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Immune response to murine cell lines of glial origin transplanted into the central nervous system of adult mice

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SUMMARY

Temperature-sensitive simian virus 40 (SV40) T antigen-transformed central nervous system (CNS)-derived murine cell lines were used to analyse the host response to transplantation in the mouse adult brain. The cell lines were shown to be susceptible to immune recognition *in vitro* by cytotoxic effector cells indicating that tissue-specific privilege was not in operation. Histological examination at time points post-implantation showed characteristic responses similar to those seen during graft rejection. Astrocytosis and up-regulation of major histocompatibility complex (MHC) class I and MHC class II activation of resident microglia and recruitment of macrophages were observed in both allogeneic and syngeneic hosts 10 days post-implantation suggesting a trauma-induced response. However, the response in allogeneic hosts was more widespread and evident when the syngeneic responses had returned to normal levels. Evidence of T-cell infiltration was also more pronounced in the allogeneic hosts. Despite quite extensive host reactions to these cellular grafts at early time-points the implants appeared to survive in the host CNS long after the responses had abated and could be detected at the maximum time-point studied of 40 days.

INTRODUCTION

Intracerebral transplantation has become important in recent years for the development of new protocols for ameliorating degenerative diseases of the central nervous system (CNS).[1–3] The nature of these diseases necessitates the use of donor tissue for transplantation derived from allogeneic sources,[4] thus there is a need for further understanding of immune processes occurring within the CNS. It is well established that immune reactivity is limited as compared with other organs and the CNS has been deemed an immunologically privileged site since early transplantation studies.[5] The advantage afforded the CNS by selective immunoreactivity presumably enables clearance of infectious agents which may invade from time to time while minimizing the damage which could result from an uncontrolled inflammatory response and constant cellular traffic. Indeed ongoing immune responses within the CNS are often associated with debilitating and life–threatening diseases such as multiple sclerosis.[6] Thus the immune damage resulting from graft rejection in the brain or spinal cord could lead to pathology. One cause for concern is the propensity for autoimmune disease associated with immunologically privileged sites[7] which could be initiated and augmented by such processes. Despite numerous studies performed to analyse graft rejection within the CNS[8–12] the events leading to immune recognition and infiltration of immune cells are not well understood. We have developed a cellular graft model to ascertain the molecular requirements for recognition within the CNS and present here initial observations made with these cell lines.

Murine cell lines derived from mixed-glial cultures and transformed with the temperature-sensitive simian virus 40 (SV40) large T antigen provide a useful tool for studying the effects of exogenous gene products *in vivo* in the central nervous system.[13] These cell lines will grow unheeded at 33° *in vitro* allowing the establishment of clonal populations to obviate complexities associated with studying cells of mixed origins, cell populations which have been well characterized before implantation, and cells easily manipulable for the purposes of gene transfer. However, when transplanted *in vivo* the transforming element is switched off at murine body temperature and the cell lines do not form tumours. Moreover, they remain stable in the host CNS for long periods allowing detailed studies over time to be carried out.

Here we show that these lines can be recognized by specific cytotoxic cells suggesting that they could be susceptible to attack *in vivo*. When grafted into the brain of adult syngeneic and allogeneic mice characteristic cellular reactions result. These reactions are qualitatively different in the two environments even though full rejection may not be effected in the allogeneic model. Thus these cell lines provide an opportunity to study molecular requirements for immune recognition and graft rejection in the CNS. The model will also allow us to

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address the efficacy of potential immunomodulatory agents which may have therapeutic value in the treatment of immunopathologies of the CNS. Furthermore, it provides us with an accessible model for examining the destructive processes not only associated with graft rejection but also with persistent infectious agents and autoimmunity.

MATERIALS AND METHODS

Cell lines

The cell lines were generated as described briefly below from neonatal CBA mice and maintained in culture at 33–34° with 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Seralab, Crawley Down, Sussex, UK), 1 mM sodium pyruvate (Gibco, BRL, Paisley, UK), 2-mercaptoethanol (5×10^{-5} M, Sigma Immunochemicals, Poole, UK) 2 mM l-glutamine and penicillin–streptomycin. Mixed glial cell cultures were prepared using a method modified from McCarthy and DeVellis.[14] Two-day-old mice were killed and their brains removed and rolled on filter paper to remove meninges. After mincing and sequential passage through 19, 21 and 23 guage needles the cell suspensions were cultured on poly l-lysine-coated flasks. Multiple passage of these cultures has been shown to enrich for an astrocyte population. After 5–6 weeks in culture the cells were infected with the tsa 58.3 recombinant retrovirus[15] and selected in 1 mg/ml G-418 and transferred to a 33° incubator. The resulting cell line was then cloned at 0·5 cells/well and eight resulting clones were characterized. All cell lines were passaged using versene/2·5% trypsin solution (Sigma) to detach adherent cells.

