Reports

Neocortical Transplants in the Mammalian Brain Lack a Blood-Brain Barrier to Macromolecules

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In order to determine whether the blood-brain barrier was present in transplants of central nervous tissue, fetal neocortex, which already possesses blood-brain and blood–cerebrospinal fluid barriers to protein, was grafted into the undamaged fourth ventricle or directly into the neocortex of recipient rats. Horseradish peroxidase or a conjugated human immunoglobulin G–peroxidase molecule was systemically administered into the host. These proteins were detected within the cortical transplants within 2 minutes regardless of the age of the donor or postoperative time. At later times these compounds, which normally do not cross the blood-brain barrier, inundated the grafts and adjacent host brain and also entered the cerebrospinal fluid. Endogenous serum albumin detected immunocytochemically in untreated hosts had a comparable although less extensive distribution. Thus, transplants of fetal central nervous tissue have permanent barrier dysfunction, probably due to microvascular changes, and are not integrated physiologically within the host. Blood-borne compounds, either systemically administered or naturally occurring, which should never contact normal brain tissue, have direct access to these transplants and might affect neuronal function.

NEURAL TRANSPLANTATION PROvides a significant tool to study the growth and development of neural tissue (*1*) and the amelioration of anatomical and physiological deficits in animal models of neural disease (*2*). Although transplantation experiments provide fresh insights into neuronal behavior, many parameters of these experiments remain undefined. This uncertainty poses a critical problem because such procedures have already begun to be used in the clinic (*3*). One of the fundamental characteristics of the mammalian brain is the blood-brain barrier (BBB). By its selective isolation of circulating compounds in the blood from those produced by the brain, the BBB effectively maintains the brain's homeostatic environment. Complete integration of a central nervous system (CNS) transplant with a CNS host would require that barrier properties that maintain neuronal (BBB) and fluid compartments [blood–cerebrospinal fluid (CSF) barrier] be completely established. Lack of barrier properties in CNS transplants could have important, although as yet undetermined, consequences for a newly developing neural system between host and graft.

Studies in my laboratory determined whether transplants from fetal neocortex have a normal BBB to macromolecules that usually are excluded from CNS compartments. Because in the fetal brain of several vertebrates including rat the blood-brain and blood-CSF barriers to protein are already established (*4*), we tested whether they were retained or altered over time, not whether they develop. It might be possible that transplant surgical procedures, or the rapid formation of a new circulation (angiogenesis) (*5*), could prevent the establishment of critical barrier properties. We found that blood-borne proteins, either systemically administered or naturally occurring, gain entry into the interstitial spaces of mature CNS transplants and the host CSF. After CNS transplantation, other circulating bioactive compounds might have comparable access to these normally impermeable compartments.

We systemically administered a glycoprotein (*6*) or glycoprotein–immune protein complex to host rats and subsequently detected the proteins histochemically. Pregnant Wistar rats were anesthetized with ether, fetuses were removed, and the fetal parietal cortex was dissected and freed of meninges in Earles' solution. Based on crown to rump length measurements, the donor fetuses ranged in age between 14 days and newborn. The wide range of donor ages was used in order to determine whether there existed any critical time for potential BBB alterations. A 1.0-mm^3 piece was either transplanted into the fourth ventricle where direct trauma to the brain is minimized (*7*) or inserted directly into the host cortex. All recipients were between 4 and 6 weeks of age; at this time the cerebral vasculature is mature. Postoperative periods ranged between at least 1 month, to ensure reconstitution of a potentially damaged BBB (*8*), to over 1 year. Horseradish peroxidase (HRP) (molecular weight, 40,000) (Sigma type VI, 1 mg per 5 g of body weight) was injected into the host femoral vein and circulated for periods between 2 and 50 minutes. Another group of hosts received a conjugated immunoglobulin G (IgG)–HRP complex (molecular weight, 190,000) (*9*) administered identically. After HRP circulation the animals were perfused first with balanced salt solution and then with 3% glutaraldehyde fixative in 0.1*M* sodium cacodylate buffer. The cortical transplants and surrounding brain tissue were removed and sectioned serially (60 μm on an Oxford Vibratome). The sections were incubated for HRP reaction with diaminobenzidine (DAB) or tetramethylbenzidine (TMB) (*10*). TMB allows significant detection of HRP in the perivascular spaces and CSF (*11*). Selected sections that had been incubated with DAB were further processed for electron microscopy by conventional methods. A third group of noninjected recipients was perfused with the same aldehyde mixture, and the transplant, together with the surrounding brain tissue, was dissected and embedded in Epon resin. Plastic sections (1 μm thick) were immunostained (*12*) with antiserum to rat serum albumin (RSA) to detect the presence of this endogenous blood protein normally excluded from the brain interstitial space.

