in individuals who are sensitized by previous cataract surgery has been recognized to be the responsible cause for this type of lens-induced uveitis.5,9

Since the antigenicity of the lens capsule...
and lens capsule polysaccharide-protein complex has been reported and the presence of glycoprotein in the lens capsule was demonstrated. \cite{1} We may consider the lens capsule also as a possible antigenic source of the lens-induced uveitis. Our present study is an attempt to compare the reactivity of heterologous antibovine whole lens, lens substance, and lens capsule antibodies to normal bovine uvea and other ocular tissues by using immunofluorescence techniques.

**Materials and methods**

*Preparations of antigens.* Fresh bovine whole lenses, lens substance, and lens capsules were obtained separately under sterile conditions. Each tissue was washed with sterile normal saline solution and ground in a sterile chilled mortar. Lens capsules were minced with sharp scissors before grinding and emulsified in a Virtis homogenizer. The ground tissues were suspended in sterile normal saline solution. The wet weight of each tissue suspended in 10 c.c. of saline solution is as follows: whole lens, 200 mg.; lens substance, 200 mg.; lens capsule, 115 mg. Methanol, 1:10,000, was added to each suspension as a preservative and all specimens were stored at -20° C.

*Preparation of antibodies.* Three groups of chinchilla rabbits were immunized. All antigen injections were made intraperitoneally with equal amounts of incomplete Freund adjuvant twice a week for 4 weeks. Each rabbit of the first group received a total amount of 800 mg. (wet weight) of whole lens antigen, the second group received 800 mg. of lens substance antigen each, and the third group received 400 mg. each of lens capsule antigen. The animals were bled a week after the last injection. The sera—rabbit antibovine whole lens antisera, rabbit antibovine lens substance antisera, rabbit antibovine lens capsule antisera—were stored at 4° C. until used.

*Conjugation of the antisera with fluorescein isothiocyanate.* The globulin fraction of these antisera was separated and conjugated with fluorescein isothiocyanate by the methods previously described.

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*The term "lens substance," is intended to mean the lens cortex and nucleus minus the lens capsule.*
The fluorescein conjugated rabbit antibovine lens capsule gamma globulin (F-CG), rabbit antibovine lens substance gamma globulin (F-SG), and rabbit antibovine lens capsule gamma globulin (F-SG) were stored at -20 °C. Fluorescein conjugated normal rabbit serum globulin (F-Normal G) was also prepared for control staining.

**Fluorescein staining and examination of sections.**

Frozen sections of fresh bovine eyes (iris, ciliary body, lens, retina, choroid, and optic nerve) were obtained in a cryostat. The thickness of sections were 6 to 7 μ for optic nerve and 8 to 10 μ for iris, ciliary body, retina, choroid, and lens. These sections were stained with the fluorescein conjugates (F-WG, F-SG, F-CG, and F-Normal G) and examined in the manner previously reported. The staining period was 1½ hours with the use of a few drops of the labeled globulin in a moist Petri dish chamber at 37°C. Microphotography was carried out by using high-speed Ektachrome 35 mm. film and Ansco Super Hypan 35 mm. film. Exposure time was 120 seconds for high-speed Ektachrome film and 30 to 90 seconds for Ansco Super Hypan film.

**Results**

As shown in Table I, the sections of tissues which were treated with the fluorescein conjugated rabbit antibovine lens capsule gamma globulin (F-CG) showed marked specific staining in the iris (Fig. 1), ciliary body (Fig. 3), retina, choroid (Fig. 5), optic nerve (Fig. 7), and lens capsule (Fig. 9) but no specific staining in the lens cortex. The sections treated with F-WG also showed specific staining in iris, ciliary body, retina, choroid, and optic nerve but less than the staining obtained with F-CG. Sometimes the former showed doubtful staining. In lens sections, both lens capsule and lens cortex were specifically stained by F-WG (Fig. 11). Sections
treated with F-SG did not show specific staining in any tissues except in the lens cortex (Fig. 10). No specific staining was observed in any tissue sections treated with the control reagent F-Normal G (Figs. 2, 4, 6, and 8).

**Lens.** In lens sections treated with F-CG the lens capsule showed marked specific staining (Fig. 9), while no specific staining was observed in the lens cortex. The lens cortex was specifically stained only with F-SG (Fig. 10). Both lens capsule and lens cortex showed specific staining by F-WG (Fig. 11). However, this specific staining of lens capsule was less if compared with the specific staining by F-CG.

**Iris.** The vascular walls in the iris were stained specifically with F-CG (Fig. 1). A slight specific staining was observed in vascular walls of the iris by F-WG. No specific staining was seen in the iris sections treated with either F-SG or F-Normal G (Fig. 2).

**Ciliary body.** The vascular walls showed marked, specific staining by treating with F-CG (Fig. 3). These vascular walls were slightly stained with F-WG. There was no specific staining in the tissue by treating with F-SG or F-Normal G (Fig. 4).

