Glial Fibrillary Acidic Protein in Radial Glia of Early Human Fetal Cerebrum: A Light and Electron Microscopic Immunoperoxidase Study

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Abstract. In order to assess the nature of glial fibrillary acidic protein (GFAP) immunoreactivity in the radial glia of early human fetal cerebrum, full thickness blocks from the midconvexity of the frontoparietal region of the cerebrum of 25 human fetuses ranging from ten to 20 weeks of ovulation age were studied by light and electron microscopic (EM) immunoperoxidase methods. The presence of GFAP within radial glia was demonstrated in vibratome sections, in de-eponized 1 μm sections and in paraffin-embedded sections both at light and EM levels in suitably fixed human fetal cerebral tissue. The results indicate that the pattern of GFAP immunoreactivity observed in “routinely” processed autopsy brains, in which fixation is suboptimal, must be interpreted with care.

Key Words: Cerebrum; Fetus, human; Glia, radial; Glial fibrillary acidic protein; Immunoperoxidase stain.

INTRODUCTION

Glial fibrillary acidic protein (GFAP) is now well established as the major protein constituent of glial filaments, which are distinct from other intermediate filaments (1). Both immunofluorescent and immunoperoxidase methods have been used for its localization in normal as well as in pathological tissues (2–7). The highly sensitive, widely used peroxidase-antiperoxidase (PAP) method of Sternberger (8) has been very effective for the demonstration of GFAP in developing as well as in neoplastic glial cells (9–12). Furthermore, the immunoreactivity of GFAP appears to be highly resistant to fixation in formalin or glutaraldehyde or to embedding in paraffin or epon. Thus the opportunity became available for retroactive studies using either autopsy or surgical material.

The presence of radial glial cells in the developing fetal brain was first revealed by the use of Golgi methods (13–15) and later confirmed by electron microscopy (EM) (16, 17). More recently the use of these methods in combination has permitted the identification of radial glial processes in the developing rat cerebrum (18) and in the human fetal cerebrum (19, 20). Using an indirect immunofluorescent technique, our laboratory first reported the presence of GFAP within the radial glia of human fetal cerebrum at ten to 18 weeks (wk) of ovulation age (9). This study was further extended into a correlative Golgi, EM and immunofluorescent analysis of 36 human fetal cerebra at seven to 20 wk (20), which led us to conclude that radial glial fibers at this stage of development already contain GFAP and possess immunocytochemical and morphological characteristics indicative of astrocytic differentiation. Rakic and his colleagues (21–24) also demonstrated the existence of GFAP...
TABLE 1

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in radial glial cells within embryonic monkey cerebral cortex, cerebellum and hippocampus during the first trimester with the aid of light and EM immunocytochemical methods. Valentino et al (25) described immunoreactivity for GFAP within radial glial fibers of the developing rat cerebral cortex by embryonic day 18. Glial fibrillary acidic protein has also been visualized in radial glial fibers in newborn rat, but only in response to injury (26). The presence of GFAP within the radial glial cells of the developing brain appears, therefore, to be well established.

Although GFAP immunoreactivity is highly resistant to routine formalin fixation and paraffin embedding, its stainability can be greatly influenced by delay in fixation or by variations in tissue processing. We attempted to use routine autopsy brains derived from spontaneously aborted or stillborn infants for the demonstration of GFAP using immunoperoxidase methods, but the results were inconsistent. Staining intensity varied considerably even within the same specimen. This prompted us to reassess the nature of GFAP immunoreactivity in the radial glia of the early human fetal cerebrum, using light and EM immunoperoxidase methods. In this study we have used only those specimens which were fixed within a short period of time after they were obtained.

MATERIALS AND METHODS

The material consisted of full thickness blocks from the midconvexity of the frontoparietal region of the cerebral of 25 human fetuses. The estimated ovulation ages varied from ten to 20 wk. The crown–rump lengths and estimated ages of the specimens are listed in Table 1. In man ovulation age is generally about two wk shorter than gestational age based on the last menstrual period. The fetuses were received as surgical specimens following abortion by hysterotomy. The brain was removed under sterile conditions within 15 to 20 minutes (min) of surgery. Finely dissected slabs of cerebral cortex were fixed in 4% glutaraldehyde for 18 to 24 hours (h) and placed in Sorenson's buffer prior to epon embedding or vibratome sectioning. Paraffin-embedded sections were prepared from intact cerebral hemispheres or portions thereof that had been fixed in Bouin's fluid for 18 to 24 h and then placed into 80% ethanol.

Immunoperoxidase staining for GFAP was carried out using vibratome sections (10 to 30 μm), de-epoxiized 1 μm sections and paraffin-embedded sections (6 to 8 μm). Thin sections were also examined which were obtained from epon-embedded vibratome sections that had been processed for GFAP immunocytochemistry. The GFAP antisera used came from three

sources (our own, some generously provided by Dr. L. Eng and some purchased from Dako). The dilution of the primary antisera was 1:500 in most instances. Vibratome sections were incubated for 48 to 72 h in primary antisera. De-eponized 1 μm sections and paraffin-embedded sections were incubated for two to 24 h.

