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Short Contribution

Astroglia-microvessel relationship in the developing human telencephalon

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ABSTRACT The telencephalon of 12 and 18 week-old human foetuses was examined for evidence of astroglia-microvessel relationship. Immature astroglia cells (radial glia and astroblasts) and astrocytes were immunostained using antibodies to the cytoskeletal proteins vimentin (VIM) and glial fibrillary acidic protein (GFAP). The microvessels were detected using an antibody to the blood-brain barrier (BBB)-specific glucose transporter GLUT1. Two extracellular matrix (ECM) glycoproteins, laminin (LM), an endothelial-derived molecule, and tenascin-C (TN-C), a glia-derived molecule, were also analyzed. In the two stages examined, VIM- and GFAP-positive fibers of the radial glia establish close relationships with the radial and periventricular microvessels, which are GLUT1-positive and lined by an LM-positive basal lamina-like matrix. At the 18th week, also radial glia transitional forms and immature astrocytes exhibit extensive contacts with the microvasculature. A TN-C-rich ECM is revealed around the vascular plexus of ventricular zones at the 12th week, and around the newly growing radial microvessels and the microvessel branching sites at the 18th week. The observations taken as a whole, suggest that during the telencephalon morphogenesis the immature astroglia cells play a role in the early establishment of the distribution pattern of the neural microvessels and in their growth and maturation.

KEY WORDS: *astroglia cells, radial glia, microvessels, extracellular matrix molecules, human telencephalon*

The precursors of the astroglial cell lineage are radial glia cells which originate within the proliferative, ventricular zone (VZ) and subventricular zone (SVZ) of the telencephalic vesicles during the early stages of neurogenesis. The radial glia cells are characterized by a long, radial process contacting the basal lamina at the pial surface and express the astroglial cell cytoskeletal proteins, vimentin (VIM, primary radial glia cells) and glial fibrillary acidic protein (GFAP, mature radial glia cells) (Choi and Lapham, 1978; Cameron and Rakic, 1991; Rakic, 1995). At the end of neurogenesis, these transient cells retract their processes, migrate, and finally transform into multipolar astrocytes of the cortex and subcortical white matter (Schmechel and Rakic, 1979). Astrocytes are well known to establish close anatomical and functional relationships with the adult brain microvessels and to induce the expression and maintenance of the blood-brain barrier (BBB) phenotype in the brain endothelial cells (Janzer and Raff, 1987; Tao-Cheng *et al.*, 1987; Bertossi *et al.*, 1993). The aim of this study was to investigate the developmental roles played by the astroglia cells during vascularization of the human brain. Glial, endothelial, and extracellular matrix (ECM) markers were used for immunohistochemical analysis in the telencephalon of human foetuses at 12 and 18 weeks. Antibodies to VIM and GFAP were used to reveal the astroglia cells. The BBB-endothelial phenotype was detected using an antibody to the isoform 1 of the glucose transporters (GLUT1) (Pardridge and Boado, 1993). The presence and distribution of two ECM molecules, laminin (LM), an endothelium-derived molecule of the brain microvessel basal lamina (Risau and Lemmon, 1988), and tenascin-C (TN-C), a glia-derived molecule (Faissner and Schachner, 1995), were also analyzed.

Abbreviations used in this paper: MZ, marginal zone; CP, cortical plate; SP, subplate; IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone; VIM, vimentin; GFAP, glial fibrillary acidic protein; GLUT1, glucose transporter isoform 1; LM, laminin; TN-C, tenascin-C; ECM, extracellular matrix; BBB, blood-brain barrier.