A total of 34 host animals was used for this study. Of these, 24 received intraventricular grafts and 10, intraparenchymal grafts. Protein reaction products were detected in each specimen examined (HRP, $n = 17$; IgG, $n = 12$; RSA, $n = 5$) regardless of the postoperative time or the age of the donated tissue. Only the intraparenchymal grafts from younger fetal donors (embryonic day 14 to 18) were found consistently to survive, whereas all intraventricular grafts flourished. A similar pattern of protein exudation was seen in every specimen although there was slight variability even when identical experiments were repeated. The heaviest amount of protein exudation was present invariably at the transplant-host interface zone, whether the contact occurred at the choroid plexus, at the dorsal medullary surface, or within a wound cavity. When the transplant did not intimately contact a host surface in the intraventricular model, the BBB to HRP appeared to remain intact (Fig. 1, A and D). It is likely that the graft did not receive a blood vessel source from that area. A second feature was the presence of petechial leakages of protein from individual vessels within the transplant. These discrete exudations usually

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could only be visualized after short circulation times such as 2 minutes (Fig. 1A), because after this time they were obscured by the continued diffusion of the protein. When the transplant was situated within the ventricle contacting choroid plexus, cerebellum, and medulla, it was entirely filled with HRP by 50 minutes, the longest period of examination (Fig. 1B). At intermediate times the transplants were only partially filled. These findings suggest a time-dependent interstitial protein diffusion emanating from vessels at both the interface zone and within the transplant.

The great sensitivity of the TMB method allowed visualization of the microvascular patterns of the brain. This observation was possible because the injected protein traversed the graft and entered and circulated in the CSF (*11*). In specimens where the graft was filled, HRP or IgG could also enter the adjacent host brain for a distance of 100 to 400 μm (Fig. 1, A and B). Previous studies (*6*) suggest this leakage might be due to protein movement along extracellular channels. Electron microscopic examination of transplant capillaries showed abnormally high numbers of HRP-containing transporting organelles within endothelial cells (Fig. 1C). Fenestrated choroidal vessels that might account for the permeable areas were not observed.

After administration of the IgG-HRP complex, a distribution nearly identical to that of the smaller HRP was evident. Perhaps owing to its larger size, the IgG-HRP molecule was not as prominent at short circulation times, but by 50 minutes it filled large portions of the intraventricular transplants (Fig. 1D).

To determine the presence of endogenous protein within the grafts, we applied antiserum to RSA to brain sections from uninjected animals. In these, the serum protein was within the transplant interstitial spaces surrounding neurons and blood vessels (Fig. 1E). The general distribution of RSA was comparable, although not as extensive as the diffused systemically administered protein; it was located predominantly near the interface region, but very little was present in the host.

Intriguing results were obtained from cortical grafts that were inserted directly into host cortex. These grafts merged with the host but occasionally were not completely intraparenchymal. After 30 minutes of HRP circulation, a 3-month-old graft was mostly filled by HRP reaction product, some of which extended into the host (Fig. 1F). Grafts located near the surface of the host brain generally contained the most reaction product. This suggests that direct access to host pial vessels or CSF might contribute to the observed permeability even though the BBB should normally be reconstituted after mechanical trauma.

As described recently (*13*), the BBB to protein in the fetus is not immature but is in fact well developed. The cerebrospinal and brain extracellular fluids are already separate compartments, and tight junctions between cerebral endothelial cells, the anatomical basis of the BBB, are present in the fetal vertebrate brain (*14*). Accordingly, it should be expected that a "mature" CNS transplant would possess barrier properties. After the formation of a new host-transplant circulation, is the tissue completely integrated physiologically within the host? These results show that, at least for large proteins known to be excluded from brain parenchyma (*15*), it is not. The lack of a significant barrier to circulating protein, either injected or endogenous, suggests a chronically incomplete host-transplant integration. CNS transplants may thus contact circulating compounds, such as immunoproteins or antibodies, to which CNS tissue should normally never be exposed. Even if the exposure is transient (although it appears to be permanent), changes in neuronal activity and maintenance could follow.

