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The status and organization of astrocytes, oligodendroglia and microglia in grafts of fetal rat cerebral cortex

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Abstract

Immunohistochemical methods were used to study the status and organization of astrocytes, oligodendroglia and microglia in fetal cerebral cortical tissue grafted on to the dorsal surface of the midbrain in newborn host rats. Grafts were examined 1–6 months post-transplantation. All grafts contained large numbers of hypertrophied, intensely glial fibrillary acidic protein-positive astrocytes. Microglia were also activated, displaying slightly increased levels of OX-42 immunoreactivity. The grafts consisted of lobules of gray matter which were separated by bands of myelinated fibres associated with large numbers of Rip-positive oligodendroglia. These glial cells had a relatively normal morphology. The density of astrocytes and microglia was reduced in these white matter-like regions. In association with chronic changes in glial reactivity, transplants also expressed increased levels of chondroitin sulphate proteoglycans (CS-56 antibody). The observed changes in glial cell phenotype and extracellular matrix in cortical transplants are likely to affect neuronal physiology and connectivity in a number of ways, and highlight the importance of studying both glia and neurons in order to gain a more comprehensive picture of the long-term functional potential of fetal brain grafts. © 1997 Elsevier Science Ireland Ltd.

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Transplants of fetal cerebral cortical tissue have been widely used to study aspects of development, plasticity and regeneration in the mammalian central nervous system (CNS). Issues examined include (1) the regional specification of cortical areas [1,21], (2) the development of neuronal phenotype [15], (3) the formation of specific axonal connections [6], (4) trophic interactions between cortex and other regions [13], and (5) the possible beneficial effects of cortical grafts in regenerative repair after ischemic or traumatic injuries in neonatal [8] or adult [26] hosts.

The majority of studies have focused on neuronal function within fetal cortical grafts, relatively few on the status and organization of macroglia and microglia within the transplanted tissue. Those reports that have examined glial cells have concentrated mainly on the characterization of astrocytes. An interesting finding, in both intraocular and intracranial cortical grafts, is that astrocytes are often hypertrophied and express high levels of glial fibrillary acidic protein (GFAP) [2,3]. Such reactive changes are likely to impact upon the maturation and function of transplanted cortical neurons as well as other CNS glia [4,7,20,23].

In the present study we have used immunohistochemical techniques to compare the status and distribution of astrocytes, oligodendroglia and microglia in fetal cerebral cortex transplanted to the midbrain of newborn rats of the same strain. Grafts were examined from 1 to 6 months after transplantation. Glial organization in grafts was compared to that seen in normal host neocortex. Because hypertrophied, reactive astrocytes can express increased amounts of various extracellular matrix molecules [5,10,19], we also studied the expression of chondroitin sulphate proteoglycans (CSPG) in these grafts and compared the level of immunoreactivity with that seen in host cortex.

Time-mated donor and host inbred PVG/c rats were used.

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Pregnant rats of E15 gestation (day after mating, E0) were anaesthetized with halothane, the embryos removed and placed in cold Hams F-10 (Gibco) medium. Neocortical neuroepithelium (150–200 μm thick) was dissected out from the fetal cerebral hemispheres and freed of adhering membranes. The pieces of embryonic tissue were trimmed (to a mean area of about 2 mm^2) and were then transplanted via a glass micropipette onto the midbrain of ether-anaes-

