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Immune parameters relevant to neural xenograft survival in the primate brain

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Abstract: The lack of supply and access to human tissue has prompted the development of xenotransplantation as a potential clinical modality for neural cell transplantation. The goal of the present study was to achieve a better understanding of the immune factors involved in neural xenograft rejection in primates. Initially, we quantified complement mediated cell lysis of porcine fetal neurons by primate serum and demonstrated that anti-C5 antibody treatment inhibited cell death. We then developed an immunosuppression protocol that included in vivo anti-C5 monoclonal antibody treatment, triple drug therapy (cyclosporine, methylprednisolone, azathioprine) and donor tissue derived from CD59 or H-transferase transgenic pigs and applied it to pig-to-primate neural cell transplant models. Pre-formed αGal, induced αGal and primate anti-mouse antibody (PAMA) titers were monitored to assess the immune response. Four primates were transplanted. The three CD59 neural cell recipients showed an induced anti-αGal response, whereas the H-transferase neural cell recipient exhibited consistently low anti-αGal titers. Two of these recipients contained surviving grafts as detected by immunohistochemistry using selected neural markers. Graft survival correlated with high dose cyclosporine treatment, complete complement blockade and the absence of an induced PAMA response to the murine anti-C5 monoclonal antibodies.

F. Cicchetti,[1,*] W. Fodor,[2] T. W. Deacon,[1] C. van Horne,[1,3] S. Rollins,[2] W. Burton,[2] L. C. Costantini[1] and O. Isacson[1]

[1]Neuroregeneration Laboratories, Harvard Medical School/McLean Hospital, Belmont, MA, USA, [2]Alexion Pharmaceuticals Inc., Cheshire, CT, USA and [3]Department of Neurosurgery, Brigham and Women's Hospital, Boston, MA, USA

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Address reprint requests to Francesca Cicchetti, Neuroregeneration Laboratories, MRC 130 McLean Hospital/Harvard Medical School, 115 Mill Street, Belmont, MA 02478, USA (E-mail: fcicchetti@mclean.harvard.edu)

*Present address: F. Cicchetti, Center de Recherche du CHUL, Unité de Recherche en Neuroscience, RC-9800, 2705 Blvd. Laurier, Ste-Foy, Québec, Canada GIV 4G2. E-mail: francesca.cicchetti@crchul.ulaval.ca or to W. Fodor, Alexion Pharmaceuticals, Inc., 352, Knotter Drive, Cheshire, CT 06410, USA. E-mail: fodor@alxn.com
F. Cicchetti and W. Fodor contributed equally to this article

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Introduction

An emerging novel treatment for neurodegenerative diseases is neural cell transplantation [1]. In Parkinson's disease (PD), dissociated human fetal ventral mesencephalon (VM) tissues have been grafted to the caudate-putamen resulting in clinically meaningful improvements that extend 10 years beyond transplantation [2, 3]. However, this experimental procedure using fetal human tissue is restrained by ethical considerations and limitations of supply associated with human fetal donor tissue. In an effort to find alternative and appropriate donor neurons for PD and Huntington's disease, xenogeneic porcine tissue sources have been explored [4–11]. However, xenotransplantation introduces additional problems of immune rejection that are more formidable than those encountered with allografts [12].

Humoral and cellular immune mechanisms contribute to the destruction of pig xenografts [12]. The humoral immune response includes xenogeneic natural antibody (XNA) reactivity and complement activation [12]. In addition, induced antibody responses contribute to humoral immunity [13]. Furthermore, direct and indirect T-cell activation lead to delayed graft rejection [12].

Pig VM donor cells have been tested in pilot trials for patients with PD. The initial findings from post-mortem studies [4], PET and clinical [11] observations indicate that neural cell survival in patients and primates was absent or notably less robust when compared with survival in rodent models. It is well documented that pig cells are lysed via an antibody-dependent complement-mediated mechanism when they are exposed to human and primate serum [14–16].

Therefore, we hypothesized that pre-formed antibody reactivity- and complement-mediated cytolysis pose a significant graft rejection risk for fetal neural pig cells implanted in primate brains during the post-implantation period, particularly when the blood–brain-barrier (BBB) is compromised with leakage of immunoglobulin and serum complement. For this reason, we investigated additional modalities of immune suppression to control humoral attack of fetal donor cells during the early period of graft maturation. We have developed murine monoclonal antibodies (mAbs) that block complement activation at the point of C5 cleavage. In addition, transgenic animals expressing a human primate complement inhibitor (CD59) or human α-1,2-fucosyltransferase (HT) were utilized as a source of donor tissue to provide an added barrier to humoral-mediated cell lysis [17]. This report describes results from four monkey transplant recipients that received porcine neural cell xenografts and were treated with a combination of immunosuppression and complement inhibition as models of neural transplantation therapy for neurodegenerative diseases.

