Original

**Glial Fibrillary Acidic Protein in the Perivascular Glia
of the Developing Human Retina**

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Abstract: The expression of glial fibrillary acidic protein (GFAP) in the perivascular glia of the developing human retina was studied immunohistochemically in 22 normal eyes. Retinas were stained with anti-GFAP antibody using the labelled streptavidin-biotin method. The GFAP-positive perivascular glial cells were first observed in the human retina at 24 gestational weeks near the optic disc. As the retina matured, the intensity of GFAP immunoreactivity increased until full term. The GFAP immunoreactivity was most intense around large vessels near the optic disc and diminished with decreasing vascular caliber and increasing distance from the optic disc. Our results support the concepts that in the developing human retina, GFAP-positive perivascular glial cells first appear at 24 gestational weeks and that the human retina contains perivascular glial cells.

Key words: glial fibrillary acidic protein, immunohistochemistry, perivascular glia, retina, human

Introduction

In the human retina, astrocytes completely sheathe small blood vessels with their sucker-like end-feet. The special cellular element between this sheath and the vascular structures has been described as perivascular glia^{1,2)}. A close relationship is believed to exist between the blood vessels of the retina^{1,2)} and brain³⁾, and the glial cells. Despite numerous investigations, the significance of this association is still unclear. Some investigations, the significance of this association is still unclear. Some investigators have shown that the astrocytes and their perivascular end-feet take part in the formation of the blood-brain barrier in the central nervous system (CNS)^{4,5)}.

In previous immunohistochemical and biochemical studies the various aspects of astrocytes have been described including their morphologic classes, development, functions, and distribution in the CNS and retina⁶⁻¹⁸⁾. Glial fibrillary acidic protein (GFAP) is reported to be a major protein component of the glial filaments that are found in high concentrations in astrocytes^{19,20)}. However, expression of GFAP in perivascular glial cells has not been well studied. The GFAP in the perivascular glia of the rat retina has been described by some authors²¹⁾ and in the cortical perivascular glia of the cat by others²²⁾. In this study, an anti-GFAP antibody was used as a glial marker and the development and distribution of GFAP-positive perivascular glial cells was investigated in the human retina by utilizing the labelled

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Table 1. Perivascular GFAP immunohistochemical reaction patterns of the retina.

Age	NFL*	GCL*	IPL*
13–22 GW†	No	No	No
24 GW†	Weak	Weak	No
25–28 GW†	Larger area	Larger area	No
30–37 GW†	Strong	Strong	No
40 GW†	Larger area	Larger area	No
4 Mo†-Adult	Larger area	Larger area	Weak

* NFL, Nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer

† GW—Gestational weeks, Mo—month.

streptavidin-biotin (LSAB) method.

Materials and Methods

Twenty-two formalin-fixed normal human eyes ranging in age from 13 gestational weeks (GW) to adulthood were collected from the files of the First Department of Pathology, Kobe University School of Medicine, for immunohistochemical analysis (Table 1). The tenets of the Declaration of Helsinki were followed. All persons and authorities gave their informed consent prior to inclusion in this study. Eyes were cut anteroposteriorly parallel to the optic axis, and a strip of retina was taken from the temporal half of the globe excluding the macula and fovea. The strip was then transferred to cold phosphate-buffered saline (PBS) until processing for paraffin embedding was begun. Tissues were sectioned at 5 μ m parallel to the axis of the optic nerve. Sections were mounted on clean slides utilizing a gelatin water bath and dried overnight at 45 to 47°C.

Rabbit anti-GFAP (primary antibody), purified from bovine spinal cord, was supplied by Dako Corporation (Carpinteria, CA, USA). This antibody reacts strongly with human GFAP and cross-reacts with GFAP in several other mammalian species. Biotinylated anti-rabbit and anti-mouse immunoglobulins were also supplied by Dako Corporation. Tissue sections were stained for anti-GFAP using the LSAB method (Dako LSAB kit, peroxidase). Endogenous peroxidase activity was abolished by incubation in 3% hydrogen peroxide for 5 minutes. Nonspecific staining was blocked by a 5-minute incubation with blocking reagent (15 ml PBS containing carrier protein and 15 mM sodium azide). Then, the specimen was incubated with appropriately diluted rabbit primary antibody (7 ml rabbit antiserum to bovine GFAP in 0.05 M Tris buffer, pH 7.6, containing 15 mM sodium azide), followed by successive 10-minute incubations with biotinylated link antibody (anti-rabbit and anti-mouse immunoglobulins in PBS, containing carrier protein and 15 mM sodium azide) and peroxidase-labelled streptavidin. Immunostaining ended after a 10-minute incubation with substrate chromagen solution (3% 3-amino-9-ethylcarbazole in N,N-dimethylformamide).

