Human Brain in Tissue Culture*

VI. Presence of Glial Fibrillary Acidic Protein in Subcultivated Human Fetal Brain Cells as Demonstrated by Immunofluorescent and Immunoperoxidase Staining

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Summary. Glial fibrillary acidic protein (GFAP) was present in cell cultures derived from human fetal brain tissue as determined by indirect immunofluorescence (IF) and immunoperoxidase (IP) staining using rabbit anti-human GFAP antisera. The IF and IP techniques were comparable in localizing the cytoplasmic distribution and the frequent perinuclear concentration of GFAP in brain cells. The horseradish peroxidase technique was more sensitive and a 1:20 - 1:40 dilution of anti-GFAP serum could be applied in the initial step of the peroxidase staining as compared to a 1:10 dilution of anti-GFAP serum for IF staining. Sequential studies of subcultivated human fetal brain cell lines by these techniques indicated that some brain cell lines become GFAP-negative rapidly, whereas other cell lines remain GFAP-positive for no less than ten subcultivations in vitro. GFAP protein was never present in any PML-SV40-transformed human brain cells.

Key words: Human brain – Tissue culture – Glial antigen

The most useful technique for identifying brain cells in vitro has been immunochemical staining using antibody prepared against various central nervous system (CNS) components. One of the more extensively studied proteins has been glial fibrillary acidic protein (GFAP), a normal component of human and rodent astrocytes [3]. Antisera against GFAP prepared in rabbits [12] have been utilized to identify astrocytes in vivo [2] and in vitro [1].

Most of these in vitro studies have been done on primary cell cultures derived from explanted brain tissue. We have previously reported the presence of GFAP in subcultivated human brain cells [5], but until now have not attempted to estimate the frequency of the phenomenon with which these cells remain positive during repeated passage in culture.

This current study describes a more thorough quantitation of persistence of GFAP-positive cells in subcultivated cultures of human fetal brain tissue. The techniques of indirect immunofluorescence and indirect immunoperoxidase staining were used throughout the study for localization of GFAP in subcultivated human fetal brain cells.

Material and Methods

Seven lines from four human fetal brains were prepared and maintained as previously described [4, 11]. Non-CNS cultures included one line of human fibroblast cells, mouse embryo fibroblasts, and CV1 cells (a continuous line of African green monkey kidney).

Monolayer cultures of all the cell lines were prepared on 11 x 22 mm glass coverslips, washed three times in phosphate-buffered saline (PBS) (pH 7.0) and fixed in methanol for 5 - 10 min. Indirect immunoperoxidase (IP) staining was carried out by incubation of the cells with a 1:20 - 1:40 dilution of rabbit anti-GFAP immune serum for 30 min at 37°C. The cells were then washed three times and incubated with a 1:100 dilution of horseradish peroxidase (HRP) conjugated sheep anti-rabbit immunoglobulin (IgG) (Cappell Laboratories, Downingtown, PA) for another 30 min at 37°C. Cells were then washed three times more in PBS and incubated at 37°C for 5 min with a fresh solution of 0.05% 3,3′-diaminobenzidine tetrahydrochloride (Sigma Chemical Company, St. Louis, MO) and 0.15% H2O2 in 0.1 M Tris buffer (pH 7.4). Cells were then washed three times in distilled water, counterstained with cresyl violet, dehydrated, and mounted with Permount. The mounted coverslips were scanned for labeled cells under bright field illumination.

To compare the sensitivity and specificity of the HRP-conjugated antibody technique with the indirect immunofluorescence (IF) technique, a parallel series of various CNS and non-CNS cells were also stained by indirect IF using rabbit anti-GFAP immune serum as...
previously described [5]. Among these cells were permanent lines of PML-SV40-transformed human brain cells [10]. In both the IP and IF procedures, controls were provided by the substitution of normal rabbit serum for rabbit anti-GFAP immune sera as well as by the application of rabbit anti-GFAP serum to non-CNS cells.

**Results**

GFAP was present in multiple subcultivations of cell cultures derived from human fetal brain as demonstrated by both indirect IF and IP. GFAP was always cytoplasmic and often concentrated in the cell processes and perinuclear region of the cell. At least two cell types contained GFAP. The first was a large polygonal cell with multiple processes giving rise to a fibrillar or fanning appearance (Fig. 1b, d). This cell usually had one nucleus and two to four nucleoli. The second type was a smaller cell with the nucleus occupying most of the cell. The cytoplasm was scanty and three to four

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**Fig. 1a–e.** Human fetal brain cell(s) in a second and b fourth subcultivation. Perinuclear concentration and extension of GFAP into cell processes are seen; indirect immunofluorescence using rabbit anti-GFAP immune serum and goat anti-rabbit IgG-FITC; c second and d fourth subcultivation; indirect immunoperoxidase using rabbit anti-GFAP immune serum and horseradish-conjugated goat anti-rabbit IgG; e fourth subcultivation; indirect immunofluorescence substituting normal rabbit serum for rabbit anti-GFAP immune serum. a × 93; b × 58; c, d × 250; e × 120