Methods

Primate serum-mediated cell lysis assay

Isolated VM neurons from embryonic day (E) 28 embryos from CD59 transgenic pigs were washed with Hanks' balanced salt solution (HBSS) 1% bovine serum albumin (BSA) and exposed to Calcein AM (Molecular Probes Inc., Eugene, OR, USA) (10 μM) for 20 min Cells were subsequently incubated at 37 °C for 30 min with increasing concentrations of rhesus serum (from 0 to 40%). Cell lysis experiments were performed with the inclusion of 5G1.1 (antihuman C5 inhibitor), 37B2 + 1B7 (anti-primate C5 inhibitors) and 18A10 (anti-rat inhibitor). Percentage of cell lysis (calcein release) and cell death was determined by flow cytometry on a Becton-Dickenson FACSort (San Jose, CA, USA).

Parkinson's disease animal model

The model of PD in cynomolgus monkeys (*Macaca fascicularis*) was produced by the acute administration (0.5 mg/kg intravenously, every week, for approximately 3 to 4 weeks until PD signs appeared) [18] of the mitochondrial complex 1 inhibitor 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [19]. Primate case (P) 4 was a *Macaca mulatta* and was not subjected to MPTP treatments.

Immunosuppression

The anti-C5 antibody dose level was extrapolated from an in vitro cell lysis assay prior to the transplant study. The in vivo delivery protocol was tested on a naïve animal prior to the transplant study. Each monkey recipient received daily intravenous (i.v) injections of anti-C5 antibodies, for a period of 14 days starting the day of transplant. The anti-C5 antibody solution consisted of two mouse monoclonal antibodies, IF7 and 37B2, in physiological saline. The antibody, 1F7, was produced by immunizing mice with human C5 and cross reacted to primate C5 (S. Rollins, unpublished results). The 37B2 antibody is a primate specific anti-C5 monoclonal and was produced by immunizing mice with baboon C5 (S. Rollins, unpublished results). Both antibodies were dosed at 7.5 mg/kg. The final antibody dose was 15 mg/kg/day and was delivered slowly as a single i.v. bolus injection via a previously implanted jugular catheter while the animal was lightly anesthetized with ketaset-ketamine (Fort Dodge Animal Health, Overland Park, KS, USA; 0.1 ml/kg). Serum was collected daily for the first 15–20 days for complement, αGal and the primate anti-mouse antibody (PAMA) response analysis. On the day of surgery, each animal also received methylprednisolone (2 mg/kg/day, i.m) (Sigma, St Louis, MO, USA), which was administered in declining doses over 14 days (i.e. 1.8, 1.6, 1.4, 1.0, 0.8, 0.6, 0.4). Azathioprine (Imuran, Borroughs Wellcome) was generally started on the day of surgery at 4 mg/kg/day and was then reduced to 2 mg/kg/day for the second week, to 1 mg/kg/day for maintenance levels until time of sacrifice.

Cyclosporine (20 mg/kg/day) (CsA, Sandimmune, Sandoz Pharmaceuticals Corporation, NJ, USA) administration began 2 days prior to the day of implantation surgery and continued for the entire length of the experiment. Only P1 differed in CsA regimen by receiving injections (i.m. 20 mg/kg/day) twice daily for the first 4 weeks. CsA dosing was then shifted to i.v. injections to reach higher blood levels of CsA. All remaining animals were dosed through an implanted catheter to achieve consistently stable blood levels. The jugular catheter was implanted 1 to 5 days prior to transplantation surgery. The micro infusion pump (CADD-Micro Ambulatory Infusion Pump, Model 5900, SIMS Deltec, Inc., St Paul, MN, USA) was attached to the jugular catheter and was placed in a pouch attached to a jacket worn by the animal. A microprocessor controlled the minipump which delivered the CsA continuously over a 24-h period for a final dose of 20 mg/kg/day. CsA levels

were monitored weekly and ranged between 500 and 5000 ng/ml (Table 1). None of the animals showed any signs of ill-health or adverse effects by the immune suppression treatment, and were assessed daily by veterinarian staff at the New England Regional Primate Center.

Cell preparation and transplantation

CD59 and HT transgenic pigs [17] were artificially inseminated to produce donor fetuses. Twenty-eight or 35 days post-insemination, pigs were euthanized following *Animal Care and Use Committee* (ACUC) approved veterinary procedures. Dissection of VM or LGE was performed in HBSS under 40-fold magnification as described previously [5].