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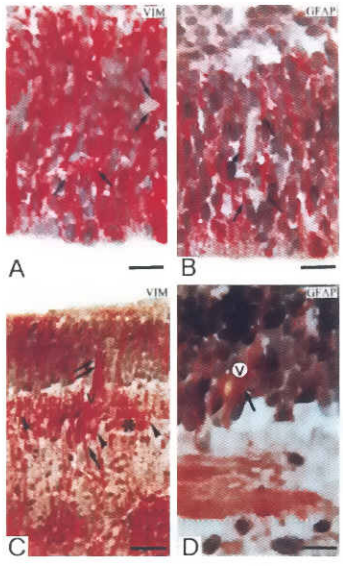

Fig. 1. VIM (A,C) and GFAP (B,D) immunostainings in the telencephalon of a 12-week foetus. (A,B) *In the VZ, thick radial glia fibers, which are VIM- and GFAP-positive, form thin septa among rows of ventricular cells and adhere to the wall of small periventricular microvessels (arrows).* **(C)** *The radial pattern of VIM-positive radial glia is recognizable throughout the neural wall. Glial fibers of the IZ (arrowheads) are crossed by tangential bundles of axons (asterisk). A radial microvessel (v) is closely paralleled by radial glia fibers both in IZ (arrow) and CP (double arrow).* **(D)** *GFAP-positive radial fibers of the CP extensively contact the wall of an obliquely sectioned radial microvessel (v, arrow). Bar, 25 μm in A,B,D; 50 μm in C.*

VIM and GFAP immunohistochemistry

At the 12th week, the telencephalic wall consists of five layers: ventricular zone (VZ), subventricular zone (SVZ), intermediate zone (IZ), cortical plate (CP), and marginal zone (MZ) (Boulder Committee, 1970). At this age, the VZ and SVZ are wide layers formed by densely-packed, strongly VIM-positive precursors of neuronal cells and bodies and processes (radial fibers) of the primary radial glia cells (Fig. 1A). GFAP-immunolabeled radial glia cells are also recognizable within the VZ and SVZ (Fig. 1B). In the poorly cellular IZ, intensely VIM-positive radial glia fibers are seen among tangential bundles of axons (Fig. 1C). VIM- and GFAP-immunostained radial fibers are situated between the parallel rows of neuroblasts in the CP, and terminate in the MZ with knob-like pial endings (Fig. 1C,D). The radial glia fibers are seen to establish contacts with the microvasculature, both surrounding the small meshes of the vascular network (periventricular plexus) located in the VZ and SVZ (Fig. 1A,B) and coursing closely to the scarce radial microvessels of the IZ and CP (Fig. 1C,D). At the 18th week, the thickness of the telencephalic wall has increased; the subplate (SP) becomes recognizable and the CP is the most prominent layer. The VZ and SVZ are constituted by many VIM- and GFAP-positive radial glia cells and fibers (Fig. 2A,B). In the IZ and SP, the ascending VIM- and GFAP-positive radial fibers give rise to short, transverse processes; here transitional forms of the radial glia are also recognizable, as mono- and bipolar cells with thick, radially oriented processes (Fig. 2C-E). In the CP, the regular arrangement of the radial glia fibers is interrupted by the presence of multipolar, VIM-positive astroblasts and GFAP-positive young astrocytes (Fig. 2F-H). At this age, the fibers of the radial glia cells extensively invest the vascular meshes of the periventricular plexus (Fig. 2A,B) and tightly parallel the numerous radial microvessels passing through the subcortical and cortical layers (Fig. 2C-G). In the CP, astroblasts and astrocytes are also seen to expand their processes on the vascular walls (Fig. 2F,H).

GLUT1 immunohistochemistry

At the 12th week, the walls of both the radial microvessels and the small meshes of the periventricular plexus are GLUT1-positive (Fig. 3A-C). At the 18th week, the CP radial microvessels are more numerous and give rise to collateral branches, which build the first large meshes of the cortex vascular network by transverse and oblique anastomoses. The vascular walls are intensely GLUT1-positive throughout (Fig. 3D-F).

LM and TN-C immunohistochemistry

At the 12th week, the microvessels of the telencephalic wall are almost continuously outlined by an LM-positive, basal lamina-like matrix that also underlines the surface of pericytes bulging out from the vessel profile (Fig. 4A,B). At the 18th week, the LM-positive basal lamina is more defined; it appears split by the pericyte bodies in two, inner and outer, distinct layers (Fig. 4C).

