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Is the adult mouse striatum a hostile host for neural transplant survival?

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Human donor cells, including neurally directed embryonic stem cells and induced pluripotent stem cells with the potential to be used for neural transplantation in a range of neurodegenerative disorders, must first be tested preclinically in rodent models of disease to demonstrate safety and efficacy. One strategy for circumventing the rejection of xenotransplanted human cells is to desensitize the host animal to human cells in the early neonatal period so that a subsequent transplant in adulthood is not immunorejected. This method has been robustly validated in the rat, but currently not in the mouse in which most transgenic models of neurodegeneration have been generated. Thus, we set out to determine whether this could be achieved through modification of the existing rat protocol. Mice were inoculated in the neonatal period with a suspension of human embryonic cortical tissue of varying cell numbers, and received a subsequent human embryonic cortical tissue cell transplant in adulthood. Graft survival was compared with those in mice immunosuppressed with cyclosporine A and those receiving allografts of mouse whole ganglionic eminence tissue. Poor survival was found across all groups, suggesting a general problem with the use of mouse hosts for testing human donor cells. *NeuroReport* 24:1010–1015

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Introduction

Preclinical testing of human donor cells requires transplantation into animal (most commonly rodent) models of disease to confirm safety and assess functional efficacy. For example, neurotoxic lesion models of Huntington's disease (HD) are commonly used as they closely model some key features of the disease, such as relevant cell loss and histological changes. These models are ideal for assessing the functional effect of transplanted cells on lesion-induced deficits observed on motor and cognitive tasks. However, genetic models such as transgenic mice may be a more relevant model for the study of neural transplantation as they more accurately reflect the progressive disruption to specific circuitry in the brain [1]. Several HD mouse models exist, expressing either insertions of the full-length HD gene or the expanded CAG repeat fragment [2–5], and these are substantially more numerous and well-established than similar models in the rat [6,7].

Six weeks after striatal allografts of wild-type mouse tissue into the adult R6/2 mouse model of HD, graft survival and marginal behavioural improvements have been observed [8], but transplants of immortalized human striatal neural stem cells into the same host yielded very small grafts and no behavioural improvement [9], suggesting that the tissue was subjected to immunorejection. Indeed, xenogeneic tissue transplanted into the rodent brain appears to be rejected from 1–2 weeks; thus, some form of immunosuppression is required to permit graft survival. Conventional methods include the use of immunosuppressant drugs, including cyclosporine A (CsA), or immunocompromised hosts, for example, SCID or *Rag2*-deficient mice. However, side effects of drugs such as CsA lead to termination of experiments before human cells have had time to fully differentiate and integrate within the host brain, thus preventing full functional assessment of donor cells. In addition, immunocompromised hosts are particularly sensitive to infection, and do not withstand a full battery of behavioural tests. We have previously demonstrated that neonatal desensitization to human tissue promotes long-term survival of human neural transplants in the rat [10], which has since been confirmed by others [11,12], although to date success has not been demonstrated in the mouse.

Our primary aim was therefore to determine whether mice could be successfully desensitized in the neonatal

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period to human foetal neural tissue to allow long-term assessment of human donor cell survival and differentiation. This would permit full behavioural assessment of transplant effects and a long-term assessment of the cells *in vivo* to determine whether they are affected by the underlying disease processes. Survival of human xenografts in desensitized mouse hosts, either quinolinic (QA) acid lesioned or unlesioned, was compared with those of CsA immunosuppressed or untreated hosts. A further group of nonimmunosuppressed or CsA-treated mice received mouse allografts. We observed poor and variable survival of transplants across all conditions. These data suggests a general problem with the use of mouse hosts for both neural xenografts and allografts, and implies the need for optimization of mouse transplant protocols before it can be determined whether desensitization may be successfully achieved in this species.

Materials and methods

Animals

All animal experiments and surgical procedures were conducted under the UK Animals (Scientific Procedures) Act 1986, and subjected to local ethical review and relevant personal, project and institutional licences. A total of 163 CD-1 mice (79 male, 84 female) were included as transplant hosts or lesion only controls. Sixty mice were purchased as adults (Harlan, Bicester, UK) and 13 pregnant dams were used to produce neonates for desensitization and embryos for mouse tissue transplants. Pregnant dams were housed individually and adult mice following weaning and on arrival were housed in same sex groups of two to four mice per cage with experiments beginning in adults weighing 20–25 g, at around 8–10 weeks of age.