In P3 and P4, the anti-C5 antibody solution (final concentration, 500 μg/ml) was added to the cell suspension after cell viability was performed. The suspension was diluted to a concentration of 60 000 to 100 000 cells/μl and 6 to 10 μl of the cell suspension was injected into each of five sites in the host brain using a 10-μl Hamilton syringe and a 45 ° bevelled needle. Each monkey underwent T_1 and T_2-weighted MR imaging prior to the first surgery [20]. Transplants were made into the caudate nucleus (two sites) and the putamen (three sites) on one side of the brain. The cell solution was injected at a rate of 1 μl per 2 min, followed by a 2-min delay before withdrawal of the needle. Animals were sacrificed approximately 8 to 12 weeks following transplantation surgery.

Histological fixation and immunostaining of brain sections

After a post-transplantation period of 2 to 3 months, animals were deeply anesthetized with sodium pentobarbital (300 mg/kg, i.v) and perfused with heparinized saline followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate buffered saline (PBS), pH 7.4. The brains were removed, post-fixed in 4% PFA overnight, transferred to increasing concentrations of sucrose in 0.1 M PBS (10, 20 and 30%) for cryoprotection and 40 μm sections were cut on a freezing microtome. The free-floating sections were processed for cresyl-violet staining or immunohistochemistry, as previously described [4,5,7,21]. Immunohistochemistry for tyrosine hydroxylase (TH) (Pel-Freez, Rogers, AR, USA) (dilution 1 : 500), dopamine and cyclic-AMP-associated receptor phosphoprotein (DARPP32) (Research Biochemical International, Natick, MA, USA) (1 : 20 000), 70 kDa neurofilament (NF70; Biodesign, Kennebunkport, ME, USA) (1 : 40), pig CD44 (clone 10–14 [4]) (dilution 1 : 2000), CD59 (Pharmigen, San Diego, CA, USA) (dilution 1 : 1000), CD3 (DAKO Corporation, Carpinteria, CA, USA; dilution 1 : 1000), CD4 (Novocastra Laboratories Ltd, Newcastle, UK; dilution 1 : 15), CD8 (Novocastra Laboratories Ltd, Newcastle, UK; dilution 1 : 80) and KP1 (DAKO Corporation, Carpinteria, CA, USA; dilution 1 : 75) was performed. Controls with omission of the primary antibody were performed on selected sections to verify staining specificity.

Table 1. Immune response

Primate case	Transplant survival	Average cyclosporine levels (ng/ml)	Transgene	Complement blockade (14 days)*	Anti-αGal Ab levels and response (2 week post-tp) (IgG/IgM)*
P1 (5.7 kg)	No (2 months, 25 days)	423.3	CD59	No	3/2
P2 (5.0 kg)	Yes (3 months)	4398	CD59	Complete	2/3
P3 (5.0 kg)	No (3 months)	2541	CD59	Partial	2/2
P4 (5.1 kg)	Yes (2 months, 8 days)	994	HT	Complete	0/0

Cyclosporine levels:
P1: 423.3 ng/ml; i.m and i.v. dosing. Average CsA levels are derived from four measurements performed after one i.m and three i.v. dosing.
P2: 4398 ng/ml; Continuous i.v. delivery via micropump. Average CsA levels are derived from 13 measurements performed after i.v. dosing.
P3: 2541 ng/ml; Continuous i.v. delivery via micropump. Average CsA levels are derived from 14 measurements performed after i.v. dosing.
P4: 994 ng/ml; Continuous i.v. delivery via micropump. Average CsA levels are derived from 15 measurements performed after i.v. dosing.
Complement blockade:
No: No blockade of the complement activity.
Incomplete: Less than 7 days of complement blockade.
Partial: Greater than 7 days of complement blockade.
Complete: 100% blockade of the complement activity.
Anti-αGal Ab levels and response:
0 = 0.
1 = 50% of human serum.
2 = equal to human serum.
3 = 50% greater than human serum.
*See Fig. 2 for detailed data.

Serum hemolytic assay

Serum complement activity from each recipient monkey was measured daily for at least 14 days following cell transplantation. A chicken red blood cell (RBC) lysis assay was used to determine complement activity as was essentially described by Rinder et al. 1995 [22], with the following changes. Primate serum was diluted to 5% final concentration. The chicken RBC/primate serum solution was incubated for 30 min at 37 °C. Following incubation, the reaction was centrifuged to pellet RBCs. The supernatant (85 μl) was then transferred to a F96 Nunc plate. Optical density (OD) at 415 nm was measured on a microplate reader (Bio-Rad Laboratories, Richmond, CA, USA). Hemoglobin release was calculated as described [22]. Complement inhibition was rated as *None (no):* no blockade of the complement activity, *Incomplete*: less than 7 days of complement blockade, *Partial*: greater than 7 days of complement blockade or *Complete*: 100% blockade of the complement activity (see Fig. 2A for detailed data).