At the 12th week, TN-C immunolabeling is absent in the MZ and CP (Fig. 4D), weak in the IZ, and strong in the SVZ and VZ; in the latter two zones, a TN-C-positive ECM fills the intercellular spaces and

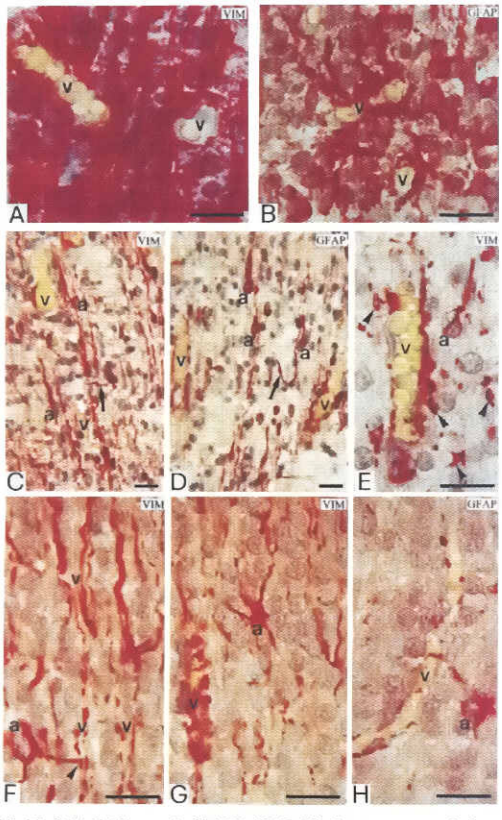

Fig. 2. VIM (A,C,E,F,G) and GFAP (B,D,H) immunostainings in the telencephalon of an 18-week foetus. (A) *VIM-positive fibers of radial glia wrap microvessels (v) of the SVZ.* **(B)** *Intensely GFAP-positive perivascular processes circumscribe microvessels (v) of the SVZ.* **(C,D)** *In the IZ, VIM- and GFAP-immunostained transitional forms of radial glia are recognizable as radially oriented unipolar and bipolar astrocytes (a). The radial glia fibers are provided with transverse and oblique side-branches (arrow). Glial fibers tightly surround the microvessels (v).* **(E)** *In the SP, short tracts of VIM-immunostained radial fibers (arrowheads) are recognizable both in the neuropil and along the microvessel wall (v). Note two postmitotic astroblasts (a).* **(F,G)** *In the CP, among rows of neuroblasts and radial glia fibers, multipolar VIM-positive astroblasts (a) are detectable. Radial microvessels (v) are surrounded by radial glia fibers. In F, an astroblasts process (arrowhead) contacts a microvessel.* **(H)** *A microvessel of the CP decked with GFAP-positive glial feet, and a multipolar astrocyte (a) sending a process to the vascular wall are shown. Bar, 25μm.*

surrounds the meshes of the periventricular plexus microvessels (Fig. 4E,F). At the 18th week, a TN-C-rich ECM surrounds the radial microvessels new-growing into the nervous wall (Fig. 4G); a perivascular TN-C is also clearly detectable both at the branching sites and along the transverse collaterals of the radial microvessels (Fig. 4H,I). In the SVZ and VZ, the intercellular and perivascular TN-C immunostaining is weaker than in the 12-week telencephalon (Fig. 4L).

The present findings contribute data on the radial glia differentiation in the human telencephalic vesicles. At the 12th gestational week, radial glia cells and fibers mainly express the cytoskeletal protein VIM, only few radial glia cells being revealed by the GFAP immunostaining. At the 18th week, more numerous GFAP-positive radial glia cells are revealed, together with unipolar and bipolar transitional forms of the radial glia. Moreover, both VIM-positive astroblasts and young, GFAP-positive astrocytes become recognizable in the CP.

As regards the astroglia-microvessel relationship, the immunocytochemical analysis reveals that the radial glia fibers are closely associated to the radial microvessels as early as the 12th week. This glia-microvessel relationship becomes even more extensive by the 18th week, when also processes of astroblasts and young astrocytes participate in the formation of the glial sheaths of the cortex primitive vascular network. The results on the close relationships between astroglial cells and vasculature suggest that radial glia, in addition to providing a scaffold for postmitotic, migrating neuroblasts (Rakic, 1971), may provide a supportive guideline to the microvessels which arise from the leptomeningeal vessels and develop within the nervous wall in a radial pattern (Bär, 1980; Duvernoy *et al.*, 1981; Norman and O'Kusky, 1986). Moreover, the observation that the glia-associated microvessels express signs of maturation, the GLUT1-positive BBB-endothelial phenotype and an LM-containing basal lamina, indicates that BBB formation is already in progress in the mid-gestation human telencephalon and suggests that also immature glial cells might be involved in the development of the BBB.