Dissection and dissociation of human embryonic cortical tissue and mouse whole ganglionic eminence

Human cortical tissue (ranging from 7–11 weeks gestation) was collected from the donation of the products of elective terminations of pregnancy. All tissue was donated through the South Wales Initiative for Foetal Tissue Transplantation (SWIFT) with ethical approval from the Bro Taf Local Research Ethics Committee. Human and mouse tissue for neonatal desensitization and transplantation was collected, dissected and dissociated as described previously [13,14]. Briefly, human foetal tissue was collected from medical terminations of pregnancy into Hibernate E medium (Gibco, Paisley, UK) before dissection and collection of human embryonic cortical (hCTX) tissue. For allograft experiments, E14 CD-1 mouse embryos were collected and mouse whole ganglionic eminence (mWGE) dissected for dissociation as with human tissue. Tissue was digested in trypsin at 37°C and washed before resuspending in Dulbecco's modified Eagle medium (DMEM)/F12 (with 5% penicillin streptomycin) to generate a single cell suspension. Viability of all cell suspensions was determined by trypan blue exclusion counting before injection and rejected if viability was lower than 80%. After counting, cell suspensions were made up to relevant concentrations for injection in 1–2 µl DMEM/F12.

Neonatal desensitisation

Pregnant CD-1 mice (10) were housed individually until they gave birth. Pups were injected neonatally into the peritoneal cavity (i.p.) with a cell suspension of hCTX between postnatal days 0–3 (P0–3). As standard, mice were injected with 1 µl of cell suspension containing 1×10^5 cells in DMEM/F12 unless otherwise specified. In the case of an injection of greater than 5×10^5 cells, suspensions were prepared in a volume of 2 µl. Pups were separated briefly from their mothers for intraperitoneal injection of cells, then returned and left until weaning at $\sim$28 days.

Surgical procedures

All surgical procedures were performed under isoflurane anaesthesia induced in an induction chamber with 5% isoflurane in oxygen at 0.8 l/min. Anaesthesia was maintained by passive inhalation of isoflurane (1.5–2.5%) in a mixture of oxygen (0.8 l/min) and nitrous oxide (0.4 l/min).

Adult CD-1 mouse hosts either received a unilateral QA lesion to the right striatum, or the host brain was left intact. The skull was exposed, a small burr hole drilled above the right striatum at 0.8 mm rostral to bregma (AP) −2.0 mm lateral to midline (L) and −3.0–2.8 mm ventral from dura (V). QA was injected through a cannula attached to a 10 µl Hamilton syringe driven by a mechanical pump. 0.75 µl of 0.09 M QA dissolved in 0.1 M phosphate buffer was injected over 6 min, the needle was then left in place for 3 min to prevent reflux of toxin up the needle tract. The incision was sutured and animals were administered subcutaneous injections of 0.5 ml saline glucose (using a 1 ml syringe and 26 G needle), 5 µl Metacam and an intramuscular injection of 30 µl diazepam (both measured and administered using a 300 µl insulin syringe) into the upper leg before transfer to a warm recovery chamber. Hosts then received transplants of hCTX or mWGE, from 2 days to 2 months post-lesion, to the same coordinates into the intact or lesioned striatum to a total of 3×10^5 or 5×10^5 cells in 2 µl.

Most animals receiving xenotransplants that were not desensitized received daily intraperitoneal injections of CsA (Sandimmun, 10 mg/kg; Novartis, Hampshire, UK) for the duration of the experiment, starting 1 day prior to transplantation.

Histology and immunohistochemistry

Mouse hosts were transcardially perfused 6–12 weeks after transplantation with 1.5% paraformaldehyde, and

brains collected for histological analysis. Brains were sectioned at 40 μm on a freezing sledge microtome and stored at 4°C in Tris-buffered saline with 0.2% azide, or at –20°C in antifreeze. 1 : 12 series were mounted for Nissl staining with cresyl violet, and additional 1 : 12 series stained as free-floating sections for immunohistochemistry using anti-HuNu (1 : 1000; Millipore, Molsheim, France) and anti-Iba1 (1 : 8000; Wako, Neuss, Germany) primary antibodies and biotinylated secondary antibodies (anti-mouse and anti-rabbit, 1 : 200; Vector, Peterborough, UK). Immunohistochemical staining protocols were the same for all antibodies with the exception of HuNu, which in some cases was biotinylated using a biotin conjugation kit to avoid the use of an anti-mouse secondary antibody and lower background staining (Lightning Link Biotin Conjugation Kit; Innova Biosciences, Cambridge, UK). Diaminobenzidine was used for visualization of all antibody staining. Small modifications were made to the protocol to attempt to improve reliable labelling of grafted cells, which is discussed further in the results section. Grafts were visualized under a Leica DRMBE light microscope (Leica Microsystems, Milton Keynes, UK). Images were captured using a Leica DFC420 camera and Leica Application Suite image analysis software (Leica Microsystems). Images were processed using Adobe Photoshop (Adobe Systems Incorporated, San Jose, California, USA).

