Individual human serum differs in the amount of antibodies with affinity for pig fetal ventral mesencephalic cells and the ability to lyse these cells by complement activation

Koopmans, J; de Haan, Aalzen; Bruin, E; van der Gun, Bernardina, Theresia, Francisca; van Dijk, H; Rozing, J; de Leij, Louis; Staal, M

Published in:
Cell Transplantation

DOI:
10.3727/000000004783983503

Publication date:
2004

Cell Transplantation, Vol. 13, pp. 631–637, 2004
Printed in the USA.

0963-6897/04 $20.00 + .00

www.cognizantcommunication.com

Individual Human Serum Differs in the Amount of Antibodies With Affinity for Pig Fetal Ventral Mesencephalic Cells and the Ability to Lyse These Cells by Complement Activation

Jan Koopmans,* Aalzen de Haan,† Elinda Bruin,† Ieneke van der Gun,† Henk van Dijk,‡ Jan Rozing,§ Lou de Leij,† and Michiel Staal*

*Department of Neurosurgery, University Hospital Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands
†Medical Biology Section of Pathology and Laboratory Medicine, University of Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands
‡Department of Veterinary Anatomy and Physiology, University of Utrecht, Yalelaan 1, 3584 CL, The Netherlands
§Department of Cell Biology, Section Immunology, University of Groningen, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands

Xenografting pig fetal ventral mesencephalic (pfVM) cells to repair the dopamine deficit in patients with Parkinson's disease is the focus of both experimental and clinical investigations. Although there have been marked advances in the experimental and even clinical application of these xenogeneic transplantations, questions regarding the host's xenospecific immune response remain unanswered. It has been shown that human serum is able to lyse pfVM tissue by both anti-gal-gal and non-anti-gal-gal antibodies by complement activation. The aim of this study was to investigate whether interindividual differences exist in the levels of pfVM cell-specific IgM and IgG subclass antibodies, their ability to lyse pfVM cells in vitro and the relationship between both. Pig fetal VM cells were incubated with heat-inactivated serum from 10 different individuals and binding of IgM antibodies and IgG subclass antibodies to pfVM cells was analyzed by flow cytometry. The ability to lyse pfVM cells was analyzed exposing ^{51}Cr-labeled pfVM cells to fresh serum or isolated IgM and IgG from the same individuals and subsequent determination of released ^{51}Cr from lysed cells. Strong differences were found between individuals in the levels of pfVM cell-specific IgM antibodies: antibody levels differed up to 40-fold. pfVM-specific IgG1 and IgG2 levels were only detectable in a few individuals. The ability to lyse pfVM cells ranged from negligible lysis up to 66.5% specific lysis. There was a strong correlation between the levels of individual pfVM-specific IgM antibodies and the ability to lyse pfVM cells in vitro. Isolated IgM, but not IgG, was able to lyse pfVM cells in the presence of complement. In conclusion, the interindividual differences in the levels of IgM with affinity for pfVM cells and their ability to lyse pfVM cells in vitro are considerable. Only few individuals possessed IgG1 and IgG2 subclass antibodies with affinity for pfVM. These findings may influence patient selection for porcine transplants and chances of graft survival in individual patients.

Key words: Xenotransplantation; Ventral mesencephalon; Parkinson's disease; Complement-dependent cytotoxicity; Porcine

INTRODUCTION

Cellular transplantation to repair the dopamine deficit in patients with Parkinson's disease using fetal dopaminergic tissue has been the focus of both experimental and clinical investigations for over three decades (3,7–9,19,24). The possible usage of pig fetal ventral mesencephalic (pfVM) tissue as a donor source has been investigated to overcome the limited availability of abortion-derived human fetal dopaminergic tissue as well as practical, ethical, and possibly infectious difficulties associated with its use (5,10–13,17,18,23). There have been marked advances in the experimental and even clinical application of these xenogeneic transplantations (4,6). However, many questions remain unanswered, especially those regarding the host's immune response and in particular the role of xenospecific "natural antibodies" against an intracerebral xenogeneic implant.