Immunofluorescence and immunohistochemistry

Cells were either stained in cell suspension or as cytospins after treatment with versene–trypsin or versene alone. Immunofluorescence was carried out by standard methods using the antibodies listed with the appropriate fluorescently labelled secondary antibody. Cell suspensions were fixed after staining in 1% formalin/phosphate-buffered saline (PBS) and analysed by flow cytometry using a FACScan (Becton-Dickinson, Cowley, Oxon, UK). Cytospins were fixed in methanol/acetone and stained by standard procedures with 10% blocking serum from the same species as the secondary reagent added to the primary antibody. After staining, the cytospins were mounted with an aqueous mounting fluid (Citifluor, London, UK) and analysed by fluorescence-microscopy. Cut cryostat sections were stained for the appropriate markers by a standard immunoperoxidase procedure. Briefly, sections were incubated overnight with the primary antibody containing 10% blocking serum from the same species from which the secondary biotinylated reagent was derived, i.e. either normal goat or normal rabbit serum. After washing, the sections were then blocked for a further 30 min at room temperature followed by the secondary biotinylated antibody. After further washing the sections were incubated with avidin–peroxidase complexes as directed by the manufacturers (Vectastain ABC kit, Vector Laboratories, Peterborough, UK).

Antibodies

Rabbit anti-bovine glial fibrillary acidic protein (GFAP) polyclonal serum obtained from Dakopatt (Älvsjö, Sweden) and A2B5 (Boehringer-Mannheim, Lewes, E. Sussex, UK) were used to detect cells of the astrocyte lineages. Rabbit polyclonal anti-bovine 2′,3′, cyclic-nucleotide 3′ phosphohydrolase (CNPase) a gift from Dr F.A. McMorris, anti-galactocerebroside (galC) a gift from Dr J. Sussman (Cambridge) and anti-myelin oligodendrocyte glycoprotein (Dr S. Piddlesdon, Cardiff) were used to detect cells of the oligodendrocyte lineage. GFAP and CNPase are intracellular antigens and were analysed using fixed cytospins of cells while the other antibodies used detect surface antigens and were analysed by flow cytometry of cell suspensions. Staining for the presence of antigens of the major histocompatibility complex (MHC) Class I and II was performed using MCA171 [mouse monoclonal antibody (mAb) against mouse class I of the H-2^k haplotype, Serotec Ltd, Oxford, UK], MCA1066 (against mouse class I of the H-2^d haplotype, Serotec), OX-6 (mouse mAb against rat Ia which also detects mouse class II of the H-2^k haplotype, Serotec) and anti-IA^d [mouse mAb detecting IA^d, American Type Culture Collection (ATCC), Bethesda, MD]. F4/80 is a rabbit polyclonal serum detecting cells of the monocyte/macrophage lineage and was a gift from Dr Siamon Gordon (Oxford). For immunofluorescence studies all rabbit primary antibodies were detected with rhodamine-labelled porcine anti-rabbit IgG (Sigma); mouse antibodies were detected using fluoroscein isothiocyanate (FITC)-labelled sheep anti-mouse immunoglobulin. For immunohistochemical staining all antibodies were detected using biotinylated secondary antibodies and the streptavidin–peroxidase method using Vectastain ABC detection kit as directed by the manufacturers. The biotinylated secondary antibody detecting rabbit sera was goat anti-rabbit IgG and that detecting mouse mAb was rabbit anti-mouse IgG (STAR11). Peroxidase activity was visualized using diaminobenzidine as a substrate (Sigma fast tablets).

Cell labelling

Cells were labelled with Sigma PKH26-GL red fluorescent dye by the method outlined by the manufacturers. Briefly, the cells were detached from the tissue culture flask when approximately 80% confluent using trypsin/versene and resuspended in serum-free DMEM. After counting, the cells were pelleted and resuspended in the diluent provided and then added to the prepared dye. After 4 min at room temperature the reaction was stopped by adding FBS and the cells were incubated for a further 1 min. The cells were washed four times in serum containing DMEM and then resuspended in an appropriate volume of PBS for implantation.

Cytotoxicity assays

Allospecific cytotoxic lymphocytes were prepared by stimulating spleen cells from a BALB/c mouse (H-2^d) with irradiated (2000 rads) spleen cells derived from a CBA mouse (H-2^k) for 5 days in a standard one-way mixed lymphocyte reaction (MLR). After 5 days the effector cells were counted and plated with ^{51}-chromium-labelled (sodium chromate^{51} ICN, Thame, Oxon, UK) target cells (glial clones) at appropriate effector to target ratios (E:T).^{51}Cr release into the supernatants was measured after 4 hr incubation at 37°. Harvested supernatants were mixed with Hisafe 3 scintillant and analysed for ^{51} Cr content using a 96-well scintillation counter (Wallac, Turku, Finland). Specific cytotoxicity was calculated by subtracting spontaneous release (SR) from targets incubated with medium alone and dividing by the maximum release from targets

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incubated with 10% Triton-X-100 after subtraction of the SR. Each E:T was carried out in triplicate.