Results

To identify surviving grafts in transplanted hosts, sections from all animals were initially stained with the Nissl stain cresyl violet (Fig. 1a, b, e, f, and i–l). To confirm the presence of transplanted human cells, tissue sections from xenografted animals were immunohistochemically stained with HuNu (Fig. 1c and g). Staining of Nissl bodies with cresyl violet showed dense staining in the grafted area on a number of sections, suggestive of surviving human grafts. However, the presence of transplanted cells could not be convincingly detected with human specific antibody staining. In these cases microglial marker staining with Iba1 (Fig. 1d and h) revealed the presence of dense activated microglia corresponding to the grafted area. Sections showing such inconsistencies between cresyl violet and HuNu staining were therefore not considered to contain healthy surviving grafts but rather grafts that were undergoing rejection.

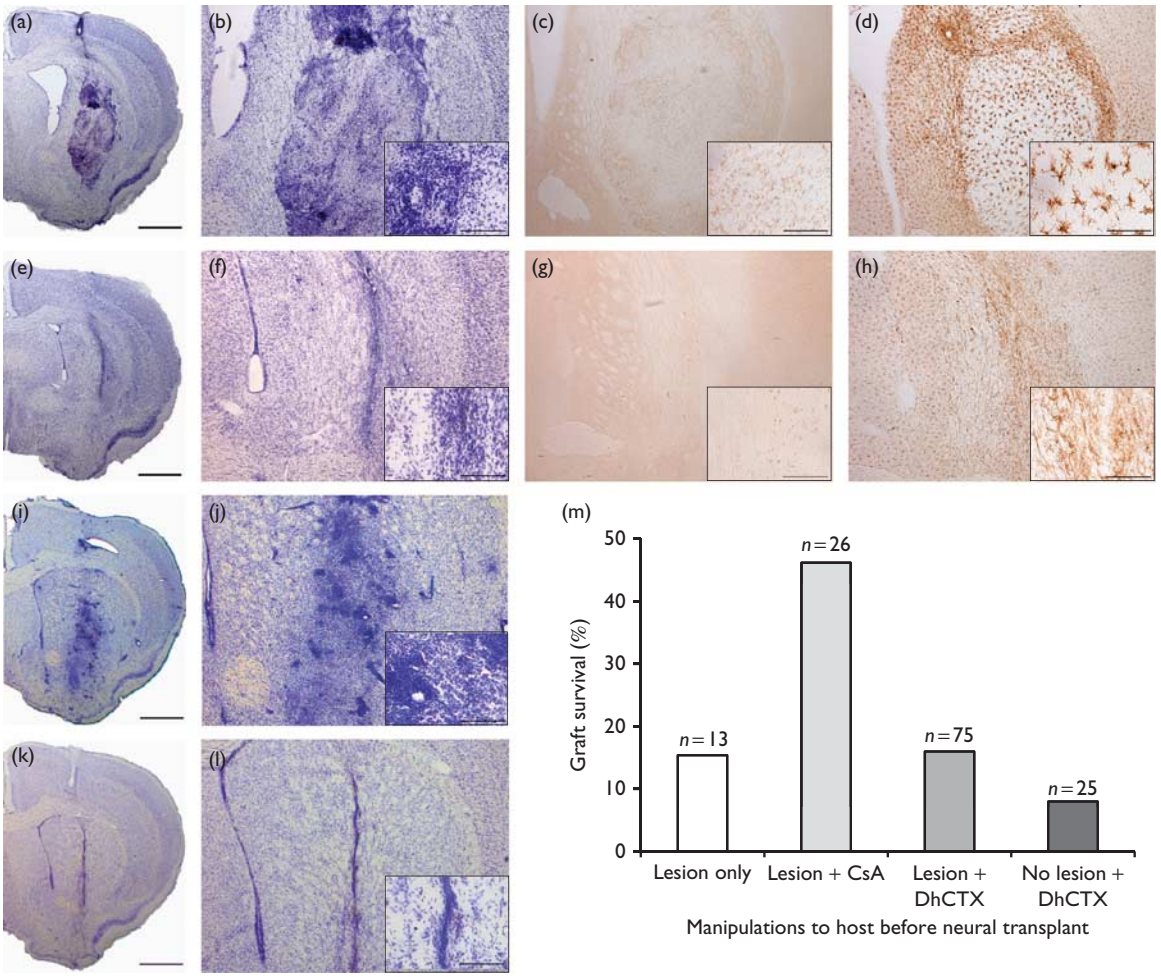
Numbers of surviving healthy transplants as detected by these criteria are outlined in Table 1. Low levels of graft survival were found across all groups in allografts as well as xenografts, even in desensitized and CsA-treated groups, irrespective of the time of assessment after transplantation, and time between lesion and transplant. A small number of large surviving human grafts could be confidently detected, mainly in CsA-treated hosts (50%), with a small number from desensitized animals (14%). A number of transplants in CsA-treated animals were very dense and vascularized, with a large amount of microglia staining (Fig. 1i and j). Surviving mouse allografts were very small, indeed most consisted of a narrow column of cells. Moreover, due to the lack of an appropriate antibody or specific donor cell label, they could not be detected with the same degree of confidence as human transplants, and therefore survival rate may have been overestimated. The majority of sections in both allograft and xenograft animals showed either no staining or an area of dead cells (with haemosiderin and nonspecific antibody uptake) and scarring. In addition, the majority of sections showed dense microglial staining with Iba1. Because of the lack of surviving grafts, statistical analysis of data was not undertaken. However, as can be seen in Fig. 1m, desensitization did not provide any improvement in promoting survival of human transplants and the percentage of surviving grafts was comparable with that of untreated animals (16 and 15%, respectively). CsA treatment provided a slight improvement in transplant survival; however, this still only reached 50%, with no improvements observed from transplants of higher numbers of donor cells (5×10^5 vs. 3×10^5).