It is unknown whether the lack of a BBB plays a role in cerebral dysfunction (*16*), although, in animal models, the converse is true—transient BBB changes can be induced by ischemia, hypertension, or edema (*17*). The establishment of both a new blood circulation and a continuity of extracellular compartments between host and graft (*6*) could permanently alter metabolism and affect capillary integrity; the presence of interstitial serum albumin could be indicative of vasogenic edema within the graft. The extensive numbers of protein-transporting organelles in graft endothelium suggest that these cells may not be metabolically normal nor have appropriate postnatal transport characteristics (*13*).

It has been suggested that morphological and enzymatic properties in transplanted capillaries are governed by the origin of the tissue and not the origin of the circulation (*18*). That the CNS transplants are readily permeable to macromolecules indicates that this concept, as applied to mammalian CNS transplants, can be questioned. It is possible that vessels arising from choroid plexus have lost their fenestrated endothelium, but that their permeability properties have not changed. Moreover, during angiogenesis, impermeable cerebral capillaries might have regressed to an early developmental stage of increased transendothelial permeability (*13*) or were rendered permeable subsequent to anoxia or ischemia. The deposition of immunological by-products or in situ immune complexes could result in the accumulation of leukocytes (*19*). Consequently, diapedesis

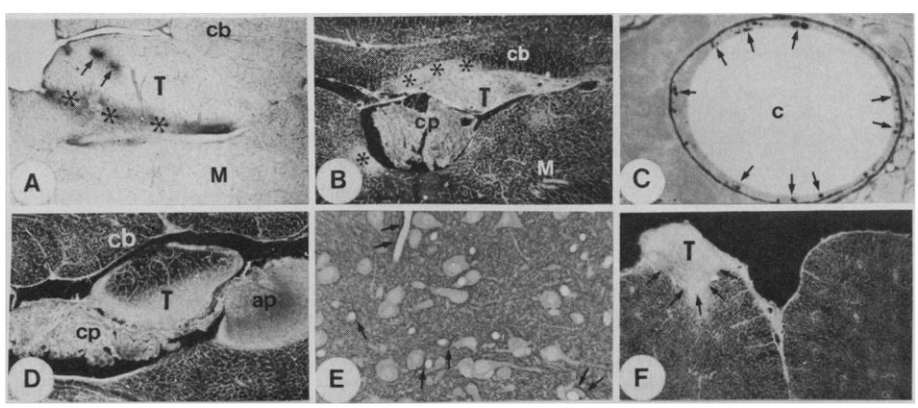

Fig. 1. (**A**) CNS transplant from fetal rat into a host rat 4 to 6 weeks of age. After 2 minutes of HRP circulation, reaction product (*) extended along the entire interface between a 3-month-old transplant (T) (derived from a donor 18 embryonic days old) and the host medulla (M). Petechial leakage from individual vessels (arrows) is evident; cb, cerebellum. The entire microvasculature is outlined by HRP that has escaped into the CSF (*11*) and has been detected by the TMB method (×18). (**B**) Cortical transplant (T) (after 5 months) (derived from a donor 19 embryonic days old) is completely filled with HRP by 50 minutes of circulation. Some protein has leaked (*) into the adjacent host cerebellum (cb) and medulla (M); cp, choroid plexus; TMB method, dark-field microscopy (×20). (**C**) Electron micrograph of a capillary (c) from a 1-year-old cortical transplant (derived from a donor 19 embryonic days old). After 20 minutes of circulation, HRP is found in many endothelial cell organelles (arrows) and adjacent neuropil (×7740). (**D**) After administration of IgG-HRP for 50 minutes a 4-month-old intraventricular transplant (T) (derived from a donor 17 embryonic days old) contacting choroid plexus (cp) and area postrema (ap) is mostly filled with reaction product. The transplant (T) does not contact the cerebellum (cb) and a portion of the transplant retains a BBB. TMB method, dark-field microscopy (×22). (**E**) Plastic section (1 μm thick) immunostained with antiserum to RSA shows reactivity throughout a 6-week-old CNS transplant neuropil (derived from a newborn donor) delineating neurons and blood vessels (arrows) (×190). (**F**) A transplant (T) (3 months) (derived from a donor 15 embryonic days old) inserted into host cortex is nearly filled by HRP after just 10 minutes of circulation. Arrows denote the interface zone. TMB method (×16).

of these cells or their released lytic enzymes might account for permeability changes in these vessels.