RESULTS

Glial fibrillary acidic protein (GFAP) immunoreactivity within the radial glial cells of human fetal cerebrum was strongest and most consistently observed in vibratome sections. These samples were the first to be fixed in glutaraldehyde during the harvesting of the tissue. As shown in Figures 1 and 2, at 12 wk of age post-migratory neurons had already begun to accumulate within the cortical plate. The ventricular zone was relatively thick, and migrating neurons were still present within
Fig. 2. Radial glial fibers extending from the ventricular zone (V) to the pia mater (PM) of a 12-wk-old human fetal cerebrum. Note brown GFAP immune reaction product within the matrix of the radial fibers (arrowheads). These fibers branch at the marginal zone and terminate as conical swellings (arrowheads) at the PM. The upper inset shows conical swellings (arrowheads) at a high magnification. The lower inset shows a mitotic figure at the ventricular surface showing positive GFAP immune reaction (arrowhead) within the cytoplasm. CP: cortical plate. I: intermediate zone. SV: subventricular zone. LV: lateral ventricle. Vibratome section. GFAP immunoperoxidase counter-stained with chloroform-methylgreen. ×900. Upper inset, ×1,800. Lower inset, ×4,500.
Fig. 3. A. Glial fibrillary acidic protein (GFAP)-positive radial glial fibers (arrows) in a 15-wk-old human fetal cerebrum. Note the delicate radial fibers extending from the ventricular zone (V) toward the intermediate zone (I). Many radial glial cells have their cell bodies (empty arrows) in the subventricular zone (SV). Note clusters of migrating neurons (large empty arrow) along the radial glial fibers. Paraffin section. GFAP immunoperoxidase stain. ×350. B. Clusters of migrating neurons (large empty arrow) along GFAP-positive radial glial fibers at higher magnification. Human fetal cerebrum. 15 wk old. Paraffin section. GFAP immunoperoxidase stain. ×700. C. GFAP-positive radial glial fibers in the intermediate zone. Human fetal cerebrum. 15 wk old. Paraffin section GFAP immunoperoxidase stain. ×700.

the intermediate zone. Radial glia extending from the ventricular surface to the pia mater were strongly immunoreactive for GFAP. The location of the cell somas varied somewhat, but most were situated within the subventricular or ventricular zones. Mitotic cells at the ventricular surface showing strong immunoreactivity for GFAP.
Fig. 4. A. Strongly GFAP-positive radial glial fibers (arrows) at the ventricular zone (V) of a 17-wk-old human fetal cerebrum. Many of the radial glial cells have their somas located in the subventricular zone (SV) as shown by empty arrows. Note extremely fine parallel arrays of radial glial fibers (small arrows) extending into the intermediate zone (I). Paraffin section. GFAP immunoperoxidase stain. ×350. B. The GFAP-positive radial glial fibers (arrows) at a higher magnification. Many radial glial cells have bipolar processes. Human fetal cerebrum. 17 wk old. V: ventricular zone. SV: subventricular zone. Paraffin section. GFAP immunoperoxidase stain. ×700.

were also identified (Fig. 2). Within the intermediate zone migrating neurons were closely apposed to radial glial fibers. The radial fibers became much finer as they entered the cortical plate. The delicate fibers then branched in fork-like fashion within the marginal zone and terminated in conical swellings (Fig. 2) at the pia mater. The configuration brought out by immunoperoxidase staining for GFAP was reminiscent of rapid Golgi images of the same fibers within the developing human fetal cerebrum.