Gal-1,3-Gal antibody ELISA

The levels of Gal-1,3-Gal antibody titers in the serum of recipient primates pre- and post-pig cell transplantation was measured by ELISA. Briefly, 50 μl of horse serum albumin (HSA, 2.5 μg/ml stock concentration) or Gal-1,3-Gal conjugated HSA (2.5 μg/ml stock concentration) was used to coat Nunc F96 Maxisorb plates in ELISA coating buffer (32 mM $NaCO_3$ + 0.1 M $NaHCO_3$) for 1 h at 37 °C. Plates were washed 3x with 200 μl of PBS + 0.05% Tween-20. Serum samples were diluted to 5% in PBS + 0.05% Tween-20 prior to use. Serum was then added and incubated for 1 h at 37 °C, followed by three washes (PBS + 0.05% Tween-20). Goat anti-monkey HRP conjugated secondary antibodies (IgG or IgM diluted 1 : 4000; Zymed) were then added (50 μl/well) and incubated for 1 h at 37 °C. HRP was developed using 0-penylenediamine tablets (Sigma). The reaction was stopped by adding 3 N HCl (50 μl/well). Colorimetric (490 nm wavelength) changes were measured on a microplate reader (Bio-Rad Laboratories, Richmond, CA, USA).

A semi-quantitative scale was derived from the calculations of the total fold increase in IgG or IgM αGal antibody levels in primates compared with pooled normal human serum. When the primate antibody levels were 50% of the human serum, the primate received a score of 1; when equal to human serum levels, a score of 2 was given; αGal antibody levels 50% greater than human serum received a score of 3 (see Fig. 2B, C for detailed data).

Primate anti-mouse antibody analysis

The levels of induced antibodies reactive to the murine anti-C5 antibodies, 1F7/37B2 were determined by ELISA. Essentially the assay is identical to the Gal-1,3-Gal ELISA assay described above, with the exception of the protein coated on the Nunc Maxisorb plates. A mixture of the two mouse antibodies, 1F7/37B2, at a final concentration of 10 μg/ml (5 μg/ml of each antibody; 50 μl/well) was used to coat the ELISA plates as described above. All subsequent steps were performed as described above in the Gal-1,3-Gal ELISA assay.

Total fold increases from the initial to the peak levels of the PAMA response for each animal were calculated. Animals that did not show an increased PAMA response were given a score of zero.

Animal care

Animals used in this study were maintained according to the guidelines of the Committee on Animals of the Harvard Medical School and Massachusetts General Hospital and those of the *Guide for Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources*, National Research Council, Department of Health, Education and Welfare. All efforts were made to minimize animal discomfort and to reduce the number of animals used. No adverse effects of ill-health related to the immune suppression protocol were observed in these animals.

Results

Inhibition of complement activation

Prior to in vivo administration of the anti-C5 antibody solution (37B2/1F7), complement mediated cell lysis experiments were performed on porcine VM cells. VM cells exposed to primate serum exhibited greater than 50% cell lysis in a 30-min assay. Treatment with the antibody combination 37B2/1F7 completely inhibited cell lysis whereas an anti-human (5G1.1) [23] or an anti-rat specific (18A10) [24] anti-C5 antibody showed no protection (Fig. 1).

In a preliminary in vivo study, i.v. infusion (15 mg/kg/day; 7.5 mg/ml of each antibody) of the anti-C5 antibodies effectively blocked primate complement activity for a period of 24 h (data