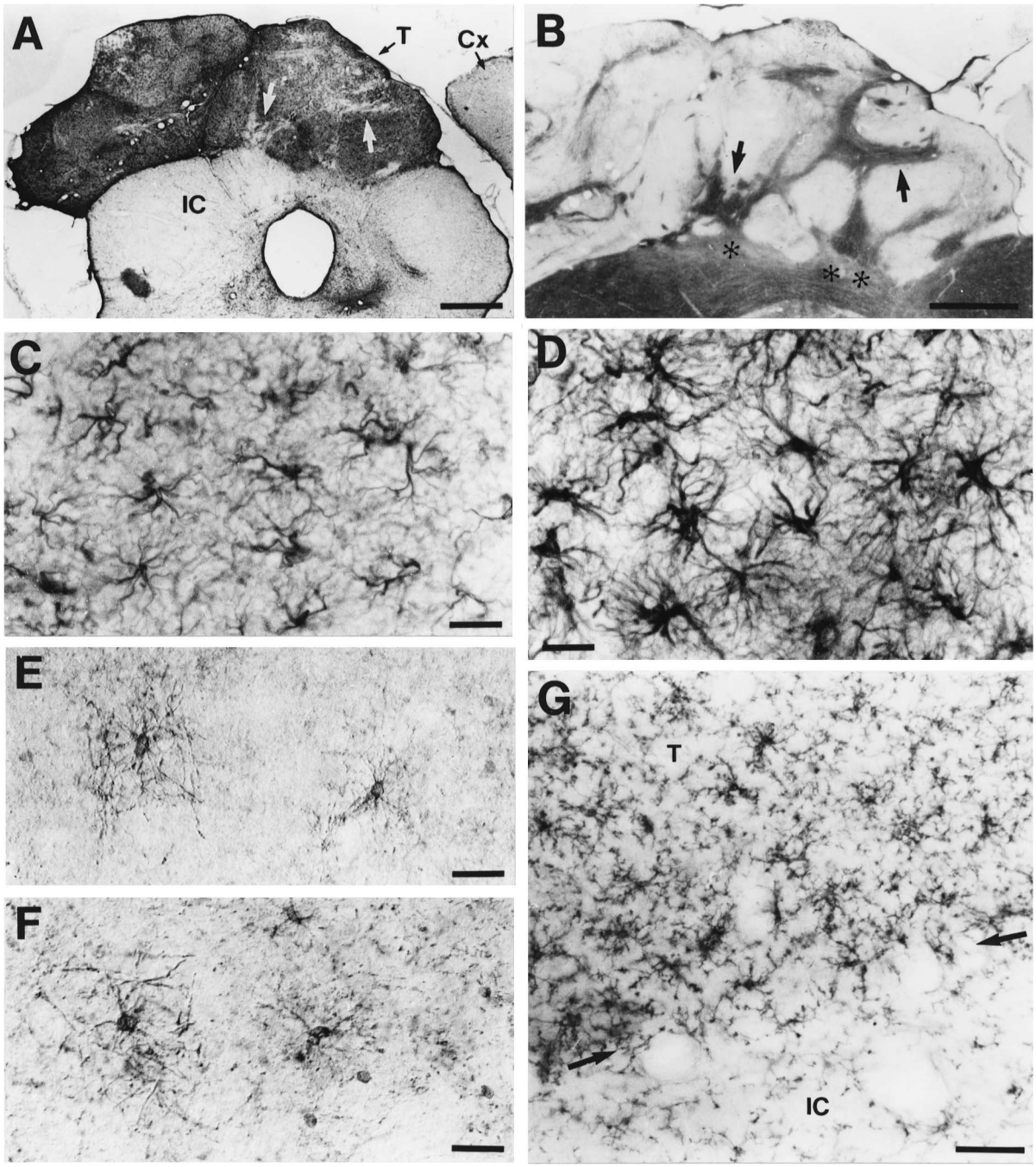

Fig. 1. (A,B) Adjacent coronal sections of a cortical graft, 88 days post-transplantation, immunostained for GFAP (A) or Rip (B). There was more intense GFAP immunoreactivity in the graft (T) compared with host cortex (Cx). Note the lobular nature of the grafts (B), and that areas of the graft containing white matter-like regions (arrows in (B)) contained comparatively few astrocytes (arrows in (A)). The asterisks in (B) show regions of fusion and fibre continuity between host inferior colliculus (IC) and overlying grafted tissue. (C,D) High power photomicrographs showing GFAP immunoreactivity in host cortex (C) and in a cortical graft (D), 88 days post-transplantation. (E,F) Rip$^+$ oligodendrocytes in grey matter of normal cortex (E) and in a grey matter-like region in a transplant (F), 102 days post-transplantation. Note the similar morphologies of oligodendrocytes in normal and grafted tissue. (G) OX-42 immunoreactivity in a cortical graft (T) and in host IC, 102 days after transplantation. Microglia in grafts generally displayed increased levels of OX-42 immunoreactivity. Scale bar, (A,B) 1 mm; (C–F) 25 μm; (G) 50 μm.