For negative control, sections were incubated with nonimmune rabbit serum instead of primary antibody. Moreover, positive controls were provided by specimens of normal human brain.

Results

From 13 to 22 GW, GFAP immunostaining could not be demonstrated anywhere in the retina. At 24 GW, the GFAP immunohistochemistry weakly labelled astrocytes and perivascular glia in the nerve fiber and ganglion cell layers near the optic disc (Fig. 1A). From 25 to 28 GW, vessels, especially those in the nerve fiber layer, were invested with GFAP-positive perivascular elements (Fig. 1B), and glial foot process also showed immunoreactivity. At locations distant from the optic disc GFAP staining was confined to perivascular elements and immature astrocytes in the nerve fiber layer. Between 30 and 37 GW, GFAP-positive perivascular glial cells were strongly immunoreactive (Fig. 1C). At 40 GW the staining pattern was similar to that described at 30 to 37 GW, with a slight increase in the concentration of GFAP-positive perivascular glia (Fig. 1D) and vascular end-feet at a greater distance from the optic disc. From 4 months onward (Figs. 1E, F), there was no significant change in the pattern of perivascular GFAP immunostaining. Most of the astrocytic processes terminated upon vessels within the nerve fiber and ganglion cell layers, and occasionally upon the inner portion of the inner plexiform layer. GFAP-positive perivascular elements were visualized at sites distant from the optic disc, but GFAP immunoreactivity of perivascular glia in the vicinity of the optic disc was so intense that the structural details were difficult to observe.

Discussion

Several glial markers are now available and among them GFAP, vimentin, galactocerebroside, O8 antigen, and S-100 protein are widely used to study the presence and location of neuroglial cells in the retina. S-100 protein labels astrocytes and ganglion cells but not the perivascular glia in the human retina (unpublished data). Findings of the present study indicate that the use of anti-GFAP antibody as a glial marker is helpful to demonstrate GFAP-positive perivascular glial cells and astrocytic end-feet in both the fetal and adult retina.

Human retinal vessels were devoid of GFAP-positive cells before 24 GW. As the retina developed, GFAP staining increased until full term. In the mature retina, vessels in the nerve fiber and inner portion of the inner plexiform layer were fully covered with a GFAP-positive sheath. This sheath was thickest around large vessels in the vicinity of the optic disc and became thinner with decreasing caliber of vessels and increasing distance from the optic disc. The GFAP immunoreactivity was found in the end-feet of astrocytes and perivascular glial cells, and their processes.

Müller cell processes are reported to terminate on blood vessels in the nerve fiber and findings are not compatible with these reports in ganglion cell layers of the rat retina^{23,24}. Our findings in the present study that the processes of astrocytes which terminate in sucker-like end-feet upon retinal vessels were labelled by anti-GFAP antibody. It has been suggested that in the monkey and human retina the astrocytic processes that join with the fascicles of the nerve fiber layer may be rich in filaments while the processes of the same cell which join with the vessels may be devoid of filaments^{24,25}. In the retina of the albino rat, it has been shown that the astrocytic end-feet upon retinal capillaries are devoid of filaments²⁶. Our findings of GFAP immunoreactivity in the vascular end-feet suggest that in the human retina, this pattern may be rare. The perivascular glial cells described in the human retina

Fig. 1. A. Weakly labelled GFAP-positive perivascular glial cells near the optic disc at 24 GW (arrows). ILM, inner limiting membrane; NFL, nerve fibers layer; GCL, ganglion cell layer. (GFAP, $\times 400$).
 B. GFAP-reactive perivascular glial cells at 28 GW near the optic disc (arrows). (GFAP, $\times 400$).
 C. GFAP-positive perivascular glial cells are seen at 30 GW near the optic disc (arrows). (GFAP, $\times 400$).
 D. At 40 GW the sucker-like end-feet are seen on a retinal vessel in the innermost layers of the retina approximately midway between the optic disc and the ora serrata (arrows). (GFAP, $\times 400$).
 E. At 4 months GFAP-immunoreactive perivascular glial cells are evident around a vessel midway between the optic disc and the ora serrata (arrows). (GFAP, $\times 400$).
 F. In the adult retina GFAP-immunoreactive perivascular glial cells are evident near the ora serrata (arrows). (GFAP, $\times 200$).

in our experiment may be compared with those reported in previous studies^{2,27}.

In summary, we have demonstrated that GFAP-labelled perivascular glial cells are first observed at the posterior pole of the globe and spread anteriorly with increasing age. Further immunohistochemical investigations may contribute to a closer understanding of the location of perivascular glial cells in the human retina.

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