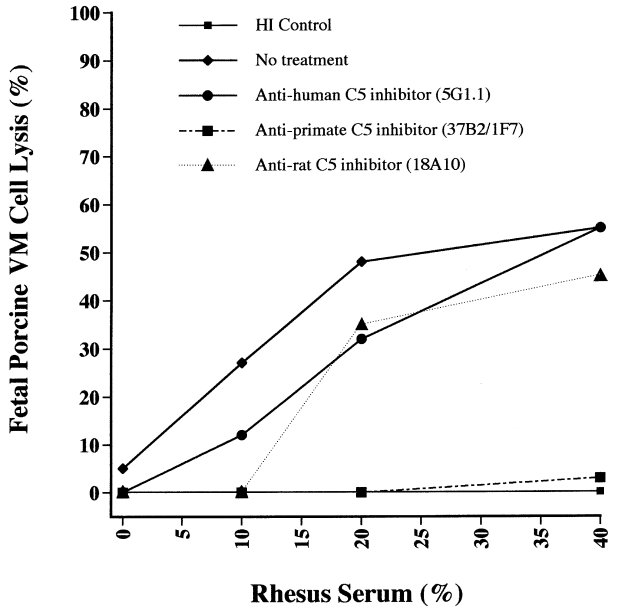

Fig. 1. Fetal porcine VM cell lysis experiment. Fetal VM cells were incubated in increasing concentrations of rhesus serum with or without the following antibody reagents: 5G1.1 (anti-human C5 inhibitor), 37B2/1B7 (anti-primate C5 inhibitors) and 18A10 (anti-rat C5 inhibitor). Cell lysis was measured as described in experimental protocol section. Heat inactivated (HI) serum was used as a control. Note the complete blockade of cell lysis with the anti-primate C5 antibody combination (37B2/1F7).

not shown). Assessment of complement inhibition was performed on serum samples drawn from each recipient using a standard chicken RBC hemolytic assay (see Experimental protocol). In all cases, pre-treatment serum samples showed complete hemolysis (100%) indicating that the animals were not complement deficient. P1 showed virtually no complement blockade (i.e. only on day three post-transplantation) (Fig. 2A). P3 exhibited partial complement blockade during the first week post-transplantation and complete blockade for the remaining of the 14-day treatment period (Fig. 2A) whereas P2 and P4 displayed complete complement inhibition (Fig. 2A).

Immune responses

Measurements of pre-formed and induced IgG and IgM αGal antibody titers were assayed on all recipients for a period of at least 14 days (Table 1, Fig. 2B,C). For example, initial IgG αGal antibody levels for P1 were greater than a pooled human serum standard and continued to increase (Table 1, Fig. 2B). The IgG antibody titers in P1 averaged 50% greater than the human serum standard over the 14-day period (score of 3). In comparison, IgM αGal antibody titers dropped below the human serum standard in the first 3 days following transplantation and then increased significantly through day 10 (Table 1, Fig. 2C). The average IgM titer over the 14 day analysis period in P1 was comparable to the human serum standard (score of 2). P2 exhibited a low IgG titer that increased over the course of the first 14 days and a high IgM titer (score of 2/3) (Table 1, Fig. 2B,C). P3 titers for both IgG and IgM were low initially and increased in the first 14 days to levels comparable to the human serum standard (score 2/2) (Table 1, Fig. 2B,C). P4 antibody titers for IgG and IgM were low throughout the 14 day analysis period (score 0/0) (Table 1, Fig. 2B,C).

We observed variability in the IgG and IgM αGal antibody titers between the individual recipients, however, if complement was completely inhibited during the first 2 weeks following transplantation it appeared that the level of αGal antibody did not affect transplant survival (compare P2 to P3). Conversely, high initial antibody titers (P1) or an induced antibody response (P3) in the absence of complement inhibition was associated with an absence of graft survival. In addition, we assayed for induced PAMA responses to the anti-C5 antibodies. The initial PAMA levels in P1 were 0.05 (O.D. 490 nm) and increased to 0.4 over a period of 14 days (Table 1, Fig. 2D), which corresponded to an 8-fold increase. P3 had a slightly less robust PAMA response than P1, showing only a 5-fold increase (Table 1, Fig. 2D). PAMA responses in P2 and P4 were negligible and zero, respectively (Table 1, Fig. 2D). The lack of an induced PAMA response in P2 and P4 suggests that our immunosuppression protocol was effective at inhibiting a primary response to the foreign antigens and correlated with graft survival. In all other cases, an induced PAMA response correlated with graft rejection (Table 1, Fig. 2D).

Macrophage and T-cell infiltration was evaluated with KP1, CD3, CD4 and CD8 immunohistochemistry, respectively. There was clear KP1 (macrophage marker) expression in the needle tract of rejected grafts. No T-cell infiltrates were detected utilizing CD3 (T-cell marker), CD4 (T-helper cell) and CD8 (cytotoxic T-cell) (data not shown).