In experiments where the cortical transplant was placed within the host cortex, it appears that new vessels formed at the interface were permanently altered and thus prevented the normal reconstitution of the BBB after trauma. Although the precise mechanism for CNS transplant permeability is not known, it seems likely that neovascularization and certain changes in capillary phenotypic expression are involved. In fact, fetal CNS graft survival may depend on an incomplete BBB. The early metabolic needs of young displaced neurons might best be met by an immersion in unidentified, blood-borne growth factors from the host. This permeability, however, would continue long after the cessation of transplant growth.

Because neural grafts can produce beneficial effects in certain situations, it remains to be determined if a deficient BBB has significant effects on either host or graft. These results suggest that CNS grafts could be directly affected by systemic administration of modulating compounds such as peptides or hormones. In addition, the transplanted tissue may be affected by both humoral and cellular constituents within the host's blood that characteristically are excluded from the brain.

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Identification and Isolation of a Variant Surface Glycoprotein from *Trypanosoma vivax*

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The protozoan *Trypanosoma vivax* is one of the most important agents of African trypanosomiasis, a disease that hinders the productive use of livestock in one-third of the African continent. *Trypanosoma vivax* is also present in the Caribbean and in South America, posing a threat to the livestock industries of the tropical and subtropical world. Much less is known of the biology of this trypanosome than of the better studied *T. brucei* and *T. congolense*. One of the variant surface glycoproteins (VSGs) of a West African stock of *T. vivax* was identified, purified, and partially characterized by the use of a combination of highly resolving techniques to maximize information from the relatively small amount of parasite material available. The molecular weight of the isolated protein (46,000) is smaller than that of VSGs from other species. As with *T. brucei* VSGs the protein from *T. vivax* is complexed with sugars and incorporates ^{3}H when living trypanosomes are incubated with [^{3}H]myristic acid, but the *T. vivax* molecule is more hydrophobic than the *T. brucei* molecule. The small size of the *T. vivax* VSG may have a bearing on the functional and evolutionary relationships of variant antigens in trypanosomes.

Trypanosoma (Duttonella) vivax, LIKE the other Salivarian trypanosomes, exhibits antigenic variation (*1*). The surface coat, the site of the variant surface glycoprotein (VSG), has traditionally been thought of as less compact in *T. vivax* than in other trypanosomes on morphological grounds (*2*). Furthermore *T. vivax* parasites are susceptible to the action of the bacterial toxin aerolysin to which *T. brucei* and *T. congolense* are resistant (*3*). Detailed studies of *T. vivax* have been hampered by the refractoriness of most laboratory hosts to this parasite of cattle as well as by the fragility of the organism and its apparent high rate of switching variable antigen types (*4*). For these reasons, it is difficult to obtain high parasitemias of organisms homogeneous for variable antigen types, which are required for biochemical investigation. We therefore used clones of a stock of *T. vivax* from West Africa that naturally infects rodents (*5*), and we sought techniques for VSG characterization and isolation that can be used with smaller numbers of trypanosomes (10^8 to 10^9) homogeneous for variable antigen types than are required for the purification of *T. brucei* VSGs (10^{11}) (*6*).

Most of our investigations were conducted with a clone, ILDat 1.2 (ILDat standing for ILRAD *Duttonella* antigen type), which was raised in irradiated C3H/He mice. We used two other similarly raised clones (ILDats 1.1 and 1.9) of the same serodeme (*4*) for comparison. Trypanosome populations were tested for homogeneity of variable antigen types by lysis tests with reference antisera (*4*). When samples of these populations were simultaneously run in two-dimensional polyacrylamide gels by use of the ISO-DALT system (*7*), only one protein train or cluster in the molecular weight range 41,000 to 50,000 differed among the clones in gels that had been stained with Coomassie brilliant blue. To determine

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