Functional capacity of solid tissue transplants in the brain: evidence for immunological privilege

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The capacity of the mammalian brain to support the physiological function of allografts was assessed in parathyroidectomized Fischer strain rats bearing either isografts or immunogenic DA allografts of parathyroid glands implanted in their cerebral cortices. Established isografts and allografts survived indefinitely in the brain, maintaining normal serum calcium levels, with equal numbers of spontaneous failures (18–21%) in each group. Similarly, both MHC-compatible and incompatible skin allografts survived and were ‘functional’ at 40–50 days postgrafting as assessed by: continued formation of keratin; the presence of differentiated hair follicles and sebaceous glands; and frequent mitotic figures. No serum alloantibodies were induced by either MHC-incompatible parathyroid glands or skin in this site. However, both types of allografts were promptly rejected or failed to become established in the brains of specifically presensitized hosts. Furthermore, when Fischer hosts with long-established intracerebral DA parathyroid grafts received orthotopic DA skin grafts, their parathyroid grafts were rejected along with first-set rejection of the skin grafts. The tempo of this cellular immune response and the primary alloantibody response that accompanied it indicate that although the intracerebral grafts failed to induce detectable host sensitization or suppression, they remained susceptible to immune effectors. Thus, by using strongly immunogenic, adult tissues, we have established that the rat cerebral cortex is an immunologically privileged site, and the privilege is not dependent on lack of graft immunogenicity or alterations in host responsiveness. Furthermore, Ia+ (possible antigen-presenting) cells were rare in the cortical parenchyma sites used for transplantation though numerous in the choroid plexus of the ventricles and in certain areas of white matter. Therefore, privilege probably reflects deficient graft antigen presentation related to the paucity of Ia+ cells as well as to the brain’s poor lymphatic drainage.

Introduction

Interest in the brain as a possible site for foreign tissue engraftment has been renewed by two recent developments that necessitate thorough investigation of the brain’s capacity to support allogeneic grafts. Firstly, with improved techniques for obtaining and maintaining therapeutically important tissues, there is a need
for graft sites that will ensure allograft survival without the need for host immunosuppression. For example, in animal models the immunogenicity of endocrine grafts can be reduced by removing highly immunogenic passenger cells (Lafferty et al. 1983). However, such regimens have not proved sufficient to prevent rejection without immunosuppression when using either outbred animals (K. J. Lafferty, personal communication) or donors and hosts that share major histocompatibility antigens (Bartlett et al. 1983). Secondly, transplants of certain neuronal and neuroendocrine tissues into relevant areas of the brain establish appropriate contacts with host 'target' cells (Bjorklund et al. 1979; Olson et al. 1980) and can to some extent correct neuroendocrine function (Gash & Scott 1980) and motor (Freed 1983; Perlow et al. 1979) and learning deficits (Labbe et al. 1983).

The brain was among the first sites recognized to be immunologically privileged, that is, affording foreign tissue grafts (allografts) prolonged and, occasionally, indefinite survival (Barker & Billingham 1977); nevertheless, it remains little characterized. More importantly, the reason, or reasons, for its privileged status have not been elucidated. Immune privilege in the brain was originally claimed by early investigators, who used transplantable tumour allografts and xenografts, and found that whereas a tumour grafted subcutaneously was routinely rejected, the same tumour grafted intracerebrally usually grew readily and was not rejected (Shirai 1921; Murphy & Sturm 1923). Moreover, tumour grafts were reported to survive for long periods in the brains of presensitized recipients (Murphy & Sturm 1923). However, since tumours often grow very rapidly, they may escape what would otherwise be an effective immune response. Thus, tumour growth is a relatively insensitive measure of host immune response capacity.