The pattern of expression of TN-C points out a further possible developmental role of the radial glia in the early vascularization of the human telencephalon. TN-C is a multifunctional glycoprotein of the ECM which in the CNS is expressed by radial glia cells and is involved in many neurohistogenetic events (Faissner and Schachner, 1995). TN-C expression also correlates with vasoproliferative events, such as angiogenesis in tumoral tissues (Zagzag *et al.*, 1996) and migration and proliferation of endothelial cells *in vitro* (Canfield and Schor, 1995). In the human telencephalon, TN-C is strongly expressed around the growing microvessels of the proliferative ventricular zones at the 12th week, and around the newly penetrating radial microvessels and the microvessel branching points at the 18th week. These findings seem to suggest that the brain vessel growth, though stimulated by angiogenic factors (Breier *et al.*, 1992) and controlled by local metabolic requirements (Bär, 1980; Roncali *et al.*, 1985; Norman and O'Kusky, 1986; Edvinsson *et al.*, 1993), could also be mediated by the radial glia cells *via* TN-C production.

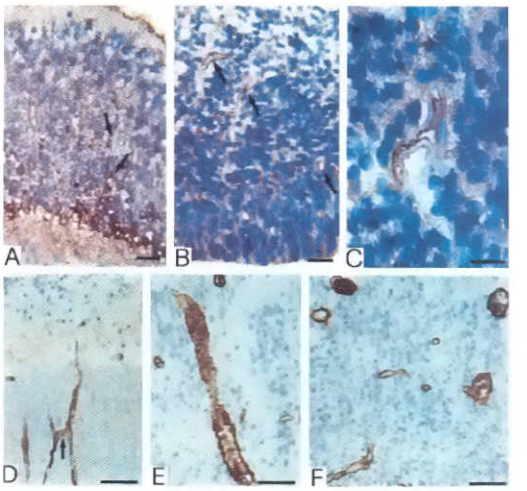

Fig. 3. GLUT1 immunostaining in the telencephalon of 12- (A-C) and 18- (D-F) week foetuses. (A) *A radial microvessel of the CP is stained by GLUT1 (arrows).* **(B)** *In the VZ and SVZ most of the microvessels of the periventricular plexus are GLUT1-positive (arrows).* **(C)** *At high magnification, the vessel endothelial layer appears GLUT1-labeled.* **(D)** *The radially oriented microvessels of the CP are GLUT1-positive, two of them are connected by a GLUT1-positive anastomosis (arrow).* **(E,F)** *A radial vessel of the IZ (in E) and microvessels of the periventricular plexus (in F) appear strongly GLUT1-positive. Bar, 25 μm.*

TABLE 1

LIST OF THE ANTIBODIES USED IN THE PRESENT STUDY

ANTIBODY	IMMUNOGEN	CLONE	DILUTION	PRETREATMENT
Mouse monoclonal IgG anti-VIM[1]	VIM from porcine eye lens	V9	1:10	Microwave
Mouse monoclonal IgG anti-GFAP[2]	GFAP from porcine spinal cord	GA-5	1:200	Microwave
Rabbit polyclonal anti-GFAP[3]	GFAP from bovine spinal cord	-	undiluted	Microwave
Rabbit polyclonal ant-GLUTI[4]	Synthetic peptide based on Rat brain GLUTI	-	1:1000	-
Rabbit polyclonal anti-LM[5]	LM from EHS mouse sarcoma	-	1:25	Ficin
Mouse monoclonal IgG anti-TN-C[1]	Human TN from U251 glioma cells	Tn2	1:15	Ficin

[1]Dako Italia, Milan, Italy; [2]BioGenex Laboratories, San Ramon, CA, USA; [3]Incstar, Stillwater, MN, USA; [4]East Acres Biologicals, Southbridge, MA, USA; [5]Euro-Diagnostica, Malmö, Sweden.