Intracerebral grafts

PKH26-labelled cell lines were implanted into the brains of 4–6-week-old BALB/c (allogeneic, haplotype H-2^d) and CBA (syngeneic, H-2^k) mice under anaesthetic (metafane, C-Vet, Leyland, Lancs, UK). Approximately 2×10^6 cells in a volume of 5 μl were injected intracerebrally using a 500 μl hamilton syringe fitted with a 25-gauge needle. A length of rubber tubing was fitted onto the needle to standardize the distance (5 mm) the graft was placed into the midbrain. 10, 30 and 45 days later the mice were deeply anaesthetized (halothane) and perfused with PBS followed by paraformaldehyde, lysine, periodate solution in PBS (PLP) into the left ventricle. The brains were removed and fixed for a further 4–8 hr in PLP and then stored in 20% sucrose until frozen and cut. Sagittal or coronal sections of approximately 0–15-μm thickness were cut using a cryostat. Slides were air-dried, analysed and noted for the presence of the graft by fluorescence microscopy and then stored at $-80°$.

RESULTS

Characterization of temperature-sensitive CNS-derived murine cell lines

Temperature-sensitive CNS-derived murine cell lines were generated and cloned as described in the Materials and Methods. Eight clones were characterized for the expression of glial-cell markers and MHC molecules. None of the cell lines expressed markers associated with the oligodendrocyte lineage, i.e. galactocerebroside, MOG and CNPase (Table 1). Expression of GFAP is associated with cells of the astrocyte lineage, both type I and type II. The observation that some of these cell lines express GFAP is consistent with the idea that the protocol used for generating these cells enriches for astrocytes. However, none of the lines was 100% positive and only weak expression was observed in the majority. This variation in expression of GFAP has been observed in similarly derived rat clones and may relate to cell cycle. Treatment with interleukin-1, which has been shown to up-regulate GFAP in primary astrocytes[16] had no effect on intensity or percentage of cells reactive with the GFAP antibody (data not shown). A2B5 is expressed by progenitor cells of the astrocyte type 2 lineage and only found on a minority of cells of type I. Expression of both GFAP and A2B5 by these cells suggests that they are of glial origin. All of the clones expressed MHC class I but substantial variation in levels of expression was observed from clone to clone. Some clones varied the expression of MHC class I after long periods in culture, decreasing with time. By contrast none of the clones expressed MHC class II which was not inducible in the presence of interferon-γ (IFN-γ; data not shown).

Recognition of the glial clones by specific cytotoxic T cells

Since these cells expressed different levels of MHC class I (Fig. 1 c) which may affect their recognition *in vivo* it was important to establish whether they could be recognized by specific cells outside the CNS where environmental factors may influence their recognition. These cells were used as targets in a standard chromium-release assay using *in vitro*-activated allogeneic cells as effectors of cytotoxicity. The data from four of the clones shown in Fig. 1 suggest that the ability of these cells to be recognized and killed by alloreactive cells is dependent on their level of MHC class I expression. Indeed up-regulation of MHC class I by IFN-γ increases the percentage cytotoxicity for all four clones shown. However, where levels are almost optimal in the absence of IFN-γ as for MGC3 (Fig. 1 a and c) the increase in cytotoxicity in the presence of IFN-γ is proportionally less. These results illustrate that the CNS-derived clones are susceptible to normal killing mechanisms and do not display tissue-specific privilege.

Host response to syngeneic and allogeneic glial clones transplanted into adult mouse brain

MGC3 and MGC7 were chosen for *in vivo* studies because they represent the extremes of expression of class I *in vitro*

Table 1. Summary of phenotypic analysis of eight temperature-sensitive SV40 large T antigen-transformed CNS-derived cell lines

	Antibody (% positive)						
Specificity	MCA171 MHC I	OX-6 MHC II	A2B5 Astro	GFAP Astro	CNPase Oligo	MOG Oligo	GALC Oligo
Cell line							
MGC1	52	0	6	11	0	1	1
MGC2	98	0	20	15	0	1	1
MGC3	96	3	9	0	0	6	9
MGC4	67	0	7	0	0	0	1
MGC5	94	0	1	42	0	1	0
MGC6	71	0	12	9	0	0	1
MGC7	62	2	14	32	0	0	5
MGC9	74	0	27	3	0	0	2

Cell lines were stained by indirect immunofluorescence either in suspension for flow cytometry or as cytospins for cytoplasmic staining and fluorescence microscopy. The abbreviations Astro and Oligo refer to the glial lineages astrocyte and oligodendrocyte respectively. Mean percentages are given of three or more separate experiments. Ranges are omitted for simplicity.

