Glial Fibrillary Acidic Protein Is Localized in the Lens Epithelium

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ABSTRACT The epithelium of the mouse lens stains intensely with antisera to glial fibrillary acidic protein (GFAP). A protein co-migrating with GFAP and immunoreactive with antisera to GFAP can be demonstrated in lens epithelium protein extracts by immunoblots. GFAP has previously been considered unique to cells of neural origin, but this study demonstrates that ectodermally derived cells express GFAP or a highly similar protein.

Glial fibrillary acidic protein (GFAP),[1] isolated from central nervous system tissues and multiple sclerosis plaques, is the major protein of astroglial filaments (1). The glial filament is an intermediate filament and is chemically and immunologically distinct from neuronal intermediate filaments (1–4). Antisera prepared against GFAP immunostain only certain types of neuroglial cells in the central nervous system (5, 6). Recently, GFAP immunostaining has been reported in Schwann cells, glia-like cells of the myenteric plexus, pituicytes, Kupffer cells of the liver, and salivary tumors (7–13). The presence of GFAP in Schwann cells, glial cells of the myenteric plexus, and pituicytes might be predicted as they are neural in origin and share morphological features of astrocytes (14, 15). We show in this report that the lens epithelium, an ectodermal derivative, is intensely immunoreactive for GFAP or a protein that is immunologically and biochemically indistinguishable from brain GFAP.

MATERIALS AND METHODS

Eyes were removed from young adult B6CBA mice, snap frozen in liquid nitrogen, and sectioned transversely or sagitally in a cryostat at −20°C. The mounted 10-μm sections were postfixed in absolute acetone for 15 min and stored in a refrigerated desiccator. The indirect immunofluorescence technique (16), the indirect immunoperoxidase technique (17), and the peroxidase-antiperoxidase (PAP) technique (18) were employed to localize GFAP. Tissues were rehydrated in 0.1 M phosphate-buffered normal saline (PBS), pH 7.4, preincubated for 1 h with 10% sheep serum, and incubated with one of three different rabbit anti-GFAP sera at dilutions of 1:100 to 1:50,000 for 1 to 24 h. For the immunofluorescence procedure, fluorescein isothiocyanate-conjugated goat anti-rabbit IgG serum was applied in a 1:100 dilution for 1 h. Slides were examined with a Zeiss Standard research microscope using a tungsten light source and 530-nm barrier filter. In the indirect immunoperoxidase procedure, peroxidase-conjugated goat anti-rabbit IgG serum was applied in a 1:100 dilution for 1 h. For the PAP procedure, goat anti-rabbit IgG serum was applied in a 1:20 dilution for 30 min, followed by the rabbit PAP complex at a 1:80 dilution for 40 min. The antibody complex was visualized by the formation of immunoreaction product during a 10-min incubation with Hanker-Yates reagents (phenylenediamine/pyrocatechol (1:2) in 0.1 M Tris buffer, pH 7.4, plus 0.01% H_2O_2). The tissues were given two 10-min rinses in phosphate-buffered normal saline between all steps of the procedure. Controls included omission of the primary or secondary antiserum, the substitution of rabbit nonimmune serum for the primary antiserum, and the use of GFAP-absorbed and vimentin-absorbed anti-GFAP, provided by Dr. Lawrence Eng. For the absorption procedure, aliquots of antisera were incubated consecutively with milligram amounts of bovine intermediate filament proteins and then the specificity of the antisera was tested by immunoblot until they no longer stained bovine rat GFAP proteins. The immunochemical properties and specificity of these antisera, which were produced against GFAP derived from both bovine spinal cord and human multiple sclerosis plaques, are well documented (19, 20). The specificity of the anti-human GFAP antisera used in this study to bovine and rat intermediate filament proteins was previously demonstrated by Eng and DeArmond (21). The horseradish peroxidase-conjugated and FITC-conjugated goat anti-rabbit IgG, goat anti-rabbit IgG, nonimmune rabbit serum and sheep serum were obtained from Cappel Laboratories (Cochranville, PA). Rabbit anti-vimentin serum was obtained from Transformation Research, Inc. (Framingham, MA). PAP complex was obtained from Sternberger-Meyer Immunochemicals, Inc. (Jarrettsville, MD). All antiserum dilutions were made in 0.02 M phosphate-buffered normal saline, pH 7.4.

Protein extracts of dissected mouse lens epithelium were prepared by the method of Chiu et al. (22) and subjected to SDS PAGE (23). Nitrocellulose blots were made from the gels (24) and immunostained for GFAP or vimentin by the PAP technique (18).