The results obtained in paraffin-embedded sections were more variable though most of the specimens examined in this study did show reactivity for GFAP within radial glial cells. Figure 3 represents a paraffin-embedded 15-wk-old human specimen that was processed for immunoperoxidase staining for GFAP. Delicate GFAP-positive radial glial fibers extending from the ventricular zone toward the intermediate zone can be seen. Because of section thickness it was difficult to trace specific radial glial fibers as they passed in and out of the plane of section, but the reaction product was distinctly visible in parallel array. As shown in Figure 3B and C, radial fibers were abundant in the intermediate zone often tightly apposed to neuronal clusters. In paraffin-embedded material, radial glia within the cortical plate sometimes failed
Fig. 5. A. The GFAP-positive cell bodies (dark arrows) and processes at the ventricular zone of a 15-wk-old human fetal cerebrum; GFAP-negative cells (arrowheads), presumably neuronal precursors, also line the ventricular surface. Note the bipolar configuration of the radial glial processes (empty arrows). The outlines of the cell bodies and processes are relatively smooth at this age. Paraffin section. GFAP immunoperoxidase stain. ×1,000. B. A radial glial cell in the subventricular zone of a 20-wk-old human fetal cerebrum. Note the strong immunoreactivity for GFAP within the cell body and within its bipolar processes. Irregular and bushy lamellar projection extend from both the cell body and its processes (arrowheads). Paraffin section. GFAP immunoperoxidase stain. ×1,000. C. A radial glial cell with bipolar processes in the subventricular zone of a 17-wk-old human fetal cerebrum. Note irregular
to show immunostaining for GFAP, especially in younger specimens. By 17 wk of age, however, GFAP immunoreactivity within radial glia was crisper and more intense. As the cerebral cortex increased in thickness the radial glial fibers were broken up into segments. The immunoreactive fibers were thicker at the ventricular and subventricular zones but became much finer in the intermediate zone (Fig. 4). While parallel arrays of radial glial fibers were oriented perpendicular to the pia mater, many migrating neurons were seen in horizontal rows within the subventricular and intermediate zones. At this age the somas of radial glial cells were situated largely within the subventricular zone. There were many bipolar forms with some placed either at the ventricular surface or within the subventricular zone (Figs. 4, 5). In older fetuses the pattern of GFAP immunoreactivity within radial glial cells closely resembled images obtained in Golgi preparations, with characteristic bushy lamellar projections extending from the soma and from the shaft (Fig. 5B, C).

Electron microscopy demonstrated alternating pattern of GFAP immunoreactivity within radial glial fibers in the intermediate zone (Fig. 6). This pattern was already apparent at 12 wk of age.

In addition to radial glial cells, numerous GFAP-positive astrocytes were observed in the cerebral cortex. These were prominent within long fiber tracts, beneath the pial surface and around blood vessels. A more detailed description of these findings will be the subject of a separate report.

**DISCUSSION**

By applying a highly sensitive immunoperoxidase technique to suitably fixed human fetal cerebral tissue we have been able to confirm the results of a previous study from this laboratory using indirect immunofluorescence (9) by demonstrating once again the presence of GFAP within radial glia both at the light and the EM level. The unequivocal demonstration of GFAP within radial glia in paraffin-embedded material indicates that the pattern of GFAP immunoreactivity observed in "routinely" processed autopsy brains, in which fixation is usually suboptimal, must be interpreted with care. Immunoreactivity for GFAP is a function of the method of tissue preparation or the stage of GFAP within the tissue. In suboptimally fixed material, therefore, the absence of GFAP staining in any given cell or tissue does not necessarily rule out its presence (6).

Although Golgi methods and EM studies are useful in identifying glial cells in the developing nervous system, immunoperoxidase methods currently provide one of the most reliable means of cell identification by demonstrating the presence of cell-specific proteins. Therefore, radial glial cells represent the earliest cells of astrocytic lineage that can be distinguished among the cellular elements in the developing central nervous system.

In human fetal cerebral cortex GFAP immunoreactivity within radial glial cells is demonstrable in fetuses of ten wk and older. In monkey cerebrum the 40th embryonic day is the earliest age at which these cells show immunoreactivity for GFAP (27). Expression of GFAP may not be the earliest specific feature of developing glial cells. Vimentin, an intermediate filament protein found primarily in mesenchymal cells, is also reported to be present in embryonic glial cells of the rat (28), chick (29), and mouse (30, 31). The specificity of vimentin within any given glial

![lamellar projections along the shaft (arrowheads). Paraffin section. GFAP immunoperoxidase stain. ×1,000. D. A GFAP-positive bipolar radial glial cell in the intermediate zone of a 17-wk-old human fetal cerebrum. Paraffin section. GFAP immunoperoxidase stain. ×1,000.](http://jnen.oxfordjournals.org/)
Fig. 6. Alternating GFAP immunoreactivity (empty arrows) in the radial fibers of the intermediate zone of a 12-wk-old human fetal cerebrum. Processed by pre-embedding GFAP immunoperoxidase stain. A. ×15,000. B. ×11,550.
cell type is still a matter of debate; nevertheless, its expression appears to occur very early in the developing central nervous system. We have attempted to demonstrate vimentin in our material using commercially available monoclonal antisera, but the results have so far been inconclusive. It is possible that fixation in 4% glutaraldehyde for 18–24 h may have interfered somewhat with the visualization of this protein. Further studies are needed. The studies of Levitt et al (23) in the developing fetal monkey telencephalon have convincingly demonstrated the onset of phenotypical glial expression prior to the last cell division. The presence of GFAP with mitotic cells at the ventricular zone of a 12-wk-old human fetal cerebrum as shown in this study indicates that cells of astrocytic lineage are being generated while active neurogenesis is still taking place at this site.

ACKNOWLEDGMENTS

The author thanks Ms. Teresa Espinosa, Mrs. Loan Duong, Ms. Virginia Bayer and Mr. Cyrus Choy for technical support and Ms. Diane Pelisse-Dobbs for secretarial help.

REFERENCES


(Received 19 August 1985/Accepted 12 November 1985)