thetized newborn rats (see refs. [14,17] for details). At the time of transplantation, each cortical graft consisted of tissue from the ventricular through to the pial surfaces and thus contained presumptive gray and white matter regions.

One to six months later, host rats were deeply anaesthetized with sodium pentobarbitone (i.p., Nembutal; Abbott) and perfused with 1% sodium nitrite followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Twelve animals were used in the present study (taken from five host litters). Three rats were perfused at 1 month, two at 2 months, three at 3 months, two at 4 months and two rats were perfused at 6 months. Brains were post-fixed for 1–2 h, encapsulated in egg-yolk and allowed to sink in a cold 30% sucrose buffer solution. Frozen, coronal sections (40 μm) were cut through the transplants and caudal parts of host cortex.

Parallel series of sections were immunoreacted with monoclonal antibodies to either: GFAP (astrocytes) (Boehringer), OX-42 (rat CD11b/CD18, microglia/macrophages, monocytes, granulocytes [24]) (Serotec), Rip (oligodendroglia/myelin [9]) or CSPG (CS-56 antibody; Sigma). Rip was used undiluted; other antibodies were diluted in Dulbecco's 'A' phosphate buffered saline containing 1% bovine serum albumin (Sigma) and 0.2% Triton X-100. Optimal dilutions were (GFAP) 1:20, (OX-42) 1:1500, (CS-56) 1:500. After an initial incubation in 0.6% hydrogen peroxide, floating sections were incubated with primary antibodies overnight at 4°C. Sections were washed, processed using the avidin-biotin-peroxidase complex method (Vectastain Elite) and then incubated in 0.05% DAB in 0.05 M Tris buffer (pH 7.6) and 0.02% hydrogen peroxide. Sections were mounted onto subbed slides, dehydrated and coverslipped with Depex (BDH). Control sections were prepared in which primary antibodies were omitted during the overnight incubation. Series of sections from some transplants were stained for luxol fast blue (myelin) and cresyl violet (Nissl).

Large cortical grafts were seen in all 12 host animals; in each case they were located on the dorsal surface of the host midbrain and/or rostral cerebellum (Fig. 1A,B). Two grafts had no obvious tissue continuity with the underlying host brain. Of the remaining 10 transplants, although most of the graft surfaces were separate from the host and were invested with their own pial/meningeal membranes, at least some part of the ventral surface of the grafted tissue was fused with the host midbrain (Figs. 1A,B and 2A).

Cortical grafts examined 1–6 months after transplantation were composed of a number of gray matter-like areas or lobules containing neurons with different morphologies [14,15], separated by distinct bands of Rip-positive (Rip^+) myelinated axons (Fig. 1B). Rip^+ oligodendroglia were particularly numerous in the white matter-like regions in transplants but, as in normal cerebral cortex, oligodendroglia were also found in gray matter areas. Isolated Rip^+ oligodendroglia in gray matter of host cortex are shown in Fig. 1E. The processes of these cells can clearly be seen radiating out from the cell bodies. Compared to host cortex there were more oligodendroglia scattered within gray matter-like regions in cortical grafts (Fig. 1F), nonetheless these glia had a similar appearance to Rip^+ cells in normal tissue (Fig. 1E). In Rip immunostained sections it was possible to identify localized areas of fibre continuity between grafts and underlying host tissue (asterisks in Fig. 1B).