Discussion

To promote the survival of human neural transplants in preclinical models of disease we have previously demonstrated that rat hosts may be desensitized to human foetal tissue in the neonatal period, allowing survival of a human striatal xenograft for sufficient time to permit assessment of functional efficacy [10]. To date, successful translation of this method from the rat to the mouse has not been achieved. In this study, we aimed to desensitize mouse hosts to human foetal tissue following the same protocols as used previously. Host animals were initially desensitized with 1×10^5 hCTX cells in the neonatal period, or treated with CsA daily following transplantation of 3×10^5 hCTX, or mWGE cells to the right lesioned or intact striatum. Survival in both desensitized and CsA-treated animals was low. In subsequent transplants, 5×10^6 hCTX cells were transplanted but this did not improve graft survival, and may have caused overcrowding and promoted rejection.

Neonatal (and *in utero*) tolerance induction has been described for decades using a similar method to that described here [15,16]. Previous work shows the induction of tolerance to allogeneic skin grafts following an injection of spleen [17,18], liver [19] or bone marrow cells [20], although less success has been observed in the case of xenografts [21]. Other examples of the induction of neonatal tolerance in mice have used large numbers of spleen cells (e.g. $1.5 \times 10^7 – 1 \times 10^8$) [22,23] injected neonatally and studies suggest the administration of larger cell numbers increases the potential of inducing tolerance [17,23]. As desensitization in our study did not promote neuronal graft survival in our initial experiments, we used increasing numbers of cells to induce

Fig. 1



(a–d) A rare successful hCTX graft in a DhCTX mouse, (e–h) a typical hCTX graft in a DhCTX mouse, (i, j) a hCTX graft in a CsA immunosuppressed mouse, and (k, l) a typical mWGE to mouse graft. (a, b, e, f, i–l) Nissl body staining with cresyl violet, (c, g) human neuronal tissue staining with HuNu, and (d, h) microglia-specific staining with Iba1. Comparison of graft survival in different treatment groups as a percentage of hCTX transplanted animals (m). Scale bars represent 1000 μm in the lowest magnification images and 100 μm in the highest. CsA, immunosuppression with daily injection of cyclosporine A; DhCTX, desensitization with hCTX cell suspension; hCTX, human embryonic cortex, mWGE, mouse embryonic whole ganglionic eminence.

desensitization in subsequent experiments; however, this did not result in improved graft survival. Indeed, there was a suggestion that fewer transplants survived in mice desensitized with the highest numbers of cells. Animals were transplanted at varying times post-lesion to identify whether any improvements in graft survival could be found, as previous data has suggested a shorter time between lesion and transplant could yield better results [24]. No differences were found in groups receiving earlier or later transplants; thus, data are discussed together. In addition to poor graft survival in desensitized hosts, CsA treatment did not promote survival to a reliable degree with only 50% of transplants surviving and poor, potentially rejecting, transplants observed in many hosts. However, hosts transplanted with mouse tissue from donors of the same strain did not show consistent survival, even when treated with CsA. Concurrent transplants carried out on desensitized rat hosts have yielded up to 100% survival of human striatal transplants (V.H. Roberton, C.M. Kelly, unpublished data).