Assessment of graft survival

Two primate recipients contained significant identifiable surviving grafts and two cases exhibited needle tracks with no or very few visible donor cells. The positioning of the needle tracts in

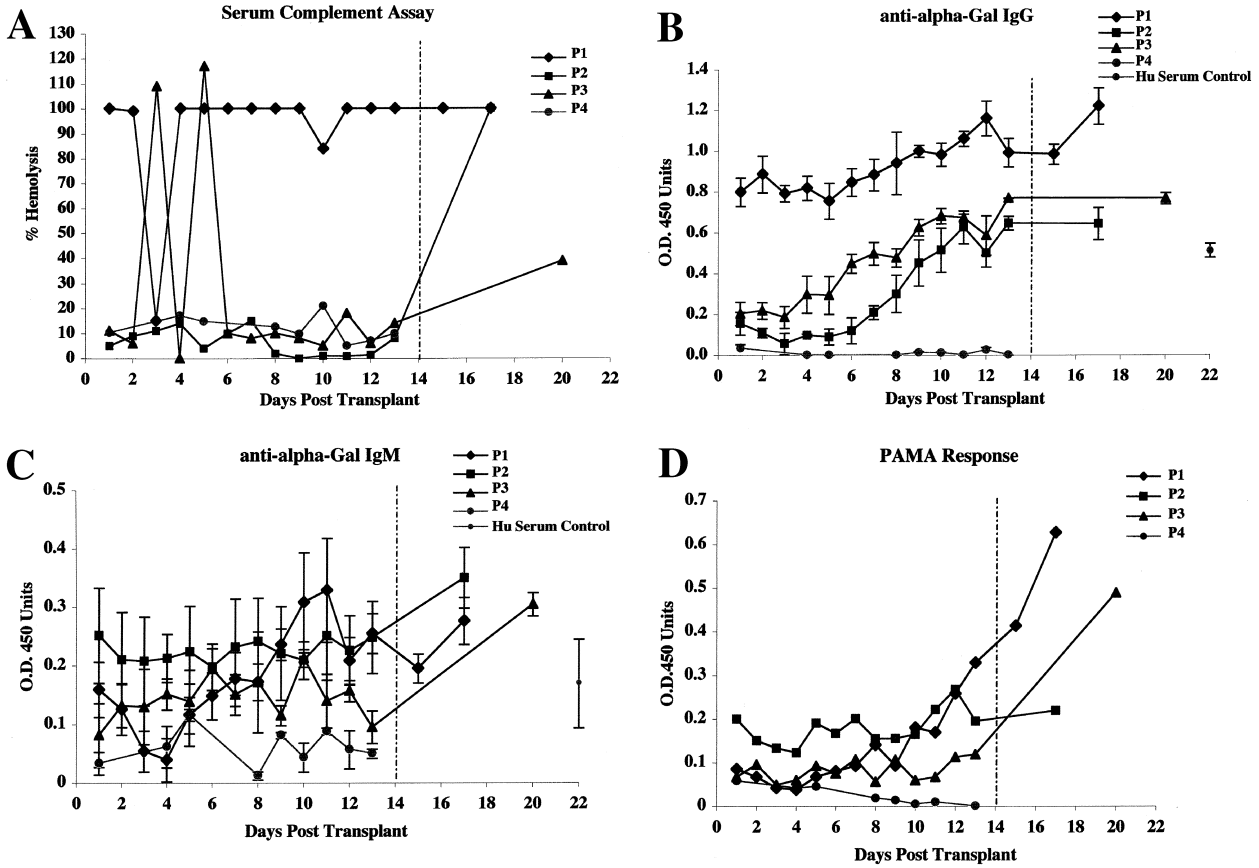

Fig. 2. Assessment of immune capacity and response in recipient animals: (A) Complement activity of recipient sera after treatment with 37B2/1F7. (B) Anti-αGal IgG antibody levels and response post-transplantation. (C) Anti-αGal IgM antibody levels and response post-transplantation. (D) Primate anti-mouse antibody response (PAMA). Dotted lines indicate end of treatment. Light blue dot represents the average levels calculated for the human pooled serum.

striatal regions confirmed accurate targeting. In P2, examination of the tissue at 12 weeks post-transplantation demonstrated one surviving graft located in the putamen. Within the graft, Nissl staining (Fig. 3A) showed cells in high density. Although quite immature, regions of the graft immunostained for the pig-specific axonal marker NF70 (Fig. 3B), TH (Fig. 3C), the pig-specific glial marker CD44 (Fig. 3D), and the CD59 human transgene (Fig. 3E). Post-mortem analysis performed at 9 weeks post-transplantation also showed a surviving graft in P4 (Fig. 3F). The LGE cell transplant stained for AChE (Fig. 3G) and was immunopositive for NF70 (Fig. 3H), CD44 (Fig. 3I), and DARPP32 (Fig. 3J).

Graft survival in P2 and P4 was associated with high levels of CsA, adequate methylprednisolone, azathioprine, complete complement blockade and the absence of an induced PAMA response. Based on our observations, graft rejection in the other recipients correlated with the failure to reach optimal efficacy in one or more of the above treatments.

