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GLIAL FIBRILLARY ACIDIC PROTEIN EXPRESSION IN THE DEVELOPING HUMAN RETINA

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INDEXING WORDS

glial cells; human retina; immunohistochemistry; LSAB method

SYNOPSIS

We have studied the expression of glial fibrillary acidic protein(GFAP), an astrocyte specific protein in the normal developing human retina using anti-GFAP antibody. GFAP positive cells were first detected at 24 gestational weeks(GW) in the nerve fiber and ganglion cell layers in the vicinity of the optic disc. Over subsequent weeks, GFAP positive cells covered larger regions of the retina. Some of the processes of these cells terminated in sucker like end-feet upon blood vessels of the nerve fiber layer. A second population of GFAP positive cells existed as perivascular glia in the nerve fiber layer in the early stage of fetal development. In the adult retina perivascular glia were found on vessels throughout the nerve fiber and in the inner portion of the inner

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plexiform layers. Staining of Müller cells end-feet was obtained only in the adult retina. These results support the views that in the human retina, GFAP positive cells first appear at 24 GW in the region near the optic disc, covering the whole retina at subsequent ages.

INTRODUCTION

Controversery still exists concerning the distribution and origin of the glial cells of the retina. However two classes of glial cells have been recognized in the mammalian retina, namely astrocytes and Müller cells. Astrocytes are predominantly found in the vascularized retina.[12] On the other hand, Müller cells are radial fibers found ubiquitously in all vertebrate retinae and are generated from the neuroepithelium of the retina.[9]

It is well recognized that GFAP is a major protein component of the glial filaments which are found in high concentration in fibrous astrocytes.[4] In our study, glial marker anti-GFAP antibody was used and the development and distribution patterns of GFAP positive cells are described in the human retina ranging in age from 13 GW(gestational weeks) to adulthood(table).

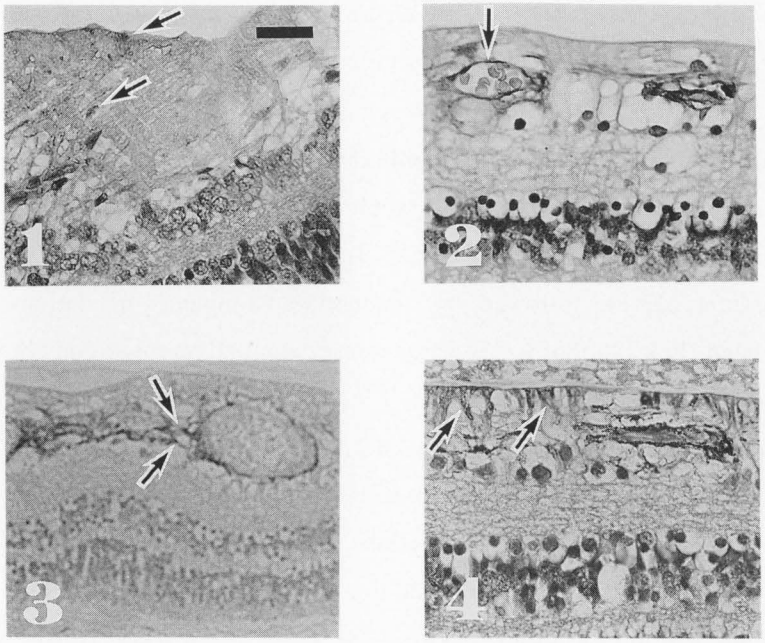
MATERIALS AND METHODS

Twenty-four human eyes were collected by enucleation and immediately fixed in 4% formaldehyde solution buffered PBS(PH 7.4). All specimens were subjected to paraffin embedding. Embedded tissues were sectioned at 4μm and mounted on slides utilizing a gelatin containing water bath and were dried over-night at 45-47°C. Sections

were processed by the labelled streptavidin-biotin(LSAB) method(DAKO LSAB Kit, Peroxidase). In this procedure, endogenous peroxidase activity was quenched by first incubating the specimen for five minutes with 3% hydrogen peroxide. Non-specific staining was blocked by a five-minute incubation with blocking reagent(15 ml phosphate buffer saline, containing carrier protein and 15 mM sodium azide). The specimen was then incubated with prediluted rabbit primary antibody(7 ml rabbit antiserum to bovine GFAP in 0.05M Tris buffer, pH 7.6, containing 15 mM sodium azide), followed by sequential 10-minute incubations with biotinylated link antibody and peroxidase-labelled streptavidin. Staining was completed after a 10-minute incubation with freshly prepared substrate-chromagen solution(3% 3-amino-9-ethylcarbazole in N,N-dimethylformamide). Negative controls for the immunostaining were carried out by incubating the sections with non-immune rabbit serum instead of primary antibody. In addition positive controls were provided by the normal human brain.