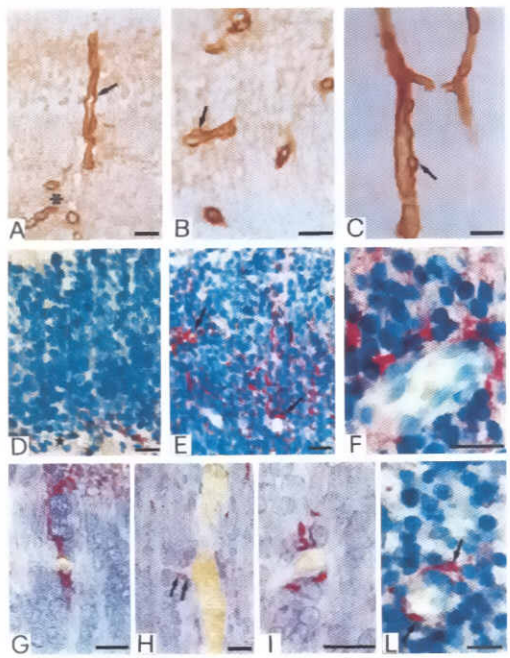

Fig. 4. LM (A-C) and TN-C (D-L) immunostainings in the telencephalon of 12- (A,B, D-F) and 18- (C, G-L) week foetuses. (A) *LM immunostaining reveals the basal lamina-like layer of a radial vessel (arrow) and vascular meshes of the periventricular plexus (asterisk).* **(B)** *Microvessels of the VZ seen at higher magnification, the arrow points a pericyte body outlined by an almost continuous, LM-positive layer.* **(C)** *Radial microvessels and their side-branches in the CP. The continuous LM-positive layer appears split in an outer and an inner lamina (arrow).* **(D)** *No TN-C staining is detectable in the CP and only a faint reactivity is seen at the boundary with IZ (star).* **(E)** *The SVZ and VZ are TN-C-positive zones. TN-C is present among the packed ventricular cells and all around microvessels of the periventricular plexus (arrows).* **(F)** *At high magnification, the strong, microvessel-associated TN-C immunostaining.* **(G)** *A vascular sprout penetrating the CP is embedded in a TN-C-positive ECM.* **(H)** *In the CP, TN-C is detected at the branching site of a radial vessel (arrows).* **(I)** *A cross-sectioned collateral of a radial vessel surrounded by TN-C.* **(L)** *The TN-C immunostaining is faint in the intercellular and perivascular ECM of the SVZ (arrows). Bar, 50 μm in A,G,H,I,L; 25 μm in B,C,D,E,F.*

Experimental procedures

Six foetuses, three at 12 and three at 18 weeks of gestation, were obtained from spontaneous abortions. The gestation age was estimated on the basis of the crown-rump length and/or pregnancy records of gestational age (based on the last menstrual cycle). The foetal brains were dissected 'en bloc' according to the procedure for human autopsy at the University of Bari School of Medicine. For each brain, the mid-lateral area of the telencephalon was fixed either in Bouin's solution or in 2% paraformaldehyde plus 0.5% glutaraldehyde in phosphate-buffered saline (PBS) for 2 h at 4°C, embedded in paraffin and sectioned at 5 μm. The sections were cut perpendicularly to the brain surface, collected on silane-coated slides, rehydrated in xylene-alcohol, placed in Tris-buffered saline (TBS), and submitted to immonohistochemical pretreatments (Table 1). The sections were treated with normal swine serum and incubated overnight at 4°C in a moist chamber with the primary antibodies detailed in Table 1. The immunostainings were performed with the alkaline phosphatase-anti-alkaline phosphatase (VIM, GFAP, TN-C) or the avidin-biotin peroxidase complex (GLUT1, LM) techniques using commercially available detection kits. Finally, the sections were counterstained with Mayer's haemalum. Negative controls were achieved by substituting primary antibodies with mouse/rabbit preimmune sera. Adult human brain tissue was used as positive control for GFAP, GLUT1, and LM; glial tumor for VIM and TN-C.

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