Discussion

Clinical studies have demonstrated that survival of human fetal DA neuronal grafts result in clinical improvements [2,3,25–27]. However, the use of human fetal tissue raises ethical and practical issues. Moreover, the lack of appropriate donor cell supply limits this therapy to an experimental treatment. Initial clinical trials using porcine VM xenografts in PD recipients have resulted in minimal survival, and clearly less than seen with allogeneic tissue [11]. Nonetheless, there exists one documented case of a porcine VM xenograft surviving 7½ months in an immunosuppressed PD patient [4]. The goal of the present study was to achieve a better understanding of the immune factors involved in neural xenograft rejection in primates. The immunosuppressive regimen utilized in the experiments reported here included clinically acceptable [28,29] triple drug therapy, and the addition of a complement inhibitor to block humoral mediated rejection during the period when the BBB is disrupted [30]

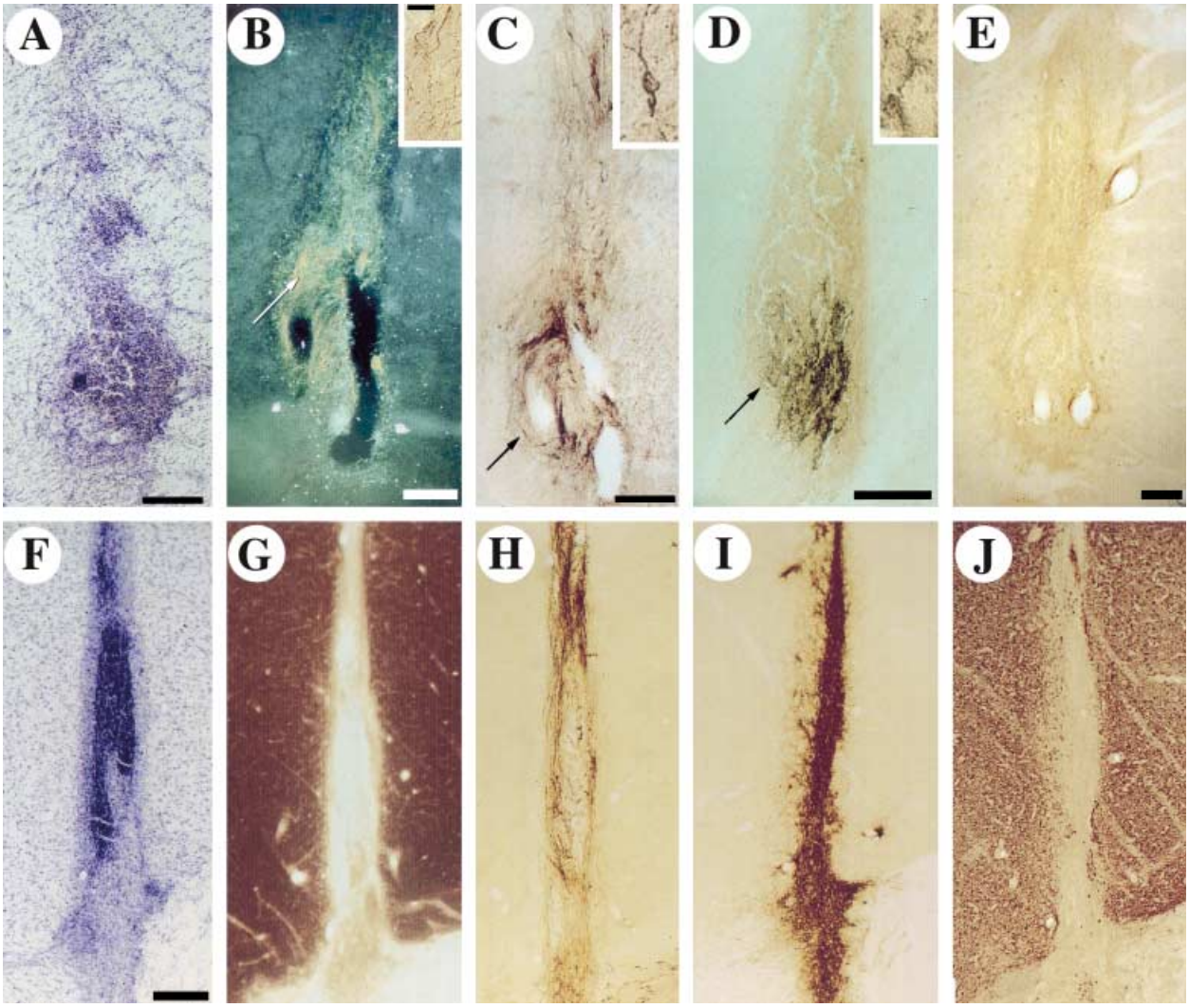

Fig. 3. (A–E) Photomicrographs of sections through a transgenic pig VM graft located in the putamen of P2, stained for (A) Nissl to distinguish graft architecture and immunostained with (B) pig axons (NF70) (C) TH to identify dopaminergic cells and processes (D) pig glia (CD44), and (E) the human CD59 transgene expression. Insets illustrate higher magnification of (B) NF70 positive fibers (C) TH positive cell and (D) an example of astrocytic cell type. Scale bars in *a* and *b* = 500 μm; *c*, *d* and *e* = 200 μm. Scale bar in insets = 20 μm (F–J) Photomicrographs of sections through a transgenic pig LGE graft located in the caudate nucleus of P4, stained for (F) Nissl to distinguish graft architecture and immunostained with (H) pig axons (NF70) (I) pig glia (CD44) (G–J) AchE and DARPP32 to identify striatal cell phenotypes. Scale bar in f (G–J) = 250 μm.

and the tissue is directly exposed to serum complement.

Several groups have investigated the immune response to central nervous system cellular transplantation [9,31]. The major conclusion is that the T-cell mediated rejection of tissues that exists across species barriers can be modulated using immunosuppressive drug therapies such as CsA (for review see Refs [9,31]). More recently, in vitro analyses reveal that human serum antibody and complement mediate cell lysis of porcine neural cells [32]. This indicates that strategies to block complement activation could improve cell transplantation outcomes. Furthermore, a humoral immune response to pig VM xenografts was documented in a rodent model [10]. We now demonstrate in vitro that porcine VM cells are sensitive to complement mediated cell lysis and that blocking complement activation can inhibit cell death. Systemic complement blockade and the use of transgenic (CD59, HT) fetal cells has provided us with the maximum barrier to humoral-mediated immune attack [17,33,34], which correlated with in vivo graft survival. These results are relevant to human therapy due to significant complement-mediated lysis of porcine neurons caused by human serum [32].