RESULTS

Immunocytochemistry

Anti-GFAP immunostaining in the lens is confined to the epithelial layer (Fig. 1, *A–D*). Examination of sagittal and frontal sections of the lens shows the staining in the epithelium is most intense in the central area of the lens and gradually becomes weaker towards the equatorial region (Fig. 1 *C*). This gradual loss of GFAP immunoreactivity correlates with the change in shape of the epithelial cells from cuboidal to elongating fiber cells (25). The nuclei of the epithelial cells are unstained but the cytoplasm is clearly immunoreactive. Short,

[1] *Abbreviations used in this paper:* GFAP, glial fibrillary acidic protein; PAP, peroxidase-antiperoxidase.

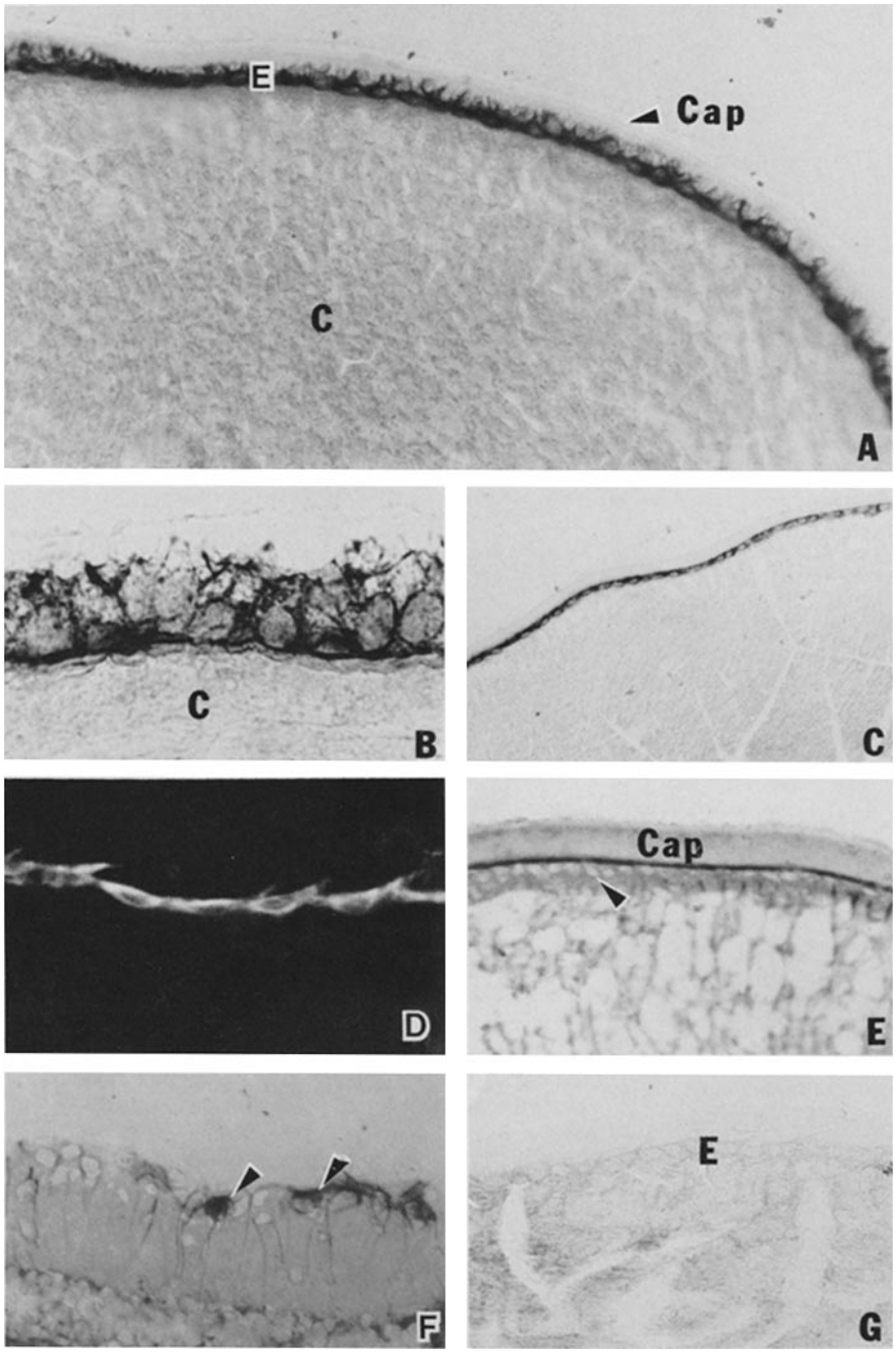

FIGURE 1 Mouse lens and retinal sections showing GFAP and vimentin immunostaining. (*A*) GFAP immunoreactivity in the lens is restricted to the epithelium (*E*). The capsule (*Cap*) and cortex (*C*) are not immunoreactive (indirect peroxidase technique). × 100. (*B*) Higher magnification shows GFAP staining in the cytoplasm of the epithelium but not cortex (*C*) (PAP technique). × 1,000. (*C*) GFAP immunoreactivity in the epithelium is most intense in epithelial cells near the rostral pole and decreases towards the equatorial region. × 200. (*D*) GFAP immunofluorescence is confined to the epithelial cells. Note the unreactive cell nuclei. × 200. (*E*) Vimentin immunoreactivity is present in radial fiber cell processes of the cortex (arrowhead) and in a dense band beneath the lens capsule (*Cap*) (PAP technique). × 100. (*F*) The cytoplasm (arrowheads) and processes of retinal glia in the nerve fiber layer exhibit GFAP immunoreactivity (PAP technique). × 200. (*G*) Control lens (GFAP-absorbed antiserum) shows absence of staining in the epithelial cells (*E*). × 200.