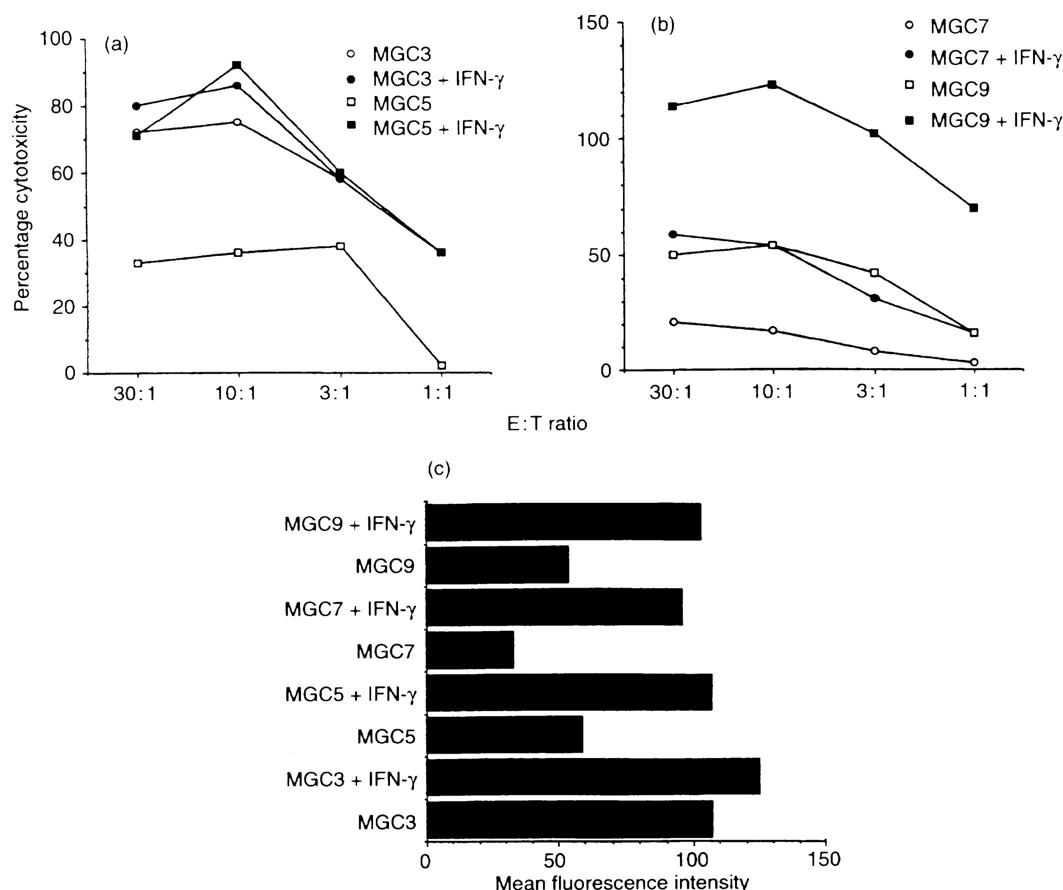

Figure 1. Cytolysis of four CNS-derived cell lines by MLR-generated cytotoxic lymphocytes. Effector cells (E) and ^{51}Cr-labelled target cells (T) were incubated for 4 hr at the ratios indicated and percentage cytotoxicity was determined as described in the Materials and Methods. Open symbols and closed symbols represent targets with and without prior incubation with 100 units ml of IFN-γ, respectively. The histogram presented in (c) shows the relative intensities (mean fluorescence intensity) of MHC class I expression of each target cell with and without IFN-γ immediately before addition to the CTL assay. The data are representative of three experiments.

(Fig. 1 c) and provide an opportunity to study the effect of this variable on graft recognition in the absence of donor class II expression. Cells were labelled using a fluorescent dye and immediately implanted into the brains of young (4–6 weeks) CBA and BALB/c mice, representing syngeneic and allogeneic hosts, respectively. Cryostat sections taken from the brains of these mice were examined for the presence of grafted cells by fluorescence microscopy and graft-positive sections were stained sequentially by immunocytochemistry for GFAP, F4/80 (a microglia/macrophage marker), MHC class I of donor and host origin and MHC class II of donor and host origin.

Detection and survival of cellular grafts

Grafted cells [MGC3 (20 mice) and MGC7 (18 mice)] were detected in 35/38 brains. This included both syngeneic[17] and allogeneic[18] transplanted animals. The grafts were generally more discrete at day 10 (Fig. 2 a) than days 30 and 45 post implantation (Fig. 2 b and c, respectively). At the later time-points cells could be observed some distance from the original site, often associated with the ventricles, having a periventricular location (Fig. 2 b and c). Despite severe inflammation in the brains of some of the allogeneic mice transplanted with MGC3 at day 10 there did not appear to be a difference between the survival of cells in the syngeneic brains compared with the allogeneic brains, although the dispersed nature of the cells made this difficult to quantify. Tumours were not detected in any of the animals and immunostaining with an antibody specific for the SV40 large T antigen showed no reactivity (data not shown).