In the in vivo transplant experiment, primate recipients were treated with two antibodies designed to block primate complement activation.

This antibody combination inhibits both C5a (a potent anaphylatoxin) as well as the membrane attack complex (MAC) (Fodor & Rollins, unpublished results). The individual antibodies alone can block C5b9 activation, however, only the combination was effective at blocking C5a and C5b9. The successful use of antibodies to inhibit complement activation has proven to be efficacious in several pre-clinical transplantation models [13,35]. The murine antibodies utilized in this study clearly inhibit primate complement in vitro as well as in vivo (Figs 1 and 2A). However, utilizing murine or xenogeneic antibodies in primate transplant recipients has limitations based on antibody clearance and induced immune responses to foreign antigens ([36], Fig. 2D). The latter problem can be overcome in clinical studies by using fully humanized anti-C5 antibodies (see Fig. 1) [37].

We and others have found that the use of CsA in primate transplantation has limitations with respect to dosing levels and absorption profiles [38]. We therefore adapted an indwelling catheter pump administering CsA at high levels to achieve optimal immunosuppressive effects. In the cohort of recipients described in this report, recipients P2, P3 and P4 received such optimal CsA dosing levels [38–41].

Of these three recipients, P2 and P4 exhibited a surviving graft 2 and 3 months post-transplantation, which was associated with complete complement blockade and an absence of an induced PAMA response. P3 received high level CsA dosing and had low initial anti-αGal antibody titers but complement was not entirely blocked during the initial post-transplant period and moreover, a significant PAMA response was detected by day 20 post-transplantation. Although recipient P2 had relatively high anti-αGal antibody titers prior to transplantation (comparable with pooled human sera) the observed complete blockade of complement in the first 2 weeks following transplantation, the absence of an induced PAMA response and the expression of CD59 appeared critical to graft survival. P4 depicted a similar profile as P2 except for the fact that the antibody titers were consistently low and that this primate recipient received HT transgenic cells. In summary, the results suggest that complement blockade is necessary for significant graft survival and that the induced B-cell mediated antibody response (as suggested by the PAMA response) can contribute to xenograft rejection of neural cells [10,42].

Our data illustrate that a significant humoral (both innate and induced) immune response can contribute to the loss of cellular engraftment. We suggest that a comprehensive immunosuppressive regimen, including complement inhibition, may improve xenograft survival in primates. These studies enhance our understanding of potential in vivo xenogeneic neural cell immune rejection mechanisms and are instructive to potential future clinical xenotransplantation therapies.

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