More recently, investigators have reported conflicting results from studies using a variety of non-malignant tissue grafts, raising questions about the extent of immunological privilege afforded grafts into the brain. Such dichotomous observations may reflect the diversity of graft immunogenicity, site, and graft preparation or implantation in these previous studies. First, tissues of different or, more often, unknown immunogenicity have been used, including skin, endocrine glands, and neuronal and neuroendocrine tissue. For example, the exciting studies showing that neuronal and neuroendocrine tissue grafts survive and function in the brain have provided important physiological information. However, they have on occasion been used inappropriately to support the hypothesis that the brain affords privilege to immunogenic tissue grafts, because the immunogenicity of the particular tissue grafted, for example, hypothalamus (Gash & Scott 1980), substantia nigra (Olson et al. 1980), superior cervical ganglion (Freed 1983), or adrenal medulla (Perlow et al. 1979) was not established by grafting the tissue into non-privileged sites. Neuronal tissues in general have been shown to contain very little histocompatibility antigen (Hart & Fabre 1979) and are likely to be poorly immunogenic. Furthermore, the grafts used were often of foetal or neonatal origin and these are usually less immunogenic than comparable adult tissues (Billingham & Silvers 1971). Therefore, the lack of immune responses to the graft in these cases may lie more with the graft than the graft site. To add to the controversy, some investigators using immunogenic adult tissue grafts have reported fairly prompt rejection (Lance 1967; Block et al. 1966; Ridley & Cavanagh 1969) whereas others.
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reported long term survival (Raju & Grogan 1977). Secondly, various intracranial implantation sites have been used, including grey matter, white matter and ventricles. It is possible that the factors influencing antigen processing and exposure of the host’s immune system to graft antigens (mainly lymphatic drainage and the presence of local antigen-presenting cells) differ among brain regions. Finally, previous studies have also varied substantially in the technical aspects of graft preparation and implantation. For example, free cellular grafts may more readily gain access to host lymphoid compartments than those within solid tissue grafts. Also, harsher techniques of implantation may alter host tissue integrity to a greater extent, increasing the possibility of host exposure and response.

With the increasing prospects for therapeutic grafting into human brain, a better understanding of the extent and nature of the brain’s immunological privilege is urgently needed, as is a resolution of previously conflicting studies. Here we report on the capacity of two highly immunogenic tissues, skin and parathyroid gland, to survive and function in allogeneic rat cerebral cortex, the impact of such grafts on the host’s immune system, and the distribution of potential antigen-presenting cells.

**Material and methods**

**Animals**

Fischer (F1; RT1^1^) and Lewis (LEW; RT1^1v1^) inbred rats were obtained commercially, while DA (RT1^a^) strain rats were raised in house. All animals were provided water and laboratory food *ad libitum*. Special dietary requirements of parathyroidectomized animals are outlined below.

**Intracerebral implantation technique**

All surgical procedures were performed on animals anaesthetized with chloral hydrate (3.6% solution injected intraperitoneally at a dose of 1 ml 100 g^-1^ body mass). Intracerebral implants were performed with the aid of a dissecting microscope on anaesthetized rats secured in a stereotaxic apparatus. After exposing the skull through a midline incision, the bone was gently scraped to remove connective tissue and rubbed with bone wax to prevent bleeding. With a dental drill, a circle 3 mm in diameter was made in the skull just lateral to the midline approximately 5 mm caudal of bregma. The bone plug was carefully removed, exposing the dura. Through a small slit made in this membrane a parathyroid gland graft was gently pushed down into the cortical parenchyma with a fine probe. Skin graft recipients received an allograft in the left visual cortex and a control isograft in the right visual cortex. Gel-foam was used to replace the excised bone, and the incision was closed with Michelle clips.

**Parathyroid gland grafts**

The use of parathyroid glands as test allografts obviates the limitations of single time point assessments of graft survival, usually based on histological criteria, since the capacity to function (produce parathyroid hormone) in the brain is readily detectable by changes in serum calcium levels, allowing longitudinal evaluation
of graft survival in individual animals. Prospective recipients of parathyroid grafts were parathyroidectomized under chloral hydrate anaesthesia, by using the technique described by Russell & Gittes (1959), and were immediately placed on a diet of deionized water and low calcium food pellets (Bio-Serv, Frenchtown, New Jersey, U.S.A.), which is essential for the development of hypocalcaemia in rats. After parathyroidectomy, the serum calcium (measured on a Calcette, Precision Systems, Sudbury, Massachusetts, U.S.A., on 40 μl serum samples) decreases from the normal level of 9–10 mg dl⁻¹ to plateau at 4–5 mg dl⁻¹ within one week (Head et al. 1983). One to two weeks after parathyroidectomy, animals with serum calcium levels in this range were used as recipients.