At all ages post-transplantation, cortical grafts situated on the host brainstem exhibited abnormally high levels of GFAP immunoreactivity compared to host cerebral cortex in the same animal (Fig. 1A). This increased GFAP immunostaining was seen in grafts contiguous with the underlying host midbrain as well as in isolated cortical grafts. The latter observation suggests that most if not all glial cells in these transplants were graft-derived and few migrated in from the host brain (see also Ref. [17]). Astrocytes with intense GFAP immunoreactivity were found throughout the gray matter-like areas in transplanted tissue. The density of these cells was significantly reduced in graft regions con-

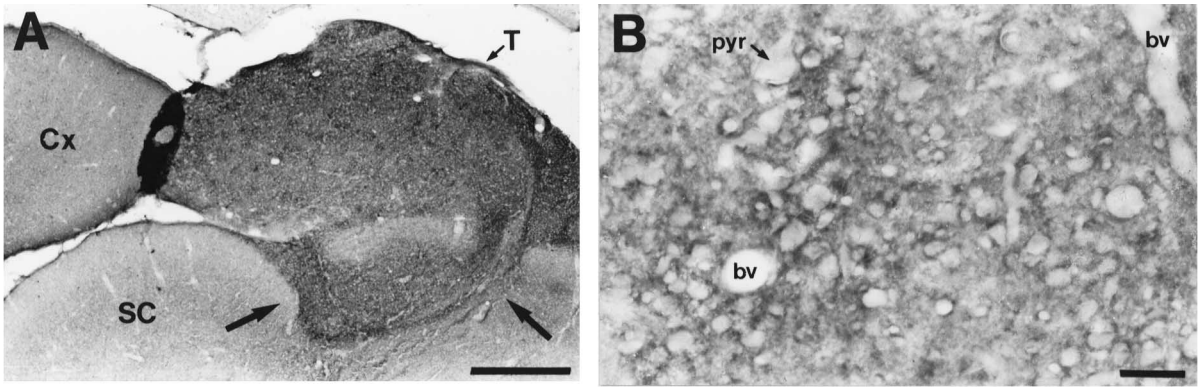

Fig. 2. Sections from a cortical graft (90 days post-transplantation) immunostained with the CS-56 antibody to chondroitin sulphate proteoglycans. (A) Low power view showing the graft (T) located on the host superior colliculus (SC). CS-56 immunoreactivity is much higher in the graft than in adjacent host cortex (Cx). Note that part of this graft is embedded in the host SC (arrows). The black area at the left hand edge of the graft is an artifact. (B) High power photomicrograph of grafted cortical tissue showing the extracellular nature of the CS-56 immunostaining. bv, blood vessels; pyr, large pyramidal neuron. Scale bar, (A) 1 mm; (B) 50 μm.

taining high levels of Rip immunoreactivity (compare arrows in Fig. 1A,B) and also in the Rip^+ tracts interconnecting graft and host midbrain (asterisks in Fig. 1B). Astrocytes in host cortex and in grafted tissue from the same animal are shown in Fig. 1C,D. Compared to host cortex (Fig. 1C), the density of astrocytes in grafts did not appear to be significantly increased, but in grafts the glia had a hypertrophied appearance with relatively large numbers of thick $GFAP^+$ processes (Fig. 1D).

Using the CS-56 monoclonal antibody, levels of CSPG were found to be considerably higher in cerebral cortical grafts compared to gray matter of host neocortex or midbrain (Fig. 2A). The extracellular nature of the immunostaining was especially obvious in transplanted tissue (Fig. 2B); note the dense immunoreactivity surrounding blood vessels (bv) and a transplanted neuron with pyramidal-like (pyr) morphology.

$OX\text{-}42^+$ cells in grafts had the appearance of ramified microglia [16] (Fig. 1G). Compared with host tissue there was generally an increase in OX-42 immunoreactivity in cortical transplants, although the level of staining varied somewhat from graft to graft. The graft shown in Fig. 1G contained the greatest increase in $OX\text{-}42^+$ staining of any graft in the study. Microglia located in the underlying host inferior colliculus can also be seen. As was the case for astrocytes, there were fewer immunostained microglia in Rip^+ white matter-like regions in cortical transplants.