tufted processes often protrude from the cell body towards the lens capsule (Fig. 1 *B*) and these processes are usually seen in the zonular region.

Although the eye contains many different types of tissues, only the glia in the retina (Fig. 1 *F*) and the lens epithelial cells were immunoreactive with the GFAP antiserum. Immunoreactivity of both types of cells was equally intense and serial dilutions of the anti-GFAP serum produced a roughly equivalent decrease of immunoreaction product. Omission of the GFAP antiserum or substitution with nonimmune rabbit serum or GFAP-absorbed anti-GFAP serum at the same dilutions entirely eliminated immunostaining of the lens and retina (Fig. 1 *G*). Our ability to demonstrate this protein in the lens is probably due to (*a*) the use of frozen sections which helps to retain the lens epithelium, whereas it is easily removed during most tissue processing, and (*b*) light fixation of the tissue in acetone retains most of the antigenicity of GFAP, which is diminished by aldehyde fixation (26).

Vimentin, a major protein component of intermediate filaments in the lens, has an immunostaining pattern different than that of GFAP (27). Incubation of the lens with antivimentin serum (Fig. 1 *E*) produced immunoreactivity in a dense band between the capsule and the epithelium and in radially oriented fibers of the lens cortex. The different staining patterns of vimentin and GFAP and the absorption studies strongly suggest that there is no cross-reactivity of the GFAP