For transplantation, whole parathyroid glands were held in cold medium for no longer than 30 min before implantation. To demonstrate immunogenicity and establish first-set rejection times, parathyroid allografts were placed in non-privileged sites such as under the renal capsules of parathyroidectomized hosts or in a subdermal pocket on the thoracic wall above the panniculus carnosus muscle. Intracerebral implantation was performed as described above. Serum was obtained from tail venous blood samples taken from recipients on days 5, 7, 9, 11, 13, 15, 20, 25, 30, and 35 after grafting, and weekly thereafter, and tested for calcium levels. After successful parathyroid transplantation, the serum calcium level rises over a period of several days to virtually normal levels (Head et al. 1983). Grafts were considered to be functional when the serum calcium level reached 7.5 mg dl⁻¹ and non-functional below 6.5 mg dl⁻¹.

For histological observation, brains of parathyroid gland recipients were fixed by perfusion with glutaraldehyde, post-fixed with osmium tetroxide, embedded in epoxy, sectioned at 1 μm, and stained with toluidine blue (Head & Seelig 1984).

Skin grafts

Skin allografts were used to test intracerebral graft survival because, although limited to morphological assessment, skin is one of the most immunogenic tissues (Hildemann 1970), being rapidly rejected when transplanted to non-privileged sites. Skin grafts prepared from shaved, washed ears as described by Billingham (1961) were used whole (approximately 1 x 2 cm) for standard orthotopic grafts or were cut into pieces 2–4 mm² for intracerebral grafts and smaller orthotopic control grafts. Plaster casts covering orthotopic grafts placed on graft beds in the thoracic wall were removed after six days, and the grafts were scored visually for epithelial survival.

The portions of brains containing skin grafts were fixed in formalin and embedded in paraffin or glycomethacrylate. Semi-serial sections of entire grafts were evaluated after staining with haematoxylin and eosin (H & E). Intracerebral skin grafts were scored for epithelial viability, differentiation, and extent of mononuclear cell infiltration. Grafts were considered healthy if the epithelium was viable with no more than slight mononuclear cell infiltration, confined to one or two small dermal foci, without epithelial involvement. Rejection was characterized by more extensive mononuclear cell infiltration, usually involving the epithelium, and evidence of epithelial destruction.
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Tests for serum antibodies

Samples of serum were obtained from tail venous blood, taken from graft recipients on days 5, 7, 9, 11, 13, 15, 20, 25, 30, and 35 after grafting, and tested for the presence of antibodies against transplantation antigens by haemagglutination assay, by using a bovine serum albumin–dextran technique (Head 1982) and by complement-dependent cytotoxicity against target lymphocytes (Amos & Pool 1976).

Detection of Ia+ cells

Because antigen-presenting cells bear substantial class II (Ia) histocompatibility antigens (Unanue 1981), cells expressing Ia antigens were detected in brain tissue by indirect immunofluorescence using the mouse monoclonal antibodies OX4 and OX3, which are directed against rat Ia determinants (Accurate Chemical and Scientific Corporation, Westbury, N.Y., U.S.A.). The OX4 antigen is present in all rat strains while that reactive to OX3 is present in the FI and LEW, but not in the DA strain. Whole coronal brain slices 2–3 mm thick containing the graft sites were frozen in isopentane cooled in liquid nitrogen, and sectioned at 8 μm. The sections were fixed for 10 min in acetone, which we have shown preserves these antigens very well in other tissues such as heart (unpublished observations). Sections were sequentially exposed to normal goat serum (1:200), OX4 antibody (1:200), and fluoresceinated goat anti-mouse IgG (1:200) from which the cross reactivity to rat IgG had been removed (Cappel Labs, West Chester, Pennsylvania, U.S.A.). Controls for non-specific staining with the fluoresceinated antibody were treated with phosphate-buffered saline (PBS) or OX3 antibody, on FI and DA tissues respectively, in place of the OX4 antibody. Non-specific binding of the fluorescent reagent was eliminated by prior absorption with guinea-pig liver powder and dilution in 35–40 % normal rat serum. Incubations of the sections were at room temperature for 30 min in a moist chamber, with 10 min PBS washes in between. The sections were mounted in 90 % glycerol, pH 9.5, and observed for fluorescent cells on a Zeiss photomicroscope III equipped with fluorescent epo-illumination.

For combined detection of Ia+ cells and cells containing non-specific esterase (a histochemical marker of macrophages), acetone-fixed frozen sections were stained for α-naphthyl butyrate esterase (kit 180-B, Sigma Chemical Co., St Louis, Missouri U.S.A.) for 30 min at 37 °C, rinsed in PBS and taken through the immunofluorescence procedure described above. Positive control slides were from spleen samples, containing large numbers of esterase+ and Ia+ cells.