These findings are not exclusive to the experiments presented here; other data from our lab reveals similar difficulty in achieving success in mouse transplants, including mouse allografts (V.H. Roberton, A.E. Evans, unpublished observations). Transplants in the mouse brain are commonly found to be substantially smaller than those surviving in the rat, even allowing for the differences in host brain size [9,13]. Promoting survival of human tissue transplants in the mouse has been shown to be a challenge even when using conventional methods of immunosuppression, that generally promote survival in

Table 1 Summary of treatment groups and graft survival

Treatment group	Number of cells for desensitization	Lesion type	Transplanted tissue type	Number of cells transplanted	Number of grafts surviving (% of grafts surviving)
DhCTX	1×10^4	QA	hCTX	5×10^5	1/6 (17)
DhCTX	1×10^5	QA	hCTX	5×10^5	2/11 (18)
DhCTX	5×10^5	QA	hCTX	5×10^5	1/16 (6)
DhCTX	5×10^5	–	hCTX	5×10^5	1/5 (20)
DhCTX	1×10^6	QA	hCTX	5×10^5	0/6 (0)
DhCTX	1×10^6	–	hCTX	5×10^5	0/5 (0)
DhCTX	2.5×10^6	QA	hCTX	5×10^5	1/4 (25)
DhCTX	2.5×10^6	–	hCTX	5×10^5	0/5 (0)
DhCTX	5×10^6	QA	hCTX	5×10^5	1/5 (20)
DhCTX	5×10^6	–	hCTX	5×10^5	0/5 (0)
DhCTX	1×10^5	QA	hCTX	3×10^5	5/27 (19)
DhCTX	1×10^5	–	hCTX	3×10^5	1/5 (20)
–	–	QA	hCTX	3×10^5	2/13 (15)
CsA	–	QA	hCTX	3×10^5	7/12 (58)
CsA	–	QA	hCTX	5×10^5	5/12 (42)
–	–	QA	mWGE	3×10^5	6/11 (55)
CsA	–	QA	mWGE	3×10^5	1/7 (14)
Total hCTX survival					27/137 (20)
Total mWGE survival					7/18 (39)

CsA, immunosuppression with daily injection of cyclosporine A; DhCTX, desensitization with hCTX cell suspension; hCTX, human embryonic cortex; mWGE, mouse embryonic whole ganglionic eminence; QA, quinolinic acid lesion.

rat transplants. Moreover, recent data suggests the mouse brain is less tolerant to cell transplants than the rat, with xenotransplant work being carried out in SCID [25] or *Rag2*$^{-/-}$ host mice [26]. In addition, transplants into neonatal SCID mice or neonates crossed with *Rag2*$^{-/-}$ mice have been used to extend survival of human cells [27,28], suggesting that the problem with survival of xenografts in the mouse brain is a widespread issue. The reason for poorer transplant survival in the mouse host as compared with the rat is currently unclear. It may be related to the mouse host response to human tissue specifically, however, poor allograft survival suggests this is not the case. Alternatively, the mouse immune system may be more sensitive than that of the rat. Without achieving consistent successful transplant survival using standard strategies it is not possible to test the suitability of neonatal desensitization as a method of preventing rejection of human donor cells in the mouse brain.

In all transplant groups small numbers of large transplants were found to survive. We cannot exclude the possibility that this is due to some hosts potentially receiving larger quantities of cells due to uneven preparation of cell suspensions or blockage of transplant syringes. However, this seems unlikely given the high levels of survival of transplants in rat hosts using the same transplant protocols [10]. Furthermore, our observations of a strong microglial response to human transplants in the mouse striatum suggest the likelihood of an increased immune response to xenografted (and possibly also to allografted) cells in the mouse compared with the rat.

Conclusion

We were unable to validate the success of neonatal desensitization of mouse hosts to human foetal tissue, specifically to striatal transplants of hCTX tissue. However, this could not be attributed specifically to the desensitization strategy and appeared to occur across all conditions, including with the use of conventional immunosuppression in the form of daily treatment with CsA. The fact that striatal allografts also showed poor survival even over relatively short survival times suggests that the adult mouse brain is a more hostile environment for graft placement than that of the rat. Indeed, the validation of the desensitization approach, as well as the use of mouse models for preclinical assessment, will have to await a better understanding of the mechanisms underlying poor graft survival, and improved strategies to address this issue.

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Conflicts of interest

There are no conflicts of interest.

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