Day 10 post-implantation

GFAP expression was not detected on either transplanted cell line *in vivo*. Donor MHC class I was detected at very weak levels on both MGC3 and MGC7 at day 10 post-implantation as shown by the haplotype-specific antibodies but not at longer time periods. MHC class II expression was never observed on either cell type *in vivo* (data not shown).

In nine out of 10 animals receiving a transplant an increase in both GFAP and F4/80 reactivity was observed at this time-point (Fig. 3 a). In the syngeneic animals this increase was restricted to areas surrounding and immediately adjacent to the grafts and where detected around the injection track (Fig. 4 a). The increase in reactivity was more widespread and

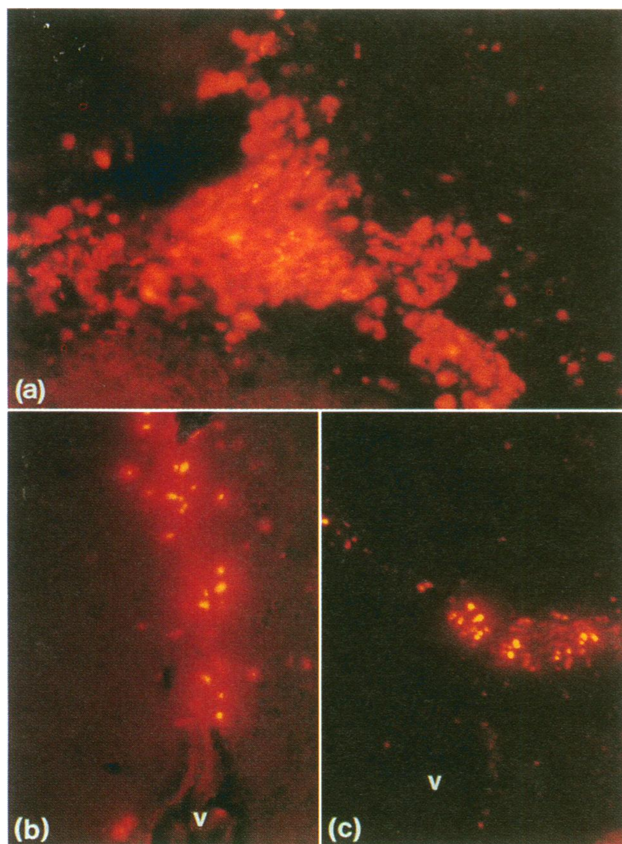

Figure 2. Representative fluorescence microscopy of grafts (a) 10 days, (b) 30 days and (c) 45 days post-implantation with fluorescent-labelled transformed glial cells. (a) and (b) are grafts from syngeneic mice while (c) shows grafted cells from an allogeneic animal. V marks the site of a ventricle. The grafted cells in (a) are more discrete while those in (b) and (c) are more dispersed. In (b) the cells appear to be periventricular; in (c) the cells appear to be following a white matter tract. Magnification for (a) × 230 (b) × 80 and (c) × 32.

more intense in the allogeneic grafts extending to areas some distance from the original implantation site (Fig. 4 b). The current study also suggests that MGC3 induces a more widespread response than MGC7 in the allogeneic animals (Fig. 3 a). A more comprehensive analysis would be required to confirm this observation.

Expression of host MHC class I and class II was observed in the brains of seven out of 10 animals. One mouse had no detectable expression of either MHC class I or MHC class II and this correlated with a lack of up-regulation of GFAP and F4/80 (Fig. 3 a). The pattern of expression in the remaining brains, however, depended first, on the clone transplanted (MGC3 or MGC7) and second, on the genetic disparity. The brains from the syngeneic mice transplanted with either clone were positive for MHC class II and the expression was restricted to the areas immediately adjacent to the graft, within the graft and blood vessels close to the graft (Figs 3 and 4 e) while MHC class I was up-regulated only in those brains transplanted with MGC7 (Fig. 3 and Fig. 4 c). The reason for this differential expression has yet to be determined and is under further investigation. The identity of the positive cells was unclear but the morphology and coincidence with F4/80 staining suggested that the majority were of the macrophage lineage (data not shown). In only one brain was mild cuffing observed. Expression of MHC class I and MHC class II was more intense in the allogeneic animals and no differential expression of the molecules was observed (Figs 3 a, 4 d and 4 f). Moreover, cells of stellate morphology in the white matter some distance from the graft were positive in addition to the infiltrating cells in and around the graft, in the meninges and in the ventricles (data not shown). In two of these brains transplanted with MGC3 heavy infiltration of leucocytes was observed along with cuffing, angiogenesis and destruction of parts of the white matter. In those transplanted with MGC7 little or no cuffing was detected in any of the transplanted brains but some mild inflammation of the meninges was noted. The identity of the infiltrating leucocytes was not clear but the