Results

First-set rejection of allografts in non-privileged sites

To demonstrate the immunogenicity of the parathyroid gland, DA parathyroid allografts were implanted under the renal capsules or in subdermal pockets of 17 parathyroidectomized FI hosts. Such grafts, as expected (Naji & Barker 1976), took readily and functioned, rendering the recipients normocalcaemic within five
to seven days. However, graft survival in these non-privileged sites was limited, with most animals rendered hypocalcaemic by 13 days after grafting (m.s.t. (median survival time), 12 ± 1.2 days). All such recipients produced alloantibodies, with peak titres of approximately 1:1000, 11–15 days after grafting.

Control DA and LEW skin grafts (2–4 mm²) were scored visually beginning seven days after orthotopic grafting to FI recipients. All were rejected by day 10 (m.s.t. of DA, 8.8 ± 1.0 days; m.s.t. of LEW, 9.0 ± 1.0 days), confirming that even at this small size, skin remains a highly immunogenic tissue. The DA grafts induced anti-DA haemagglutinating antibodies, with peak titres of 1:128 on day 15.

**Capacity of the cerebrum to sustain first-set allografts**

(a) **Parathyroid glands**

To test the capacity of the rat cortex to sustain functioning parathyroid glands, the fate of isografts transplanted into the visual cortex was determined in 15 FI recipients. Most (73 %) of the grafts took and functioned for the duration of the observation period, up to 180 days (table 1). Two of these syngeneic grafts (18 %) failed spontaneously at 9 and 35 days, a result we have not observed in other privileged sites such as the anterior chamber and testis. The incidence of successful establishment of implanted DA parathyroid allografts into FI cortex was lower than with isografts, that is, 54 % functioned. As with isografts, most established DA grafts survived throughout their observation periods, up to 96 days (table 1). None of these animals had detectable serum alloantibodies. Again, as with isografts, a small number (21 %) ceased to function. Thus, once established in the brain, the potential for long-term survival of parathyroid allografts as well as isografts is high.

**Table 1. Survival of parathyroid glands in the rat visual cortex**

<table>
<thead>
<tr>
<th>host animals</th>
<th>graft donor strain</th>
<th>number of takes (%)</th>
<th>length of graft survival/days†</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI</td>
<td>FI</td>
<td>11/15 (73 %)</td>
<td>9, 35, &gt; 30, &gt; 35, &gt; 55, &gt; 60, &gt; 120, &gt; 120, &gt; 120, &gt; 180</td>
</tr>
<tr>
<td>FI</td>
<td>DA</td>
<td>19/35 (54 %)</td>
<td>11, 30, 39, 40, &gt; 35, &gt; 35, &gt; 35, &gt; 35, &gt; 35, &gt; 37, &gt; 38, &gt; 42, &gt; 55, &gt; 56, &gt; 63, &gt; 66, &gt; 68, &gt; 69, &gt; 87, &gt; 96</td>
</tr>
<tr>
<td>FI previously sensitized to DA</td>
<td>DA</td>
<td>1/11 (9 %)</td>
<td>20</td>
</tr>
</tbody>
</table>

† Functional survival defined by serum Ca²⁺ > 6.5 mg dl⁻¹.
> 30, On this day the animal was killed or treated while bearing a functioning graft.

Functioning allografts observed histologically contained typical cords of chief cells in a highly vascular and compact connective tissue stroma (figure 1, plate 1), resembling the appearance of long-established parathyroid grafts in other privileged sites (Head *et al.* 1983).
Figure 1. A DA parathyroid gland perfusion-fixed 56 days after transplantation into the visual cortex (v.c.) of a parathyroidectomized FI host. Cords of healthy chief cells (c.) are surrounded by compact connective tissue. A retained segment of the gland's capsule (ca.) is seen. There is no evidence of immune reaction, and the host animal was normocalcaemic. Toluidine blue, 1 μm; magn. ×350.