Astrogliosis in intraocular and intracranial cerebral cortical grafts has been reported previously, and is especially prominent in grafts isolated from host tissue [2,3]. In the present study, a large increase in GFAP immunoreactivity was seen not only in isolated grafts but also in grafts with obvious fibre continuity with the underlying host midbrain (see also Ref. [12]). Others have shown that cortical tissue grafted to the neonatal midbrain develops efferent and some afferent connections with the host, but most of these connections are uncharacteristic of normal cortex [14]. Interestingly, neurons in cortical grafts do develop intrinsic (presumably cortico-cortical type) connections [14]. Thus if, as previous evidence suggests, isolation in some way influences glial cell reactivity in grafts [2,3], it must be the selective absence of afferent inputs and/or efferent connections with appropriate host targets that is paramount.

Activated astrocytes are known to produce a range of soluble growth factors, cytokines, adhesion molecules and extracellular matrix molecules [4,7,10,18–20] which can influence neuronal maturation and the extent and pattern of axonal growth into and within the graft neuropil. These influences may be positive or negative. For example, the increase in CSPG expression seen in cortical transplants might be expected to impede axonal growth [18,19] and thus reduce host innervation of the grafted tissue [14,15]. Only a few studies describing cerebral cortical transplantation have examined glial cell status in the grafts and to our knowledge the present study is the first to examine CSPG expression. If the changes we have found in fetal cortical grafts are a general phenomenon, they could have a potentially confounding effect in transplant studies concerned with the regional specification of neocortex and how specific axonal connections are established [1,6,21].

In addition to influencing synaptic organization and function [23], astrocytes also play critical roles in K^+ and pH homeostasis, and in the maintenance of the extracellular environment [27]. There are reports of abnormal electrophysiological characteristics in fetal grafts [11,22] and abnormalities in the blood–brain barrier have also been described [25], both perhaps a consequence of atypical astrocytic differentiation. Rosenstein [25] proposed that fetal neural transplants retain an immature structure and metabolism, and may be subject to a sublethal form of chronic ischemic injury. There are differences in the composition and geometry of the extracellular space (ECS) in developing CNS and in pathological situations such as ischemia or reactive gliosis after injury (reviewed in Ref. [27]). It will be of interest to determine whether such changes in ECS characteristics are seen in neocortical grafts.

Cortical grafts also contained increased levels of OX-42 immunoreactivity. These ramified microglial cells were apparently activated to some degree although they did not have a phagocytic appearance [16]. This activation in grafted tissue occurred even though attempts were made to minimize immunological effects by transplanting between genetically characterized inbred rats (PVG/c strain) and by using newborn hosts. The synergistic effects between astrocytes and microglia are well known [4,16,20] and involve a range of cytokines, growth factors and adhesion molecules. The long-lasting reactivity of these two cell populations in cortical grafts may involve some form of reverberatory interaction within the grafted tissue, perhaps similar to a low level inflammatory response. Factors released by astrocytes and microglia also affect oligodendroglial survival, proliferation and maturation [20], yet in grafts oligodendroglia were relatively normal in appearance and were distributed in a fashion similar to that seen in normal cerebral cortex. This is similar to their status in tectal grafts [12]. Why oligodendroglia do not get affected by the generally reactive environment of the grafts is unclear and is an issue that merits further attention.

Transplantation of human fetal brain material in an effort to treat human neurodegenerative disorders has met with some success. Like others however [10], we believe it is important to determine what happens to macro- and microglia in such transplants, and what impact changes in glial cell phenotype may have on the long-term functional potential of fetal brain grafts. The chronic astrocyte reactivity seen in cortical grafts may serve as an experimental model in which to study in vivo how changes in the status of these cells affect the maturation and function of CNS neurons, and may help to shed light on a variety of neuropathological conditions associated with inflammatory, ischemic and degenerative diseases.

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