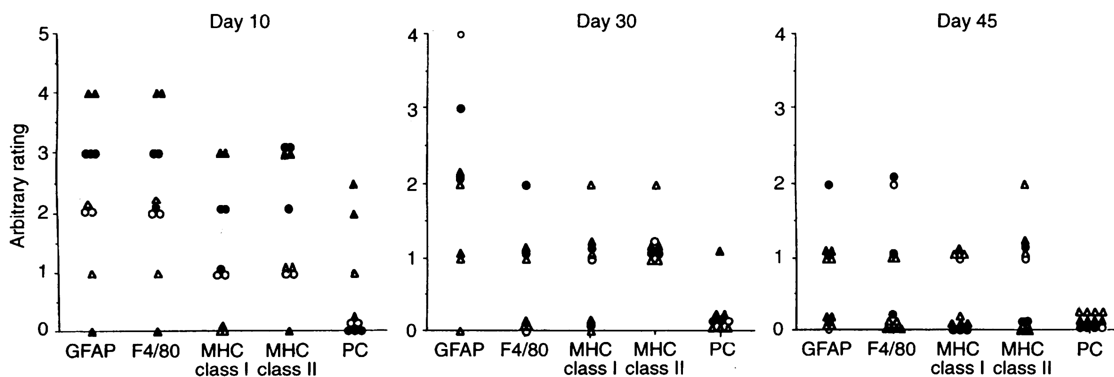

Figure 3. Rating of immunohistochemical staining of cryostat sections. Each symbol represents an individual mouse while closed symbols represent data derived from the allogeneic, BALB/c strain and open symbols derived from the syngeneic, CBA strain. The circles indicate mice implanted with cell line MGC7 and the triangles those with cell line MGC3. Sections were stained with the antibodies indicated at (a) 10 days (b) 30 days and (c) 45 days post-implantation. The MHC expression referred to is of host origin. The following categories for the purpose of rating each section were used: (0) no specific staining above background levels or in the case of GFAP and F4/80 no increase in normal staining (around ventricles and blood vessels); (1) low number of positive cells in and around grafted site and around blood vessels; (2) more positive cells with greater intensity and clustered around grafted site; (3) very intense staining in grafted areas and clustered or scattered cells some distances from graft; (4) intense staining throughout brain.

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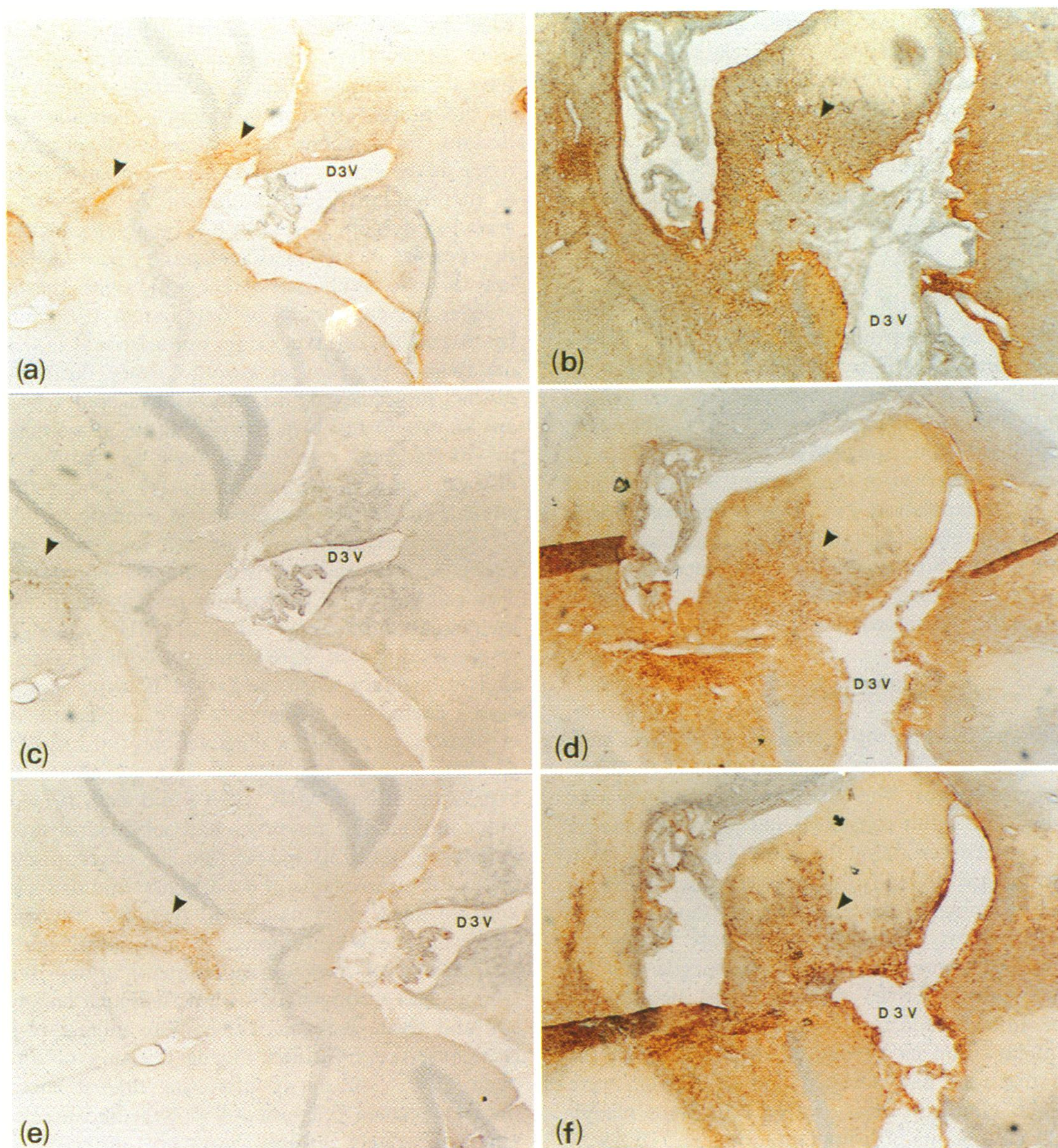