Figure 2. An FI skin graft in the right visual cortex of a syngeneic FI recipient 50 days after transplantation. A portion of the cyst is seen, much of it lined by healthy stratified squamous epithelium, with keratohyalin granules in the granular layer, and the central cavity filled with keratin. In this region, the dermis surrounds differentiated hair follicles and sebaceous glands and mitotic figures are seen in basal epithelia. H & E, 3 μm; magn. ×300.
Figure 3. A LEW skin graft 15 days after transplantation to the left visual cortex of a FI recipient. The graft is a keratin-filled cyst surrounded by healthy stratified epithelium with keratohyalin granules. A few sebaceous glands can be seen. There is no mononuclear cell infiltration or other evidence of immune reaction. H & E, 3 μm; magn. × 128.
Figure 5. A DA skin graft 50 days after transplantation to the left visual cortex of a FI recipient. The keratin-filled cavity was surrounded by viable epithelium with keratohyalin granules. On this side, sebaceous glands and hair follicles are seen, along with mitotic figures. No evidence of immune damage or mononuclear cell infiltration was found. H & E, 3 μm; magn. × 220.

Figure 6. A DA skin allograft five days after transplantation into the visual cortex of a FI rat previously sensitized to DA alloantigens. The epithelium has been destroyed, with remnants containing no keratohyalin granules. Mononuclear cell infiltration is substantial on one side of the graft. H & E, 8 μm; magn. × 125.
Figure 8. Ia+ cells in the brain detected by indirect immunofluorescence. A section of cortical parenchyma (a) contains only one positive cell (arrow), revealed in phase contrast (b) to be associated with the wall of a blood vessel. The parenchyma otherwise contains only specks of gold-coloured autofluorescent material. On the other hand, the choroid plexus (c and d) contains numerous Ia+ cells, usually subepithelial in location (arrows). Again, small specks are only gold-coloured autofluorescent granules. All magns × 467.
Skin grafts in the cerebral cortices of non-sensitized FI hosts were recovered and studied histologically 15, 20, 30, 40 and 50 days after grafting, with two to six samples at each time point. As seen in figure 2, isograft controls formed keratin-filled cysts lined by stratified squamous epithelium with a prominent granular layer, resembling heterotopic skin grafts described by Medawar (1948). In many samples, differentiated elements such as hair follicles and sebaceous glands were seen, and mitotic figures were common among the epithelial cells.

The allografts resembled their corresponding isograft controls in the contralateral visual cortex, forming epithelial-lined cysts (figure 3, plate 2). As seen in figure 4, most allografts, whether MHC-compatible or incompatible, survived well in the rat brain. All (14) Lewis allografts were healthy, most (13) without any mononuclear cell infiltration, even 40 days after transplantation. Fifteen out of 17 DA grafts into brain also escaped rejection, up to 50 days, that is, more than five times the m.s.t. in non-privileged sites. Most of these DA allografts (70%) had healthy hair follicles or sebaceous glands, or both (figure 5, plate 3), although differentiation was often not as extensive as that in isografts placed in contralateral cortex.

**Figure 4.** Prolonged survival of allogeneic skin grafts in the cerebral cortices of FI hosts. At various times after transplantation, grafts were assessed histologically for viable epithelium, judged by a healthy stratified epithelium with keratohyalin granules and evidence of mitotic activity, and for degree of mononuclear cell infiltration. Each point represents one graft. ○, DA graft into FI host; △, LEW graft into FI host; ▲, DA graft into FI host presensitized to DA alloantigens.
The majority of grafts were placed in the visual cortex, with some being deeper, that is, within the hippocampus. The location did not affect allograft survival. None were placed in white matter or the ventricles.

As observed in parathyroid gland recipients, intracerebral DA skin grafts did not induce detectable serum alloantibodies between 5 and 35 days after transplantation.

Rejection of intracerebral allografts in presensitized hosts

To test the possibility that blockade of immune effectors in the brain was occurring, FI animals were grafted orthotopically with standard DA skin grafts three weeks before receiving intracerebral DA parathyroid or skin allografts as before. The resulting sensitization of the FI hosts to DA antigens was verified by high levels of serum alloantibodies on the day of grafting. The successful functioning of parathyroid allografts seen in immunologically naive hosts did not occur in these presensitized recipients (table 1). Only one of 11 hosts manifested a slight elevation in the serum calcium level, and this declined to pregraft levels within 20 days.

Skin grafts were recovered from eight sensitized FI hosts and observed histologically 5, 8, or 11 days after intracerebral implantation. All isografts were healthy, with well differentiated epithelium. In contrast, as shown in figure 4, all of the allografts in the contralateral cortex were clearly being rejected. At five days, there was substantial infiltration by mononuclear cells, and much of the epithelium was degenerated, lacking keratohyalin granules and mitotic figures (figure 6). At the later times, epithelial destruction was complete, and mononuclear cell infiltration increased in intensity.