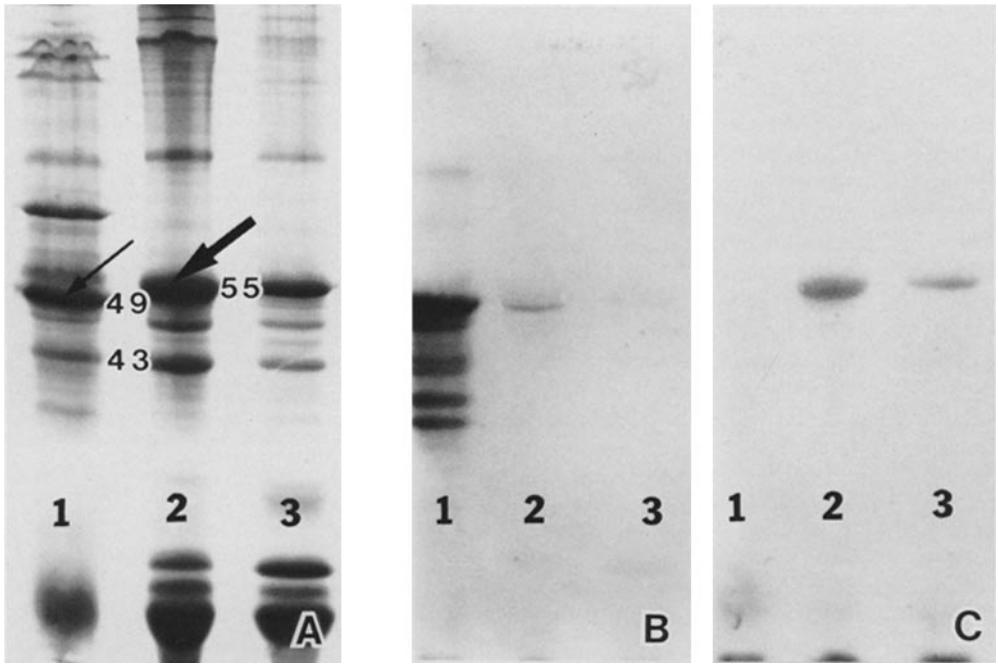

FIGURE 2 Electrophoretic gel and immunoblots of mouse lens and spinal cord extracts. (*A*) Coomassie-blue-stained SDS polyacrylamide gel of protein extracts from spinal cord (lane *1*) (35 μg protein) and lens epithelium (lanes *2* and *3*) (50 and 25 μg protein per track, respectively). GFAP band is denoted by the small arrow; vimentin band is indicated by the large arrow. Molecular weights ($\times 10^{-3}$) calculated from standards are shown: *55*, vimentin; *49*, GFAP; *43*, actin. (*B*) Nitrocellulose immunoblot of the gel in A, after reaction with anti-GFAP serum. Additional immunoreactive bands in the spinal cord track (lane *1*) are degradation products of GFAP. An immunoreactive protein band co-migrating with the major band of spinal cord GFAP is present in the lens epithelium track (lane *2*). (*C*) An immunoblot identical to *B*, but reacted with rabbit anti-chick vimentin serum. Lens epithelium (lane *2*) shows a dense immunoreactive vimentin band, while no reactivity is present in spinal cord extract (lane *1*). Gel and immunoblots are original size.

antiserum with vimentin.

Immunoreactivity of Electrophoretically Separated Lens Proteins

The presence of GFAP in lens epithelial cells was also demonstrated on nitrocellulose immunoblots of cell extracts electrophoresed simultaneously with spinal cord in SDS polyacrylamide gels. The Coomassie-Blue-stained gel demonstrates the GFAP band in the spinal cord, migrating at a lower apparent molecular weight than the main vimentin band of the epithelial fiber cell (Fig. 2). Immunoblots showed a major reactive site for GFAP in the spinal cord, with reactivity also noted in the region of breakdown products. The epithelial reaction formed a sharp narrow band, corresponding in position to spinal cord GFAP, and located immediately beneath the position of the vimentin band. The antivimentin serum did not cross-react with the spinal cord proteins. This evidence strongly suggests that GFAP or a protein virtually identical to GFAP is present in the lens epithelial cell, most likely reflecting a distinct population of intermediate filaments. Our studies also suggest that GFAP is present in many different species as immunoblots of chick lens epithelia reacted strongly with the anti-GFAP and immunostaining was observed in lens epithelial of another mammalian species (golden hamster).

DISCUSSION

Although the lens epithelial cells and astroglial cells have different embryological origins (28, 29), their shape, morphology and functions are similar in many respects. Bundles of intermediate filaments are conspicuous within the cytoplasm of both cell types. The immunocytochemistry of the lens suggests that the intermediate filaments are heterogenous, consisting of both vimentin and GFAP. GFAP appears to be distributed throughout the cell while vimentin is confined primarily to a dense band near the apical portion of the epithelium. Vimentin has also been demonstrated in astrocytes but is more prominent during early development (30, 31). Intermediate filaments in astroglia have been postulated to play a role in structural support (32) and its presence in the lens may serve a similar purpose (33–35). Injury to both cell types, such as transcorneal needle injection or a stab wound to the central nervous system, produces a hypertrophy of intermediate filaments and increased mitotic activity (36–39). Functionally, active transport mechanisms for K^+ have been demonstrated for both astrocytes and lens epithelial cells (40). Other structural similarities include many gap junctions but few tight junctions, glycogen granules, similar chromatin patterns, interdigitating processes, and apposition to a basal lamina (25, 41).

The embryologic origin of the lens epithelium is brought into question by this immunochemical demonstration of GFAP. The evidence is incontrovertible that the lens is an ectodermal derivative (28, 29), formed when the neuroectodermal optic vesicle contacts the overlying ectoderm. One explanation for GFAP in the lens epithelium is that the proximity of nervous tissue may induce GFAP synthesis in the lens epithelium by transcription from the same activated gene present in astrocytes. It is interesting to note that both the embryonic neural retina and the lens primordium are

bipotential; that is, the embryonic neural retina can be experimentally stimulated to "transdifferentiate" into lens fiber cells and, conversely, differentiation of the lens primordium into retinal tissue can be demonstrated (42–45). It is thus possible that the induction of GFAP may result from a particular combination of the same factors that produce other properties shared by astroglia and lens epithelium such as junctional complexes, etc. Still another explanation is that there are two genes, the one in the lens epithelium being a product of gene duplication of the astrocytic GFAP gene. This is an attractive hypothesis because the intermediate filament genes have undergone a number of gene duplications resulting in a complex and varied family of genes. Isolation and identification of these GFAP genes will be essential to definitively determine their origin. Whatever the cause, the GFAP protein in astrocytes and lens epithelial cells implies a common genotypic expression. Comparison of the structure and function of these two cell types may provide a better understanding of the functional role of this intermediate filament.

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