Figure 4. Immunohistochemical staining of representative brain sections at day 10 post-implantation of syngeneic grafted cells (a, c and e) and allogeneic grafted cells (b, d and f). Sequential sections from grafted brains were fixed in paraformaldehyde and stained for GFAP (a and b), host MHC class I (c and d) and host MHC class II (e and f). The grafts (arrowheads) are located in areas of the brain close to the dorsal third ventricle (D3 V). Extensive infiltration and destruction of tissue in (b) (d) and (e) can be observed. Magnification is ×40.

intense staining with F4/80 in these areas suggested that the majority were of macrophage origin although lymphocytes were also present.

Staining of sections with antibodies specific for T cells and T-cell subsets confirmed that the majority of CD45-positive cells were not T cells. In certain syngeneic hosts with discrete grafts the number of T cells present in the grafted area was less than five (in a single 15-μm section). In the brain of the grafted animal with the greatest inflammation (see above) more infiltrating lymphocytes were observed. In one section adjacent to the graft more than 100 CD45-positive cells were present of which 42% were CD3 positive and 49% of the T cells were positive for CD8 but this level of staining was unusual.

Far greater numbers of T cells were seen in the brains of the allogeneic transplants and frequently greater than 200 CD45-positive were seen in an equivalent area of which on average 41% (range 36–45%) were CD3 positive and 59% (range 42–75%) of the T cells were CD8 positive.

Day 30 post-implantation

By day 30, the host responses to implantation had diminished. The GFAP expression in the syngeneic mice had increased very weakly over the entire brain but no longer exhibited enhanced expression around the grafted site. Concomitant with these observations was a diminished reactivity with the F4/80 antibody. Enhanced expression of both GFAP and the F4/80

antigen was still apparent in the allogeneic grafted brains but with reduced intensity. Interestingly MHC class I expression was reduced more markedly in the allogeneic mice and MHC class II expression could be detected after MHC class I had returned to background levels. Some mild inflammation was observed in one allogeneic transplanted brain. Few T cells (<5) were observed in a single section from any of the transplanted brains of both syngeneic and allogeneic hosts.

Day 45 post-implantation

GFAP was reduced to background levels in six of 12 brains. Enhanced expression could still be detected around the grafted site in the remaining brains but with much decreased intensity. F4/80 staining was also minimal in the five of 12 brains which showed residual expression. Some MHC class I and class II expression was also observed in those brains which also had weak positive F4/80 expression. No obvious differences were noted between the syngeneic and allogeneic transplanted animals in that remaining host reactivity was observed in both circumstances. No additional differences between MGC3 and MGC7 transplantation were seen.

DISCUSSION

In this study immortalized murine CNS-derived cell lines were established using a temperature-sensitive SV40 T antigen-containing retroviral vector. The characterized cell lines were then transplanted into adult syngeneic and allogeneic mouse brains with the aim of ascertaining the stability and integration of the cells into the host to study immune responses following implantation. In summary, the responses in the allogeneic host were generally more pronounced than those seen in the syngeneic hosts. This was observed for astrocytic (GFAP expression), microglial (F4/80) responses and lymphocyte infitration. The differences in responses were most obvious at day 10 post-implantation as by day 30 the majority of the graft-localized reactions had declined and by day 45 were barely detectable. None the less, grafted cells were readily detectable at all time-points post-implantation.