Tests of host responsiveness and intracerebral graft vulnerability

When ten FI animals, bearing functioning intracerebral DA parathyroids for 30–90 days, were test-grafted orthotopically with standard DA skin grafts, the tempo of skin graft rejection (m.s.t., 7.7 ± 1.0 days) was typical of the first-set response (m.s.t., 7.5 ± 1.1 days) by normal animals in this strain combination (Head 1982). Additionally, as skin graft rejection proceeded, primary, not anamnestic, alloantibody responses were detected in the sera (figure 7). As the orthotopic skin grafts were being rejected, the intracerebral parathyroid glands also succumbed, with the hosts’ serum calcium levels falling below 6.5 mg dl⁻¹, seven to nine days after grafting (figure 7).

Ia⁺ cell distribution

Since intracerebral allografts were vulnerable to immune effectors but did not induce such effectors in naive animals, we investigated the possibility that this lack of the initiation of an immune response is related to the density or distribution of antigen-presenting cells in the brain. Potential antigen-presenting cells were localized in the brain by indirect immunofluorescence of the class II antigens required for antigen presentation. Coronal sections through the region used for grafting in three normal FI and DA animals revealed that much of the brain parenchyma is deficient in Ia⁺ cells. Within the graft sites, that is the visual cortex
Figure 7. Responses of ten FL hosts bearing long-surviving intracerebral DA parathyroid glands to an orthotopic DA skin graft. Shortly after the skin grafts were rejected (m.s.t., 7.7 days), a primary serum alloantibody response was detected, and the parathyroid grafts were rejected.

and hippocampus, the rare Ia+ cells observed were usually clearly associated with the walls of blood vessels (figure 8a, b, plate 4). In contrast, the ventricles were well endowed with these cells (figure 8c, d), mainly in the connective tissue beneath the choroid epithelium and in the lumen. They were also consistently seen in the meninges where they resembled the Ia+ dendritic cells in the capsules or linings of many organs (J. R. Head, unpublished observation).

In areas rich in white matter, such as corpus callosum, Ia+ cells with very long, thin processes were relatively abundant. These cells were arrayed along capillaries, sometimes scattered but often in clusters.

When the tissues were tested for non-specific esterase, occasional positive cells were seen in choroid, often near larger blood vessels, and more rarely in the cerebral parenchyma. However, most Ia+ cells were negative for esterase, suggesting that the majority of Ia+ cells observed were not conventional macrophages but rather belonged to the dendritic cell lineage.

Discussion

The present findings provide unequivocal evidence, using highly immunogenic tissues, that the visual cortex and hippocampus of the rat brain are excellent sites for prolonged survival of solid tissue allografts in naive hosts, protecting them well beyond the m.s.t. in non-privileged sites. As with other privileged sites (see Barker & Billingham 1977) the brain does not protect allografts from rejection in presensitized hosts (see Medawar 1948; Raju & Grogan 1977). Moreover, established
intracerebral parathyroid allografts were susceptible to effectors induced by donor strain orthotopic skin grafts applied weeks or months after parathyroid implantation. The immune privilege of this site is, therefore, not a result of efferent blockade of induced immune effectors. Rather, it is more probably due to deficient graft-antigen presentation to the host's immune system. Not only did intracerebral parathyroid allografts fail to induce demonstrable alloantibodies, but, when animals with long-surviving parathyroids were challenged with donor skin, they reacted in first-set fashion, producing a typical primary antibody response, indicating that intracerebral exposure to a solid tissue allograft had neither sensitized the hosts nor induced hyporesponsiveness. However, further dissection of host responses to these grafts, by using in vitro tests, will be necessary to determine whether subpopulations of T cells are selectively affected. Such an effect has been shown by grafting free tumour cell allografts across minor histocompatibility barriers into another privileged site, the anterior chamber of the mouse eye (Niederkorn & Streilein 1983); cytotoxic T cells were generated, but induced suppressor T cells prevented generation of delayed-type hypersensitivity T cells.