RESULTS

At 24 GW GFAP positive cells were first detected in the nerve fiber and ganglion cell layers in the vicinity of the optic disc(fig.1). Perivascular glia began to show GFAP reactivity in the nerve fiber layer only. During the period spanning 25 and 37 GW GFAP activity was found to extend to the larger regions of the ganglion cell layer(fig.2). From 40 GW onward(fig.3) GFAP positive cells were seen both in the vascular and avascular regions of the retina. The highest concentration of the reactivity was found in the vicinity of the optic nerve. The glial foot-process on the blood vessels was also found to be reactive for GFAP staining. GFAP reactive perivascular glial cells could be detected at



Figures are all stained with anti-GFAP antibody(LSAB method) using hematoxylin as counterstain. Scale bar=8μm for figs. 1, 2 and 4 and, 16μm for fig.3. **Fig. 1-** Showing weak GFAP positive cells in the nerve fiber and ganglion cell layers near the optic disc at 24 gestational weeks(arrows). **Fig. 2-** At 32 weeks GFAP active cells are seen in the nerve fiber and ganglion cell layers near to the optic disc. GFAP positive reaction is also evident around vessels(arrow). **Fig. 3-** At 4 months the photomicrograph showing the suckerlike end-feet ending on a retinal vessel in the region approximately midway between the optic disc and the ora serrata(arrows). **Fig. 4.** Showing GFAP positive cells in the nerve fiber and ganglion cell layers of the far periphery of adult retina. The GFAP positive Müller cells end-feet are also seen in the photomicrograph(arrows).

different sites. In the adult retina, Müller cells end-feet were seen to be well developed along the inner limiting membrane and their cytoplasm appeared to have a brownish cast following GFAP staining procedure(fig.4).

TableI. GFAP immunohistochemical reaction patterns of the retina.

Age	NFL	GCL	IPL
13-22GW	-	-	-
24GW	+	+	-
25-28GW	+ *	+ *	-
30-37GW	+ +	+ +	-
40GW-4Y	+ + *	+ + *	-
Adult	+ + *	+ + *	+

- negative, + weak, + + strong, * larger region of the layer,
GW- gestational week, Y- year, NFL- nerve fiber layer,
GCL- ganglion cell layer, IPL- inner plexiform layer.

DISCUSSION

Several types of glial cells in the human retina have been reported namely, Müller cells, perivascular glia, lemmocytes and astrocytes.[13] However the distribution of glial cells in the human retina differs from that in the rat and in the rat retina the presence of Müller cells only, has been reported.[5,10,11] In recent years the presence of GFAP positive cells in the nerve fiber layer of the mouse retina have been described.[2] Our observations that GFAP reactivity in the human retina is confined predominantly to the nerve fiber and ganglion cell layers supports previous reports that GFAP positive cells in the trout retina are confined mainly to the nerve fiber and ganglion cell layers.[3] Anderson and

coworkers[1] have reported that only occassional astrocytes are present among Müller cell footplates in the human retina while we have detected a high concentration of GFAP staining perikarya in the nerve fiber and ganglion cell layers of the adult human retina.

It was claimed that Müller cell processes were the ones ending on blood vessels in the nerve fiber and ganglion cell layers of the rat retina.[6-8] The interpretation of our results is at variance with these reports. We have been able to demonstrate GFAP reactivity within astrocyte processes which end in sucker like end-feet upon blood vessels. The perivascular glia found in the human retina in our study seem to be the same as those described by others.[14]

In summary we have demonstrated the GFAP positive cells in the human retina and these cells are first observed at the posterior pole of the globe and as the age advances these cells spread anteriorly.

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