Primary and immortalized lines have been developed previously with the aim of studying the differentiation and maturation of CNS-derived progenitor cells[17–19] and for the restoration of function as a therapeutic approach in CNS disorders.[20–22] However, limited information is available with respect to the immunological consequences of such transplantation. The interest in the immunology of CNS transplantation has increased since the advent of these new therapies but it is still generally regarded as an immunologically privileged site.[23] The two cell lines studied here were found present in the host brain for at least 40 days post-implantation, some as a discrete mass at the original site while others appeared to migrate through the ventricular walls and into the parenchyma thus displaying diffuse engraftment. This observation has been made with other grafted immortalized progenitor cells[22] without disrupting normal neurobiological processes. Thus these cell lines appeared to behave appropriately *in vivo* providing a tool for studying the immunological consequences of their transplantation. Previous reports analysing host response to cerebral grafts have documented events post-implantation and during rejection. However, the majority of these studies have used the rat as the host animal and only a few groups have used the mouse.[24] The development of a murine model will have the advantage of the availability of the substantial tools in the murine system including genetically altered hosts. Second, these studies have often used solid or dissociated tissue as the graft material. This system gives only indications as to the relative contribution of the different cell types to graft recognition. Indeed, it has been suggested by some that the presence of donor microglia and donor vascularizing cells may increase the chances of recognition since they may enhance antigen presentation.[25] In our system the complication of heterogeneous cell types does not arise and moreover we can manipulate the cells *in vitro* to study the contribution of distinct molecules. Third, others have used xenografts which are known to result in more vigorous responses and indeed the mechanisms resulting in their recognition are possibly different. Fourth, we wished to ask the questions in adult animals since this is more relevant clinically.

After implantation of the cell lines into syngeneic and allogeneic mice, characteristic cellular responses were observed. Two cell lines were used in this present study which differ in their quantitative expression of MHC class I. The host responses observed were similar in both syngeneic and allogeneic transplants when the low MHC-expressing cell line was used. These host responses were: increased local GFAP expression, increased local activation of or recruitment of cells of the monocyte/macrophage lineage, and local increased expression of both MHC class I and class II at day 10 post-implantation. The responses had subsided in the majority of animals by days 30 and 45. Similar observations have been made after transplants of solid tissue[8] and dissociated tissue[12] and are most likely associated with the trauma of surgery. However, the host response to allogeneic high MHC class I-expressing cell lines was more intense at day 10 and in some animals was accompanied with perivascular cuffing, angiogenesis and tissue damage. These more intense responses have been observed by others during rejection of cerebral transplants[8,11,25–27] thus it is interesting that at later time-points the transplanted cells are still present and no tissue damage remained. Other groups have not observed such strong responses after allogeneic transplantation[12,28] while others have reported complete rejection.[8–10] These differences have been attributed to site of transplant[8,29] (those that are positioned in the ventricular system are rejected more rapidly), to extent of surgery induced trauma, and to the nature of donor tissue.[24] Since MGC3 and MGC7 were implanted by the same method it is less likely that in this case the inflammatory response was entirely due to trauma. However, this could certainly be a contributing factor and may suggest that the lack of an inflammatory response to MGC7 above normal is in fact the more unusual situation. The hypothesis favoured is that donor MHC class I may be important for graft recognition. The observation that survival of intracerebral grafts can be increased with masking of MHC class I in donor tissue[30] is evidence for this.

However, differential expression of contributary cytokines by the two cell lines cannot be ruled out as a possibility for the different host reponses observed. Preliminary data from RNAase protection assays suggest that the cytokine profiles do not differ between the two. Indeed, both contain mRNA for interleukin-6 and tumour necrosis factor-β

(N. MacDonald, unpublished observations) which are consistent with an astrocyte phenotype.[31] The induction of other proinflammatory molecules by these cells during transplantation has not been addressed.

In this study we have developed cell lines derived from the CNS which will enable us to study some questions regarding immune triggering and modulation *in vivo*. These cell lines are clonal and appear to remain stable for at least 40 days post-implantation. The advantages of these lines are that we can study the importance of the nature of the cell type on immune reactivity, that they can be genetically manipulated *in vitro* to express putative modulatory proteins, and that we are now able to place the grafts stereotaxically to look at temporal events occurring during the early stages of immune recognition. The *in vivo* observations presented here suggest that an inflammatory response to cell lines devoid of class II can be initiated in the CNS and despite this onslaught they can survive for periods of time after the host cellular response has abated. The reasons for this are unclear but the inability to detect donor MHC class I, except very weakly, on the grafted cells *in vivo* suggests that mechanisms may be in operation to down-regulate this molecule. Other groups have shown that viruses can persist in the CNS despite detectable immune responses and similar mechanisms may account for this. This may be an advantage to those developing viral vector systems for gene therapy but may pose a clinical problem if the inflammation and adaptive immune response become deleterious to the host. Indeed the use of cell lines as vehicles for regenerative purposes in the CNS in order to correct neurodegenerative disease is already a viable prospect. The observations made here suggest that caution need always be taken in their clinical use.

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