Based on current knowledge of antigen processing in various sites, two factors are likely to influence significantly the fate of allografts implanted in the brain. The first is lymphatic drainage to regional lymph nodes. Lymphatic deficiency characterizes two well defined natural privileged sites, the anterior chamber of the eye and the hamster's cheek pouch (Barker & Billingham 1977) as well as artificial privileged sites, experimentally created to confer privileged status on conventional sites (Barker & Billingham 1968). The parenchyma of the central nervous system lacks conventional lymphatic vessels. However, tracers injected into the cerebral cortex or cerebrospinal fluid do appear to some extent in lymphatics and local lymph nodes, and ligation of cervical lymphatics causes oedema of the brain (Casley-Smith et al. 1976; Bradbury 1978). Nevertheless, most fluid flow out of the brain is via the venous vasculature. Exclusive egress by the vascular route, promoting antigenic exposure in the spleen, seems responsible for the privilege afforded free cell tumour grafts in anterior chamber (Streilein & Niederkorn 1981). Thus a paucity, though not complete absence, of lymphatic drainage probably contributes to immune privilege in the brain.

The second factor contributing to privilege is the distribution of potential antigen-presenting cells in a site of antigen exposure. These cells characteristically express abundant Ia, or class II, histocompatibility antigens necessary for effective presentation of antigen to host T cells (Unanue 1981). Included in this family of cells are Ia+ macrophages, dendritic cells in the lymphoid tissue (Steinman & Nussenzweig 1980), and Langerhans cells in the skin (Streilein & Bergstresser 1980). Local deficiencies of such cells can interfere significantly with antigen processing. For example, the cornea and hamster's cheek pouch skin, which have few Ia+ cells, are poor sites for induction of contact sensitivity (Streilein & Bergstresser 1980). Our finding that the grey matter of brain, including the cortical parenchyma containing the graft sites, was almost devoid of Ia+ cells, is probably related to privilege. We found, confirming the report of Hart & Fabre (1981), that the choroid plexus in the ventricles has substantial numbers of such cells in both the connective tissue and in the cerebrospinal fluid. Their dendritic morphology...
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and the failure of most to express a macrophage marker, non-specific esterase activity, suggest that these cells are akin to the dendritic cells which are resident in lymphoid tissue (Steinman & Nussenzweig 1980) and are scattered throughout the connective tissue of many organs (Hart & Fabre 1981). A distinct population of Ia+ cells with extremely dendritic shape was located exclusively in white matter. If these are glial elements, they may be the in vivo counterparts of cultured neonatal rat astrocytes reported to express Ia antigens, present antigen to T cells, and to produce an interleukin-1-like factor (Fontana et al. 1982, 1984).

The privileged status of the brain has been questioned as numerous studies have provided widely conflicting results. Some reported fairly prompt rejection (Lance 1967; Block et al. 1966; Ridley & Cavanagh 1969) whereas others, including the present study, found long-term survival (Murphy & Sturm 1923; Raju & Grogan 1977). Some have reported sensitization of the host via exposure in the brain (Lance 1967; Geyer & Gill 1979), but in the current study this was not the case. The observations on egress of fluid from the brain and distribution of potential antigen-presenting cells discussed above suggest that a key factor may be location of the antigenic exposure. They predict that the ventricles and subarachnoid space, with high flow of cerebrospinal fluid and substantial Ia+ cells, would be much less successful sites for highly immunogenic grafts than grey matter, and that certain white matter areas, if the Ia+ cells we observed are indeed capable of antigen presentation, might also be less hospitable. Several reports support these contentions. For example, skin allografts into the white matter lateral to the thalamus in rat brain were regularly rejected (Ridley & Cavanagh 1969), as were parathyroids grafted into white matter in dogs (Lance 1967), whereas we found little evidence of rejection of either type of tissue in cortical grey matter. Also, injection of heterologous lymphocytes into the subarachnoid space sensitized animals systematically and locally, in the choroid plexus, to respond to a second infusion (Neuwelt & Doherty 1977). Intraventricular grafts of islets of Langerhans, which contained some immunogenic lymphoid tissue, were able to alleviate diabetic symptoms in rats only if they were syngeneic and not allogeneic to the host (McEvoy & Leung 1983). The ventricles can, however, be successful graft sites, if relatively non-immunogenic tissues are used, such as free endocrine or neuronal cell populations (Perlow et al. 1980; Gash & Scott 1980; Weiss et al. 1978; Kreiger et al. 1982). Thus the present findings and other studies to date, taken together, suggest that transplantation of foreign tissues into the brain can most successfully be accomplished if: (i) potentially immunogenic tissues are placed in the grey matter, avoiding white matter and ventricles; or (ii) ventricles are used as sites for poorly immunogenic tissues such as those from embryonic donors or purified cell preparations from which leucocytes and other highly immunogenic 'passenger' cells have been removed.

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References


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