**Retina.** Retinal vascular trees showed remarkable specific staining by treating with F-CG (Fig. 5). The vascular tree was

**Table I. Reactivity of the fluorescein conjugated antibodies to ocular tissues**

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<tr>
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<th>F-CG*</th>
<th>F-WG</th>
<th>F-SG</th>
<th>F-Normal G</th>
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<tr>
<td>Lens capsule</td>
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<td>Lens cortex</td>
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<td>Vascular walls, ciliary body</td>
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<td>Vascular walls, retina</td>
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<td>Vascular walls, choroid</td>
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<td>Vascular walls, optic nerve</td>
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*Abbreviations stand for:
F-CG: Fluorescein conjugated rabbit antihuman lens capsule gamma globulin.
F-WG: Fluorescein conjugated rabbit antihuman whole lens gamma globulin.
F-SG: Fluorescein conjugated rabbit antihuman lens substance gamma globulin.
F-Normal G: Fluorescein conjugated normal rabbit gamma globulin.
Reactions to antilens capsule antibodies

Fig. 9. Lens section of bovine eye treated with F-CG. Marked, specific staining is observed in the lens capsule. There is no specific staining in the lens cortex. (×100.)

Fig. 10. Section of bovine lens stained with F-SG. Specific staining is observed in cortex, but no specific staining in the lens capsule. (×100.)

Slightly stained with F-WG, while no specific staining was observed in the sections treated with F-SG and F-Normal G (Fig. 6).

Choroid. Choroidal vessel walls were specifically stained with F-CG (Fig. 5). Only slight specific staining was observed in choroidal vessels by F-WG. Specific staining was not observed in the sections treated with F-SG or F-Normal G (Fig. 6).

Optic nerve. Capillary walls along the pia mater and septum of the nerve bundle showed marked specific staining by treating the sections with F-CG (Fig. 7). The F-WG stained section also showed specific staining in the capillary walls, but less than the staining obtained with F-CG. No positive stainings were seen in the sections treated with F-SG or F-Normal G (Fig. 8).

Discussion

In a preceding publication we were able to confirm and extend a report that an antibody produced against rat kidney glomeruli would bind to special sites in the rat eye, particularly to the lens capsule. It seemed logical to consider that the specific sites in ocular tissues responding to this antiserum could also serve as an antigenic stimulus in a heterologous system yielding antibody specific for basement membrane antigens in various body tissues. The lens capsule was best suited for this purpose. Its ability to stimulate production of antibody that reacts with not only lens capsule but also with vascular walls in the iris, ciliary body, retina, choroid, and optic nerve makes it necessary to consider its possible importance as a factor in special cases of phacoanaphylactic uveitis.
There is an interesting comparison to be drawn between the lens capsule-induced antibodies and those derived from lens substance. The result that specific staining was obtained in the lens cortex and nucleus by treating lens sections with F-SG (Fig. 1) supports the postulation that the lens substance protein is a possible antigenic factor of lens-induced uveitis, providing, of course, the lens material leaks out into the anterior chamber following damage of the lens capsule or increased permeability of the lens capsule. However, there was no specific staining in any other eye tissues when the tissues were treated with F-SG. This suggests that the lens substance antigen has no other common antigenic components in any other eye tissue. In contrast, when F-CG was used to treat other parts of the eye, specific staining occurred with vascular structures in different eye tissues. That this should occur to a weaker degree when using F-WG was to be expected since whole lens antigen suspensions contained capsular components, though in lower concentrations.

Before any speculation can be made on a possible role of lens capsule participation in induction of uveitis in man, it will have to be demonstrated that lens capsule is antigenic in an autologous system. Even a demonstration in a homologous system would be somewhat encouraging. The present study is only a type of serologic demonstration of tissue-binding capacity for antilens capsule antibody.

The precise localization of the antilens capsule antibody in vascular walls was difficult to determine because of the limited resolution of the ultraviolet light microscope. The sites of the specific staining seemed to be in the vascular wall themselves, including the cell layers of the endothelium and epithelium as well as in the basement membranes. However, it is more reasonable to suppose that the basement membrane lining the vascular tree is the site of the specific staining mainly because of the reported work on the antigenic relationship between the lens capsule and glomeruli basement membranes and the fact that the lens capsule has some similar biochemical component with basement membranes. It has been demonstrated with ferritin conjugated antibody, in conjunction with electron microscopy, that the anti-glomerular antibodies, when applied to kidney tissues, localized mostly in the glomerular basement membrane. It appears that the same technique will be necessary to locate the exact site where antilens capsule antibody is reacting in the region of blood vessels.

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REFERENCES
2. De Vree, J. A.: Bilateral endophthalmitis phacoanaphylactica; pathologic study of the lesion in eye first involved and, in one instance, the secondarily implicated, or “sympathizing” eye, A. M. A. Arch. Ophth. 49: 607, 1953.