Human serum is toxic to pig cells but different human individuals show considerable quantitative differ-

Accepted May 5, 2004.
Address correspondence to Jan Koopmans, Department of Neurosurgery, University Hospital Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands. Tel: +31-50-3613218; Fax: +31-50-3611715; E-mail: J.Koopmans@nchir.azg.nl

ences in their ability to lyse adult, nonneural porcine tissue by complement activation (21). Toxicity to pfVM tissue has been demonstrated for pooled human serum and was found to be mediated not only by anti-gal-gal antibodies but also by non-anti-gal-gal antibodies (22).

The aims of this study were to investigate: 1) whether interindividual differences exist in the ability to specifically lyse pfVM cells in vitro, 2) the serum levels of pfVM cell-specific IgM and IgG subclass antibodies, and 3) the relationship between the toxicity of sera to pfVM and serum levels of pfVM cell-specific IgM and IgG.

MATERIALS AND METHODS

Antibodies and Reagents

Affinity chromatography used anti-human IgM agarose (Sigma, A9935) and MabTrap GII kit (Pharmacia Biotech, 17-1128-01). Complements were Cedarlane CL3335 Low-tox-H rabbit complement and Biotest 824050 Complement. The medium used was AIM-V Gibco. Fluorescent antibodies used were goat anti-human-IgM-FITC (Protos) dilution 1:40; mouse anti-human-IgG1-FITC (Zymed) dilution 1:20; mouse anti-human-IgG2-FITC (Zymed) dilution 1:20; mouse anti-human-IgG3-FITC (Zymed) dilution 1:20; mouse anti-human-IgG4-FITC (Zymed) dilution 1:20; and mouse anti-human-IgG-FITC (Sigma) dilution 1:10.

Isolation and Preparation of Fetal VM Tissue

Porcine fetuses were obtained from anesthetized 28-day pregnant cross-bred Dutch Yorkshire Landrace pigs by hysterotomy and the VM was immediately microscopically dissected as previously described (11,17,23). The VM was then cut into six to eight small pieces and stored in saline for 2 h at 4°C before being processed into a single cell suspension (14). Viability was assessed using the trypan dye exclusion test and viability was expressed as percentage of viable cells with respect to the total number of cells.

Human Serum and Immunoglobulin Purification

Human serum was obtained from 10 healthy volunteers. None of the individuals was ever directly exposed to porcine antigens (i.e., porcine insulin) or worked on a pig farm. All individuals ate (pig) meat. The serum was either used fresh or heat inactivated and stored at −20°C until later use. Purified IgM and IgG was obtained from the sera by affinity chromatography using anti-human IgM agarose and MabTrap GII kit following the manufacturer's instructions. The concentration of the purified IgM and IgG in the individual solutions after purification was determined using the Dade Behring BN2 and subsequently diluted to a standard concentration of 0.5 mg/ml IgM and 4 mg/ml IgG to avoid influence of individual antibody levels.

Determination of Cytotoxicity

The ability of sera to lyse pfVM cells was analyzed by exposing ^{51}Cr-labeled pfVM cells to fresh serum and subsequent determination of released ^{51}Cr from lysed cells. To label pfVM cells with ^{51}Cr approximately 20 million cells were incubated with ^{51}Cr (200 μCi) in 100 μl PBS for 60 min at 37°C. Cells were subsequently washed with AIM-V medium and albumin (9:1) three times before being centrifuged for 5 min at 1800 rpm and resuspended in 1 ml AIM-V. The suspension was diluted to 5×10^4 cells/ml and plated at 100 μl/well in a 96-well plate (Costar). Next, 100 μl/well of prediluted serum, IgM/IgG subfractions, and/or rabbit complement was added at twofold dilutions. Alternatively, fresh human serum deficient of pfVM-specific IgM and IgG was used as a complement source. Maximum ^{51}Cr release was determined adding Triton X-100 and background release adding AIM-V. After 60 min at 37°C the supernatant was removed and released ^{51}Cr was measured using a Packard gamma counter. The percentage specific lysis was calculated as the measured minus background release divided by the difference between maximum and background release. All measurements were performed in triplets and data are expressed as mean values.

Binding of Antibodies to pfVM

To investigate the binding of different human immunoglobulin classes and subclasses to pfVM cells, cells were incubated with heat-inactivated serum from 10 different individuals. Binding of IgM antibodies and IgG subclass antibodies was analyzed by flow cytometry. More specifically, cells were incubated with serum for 30 min at 37°C and subsequently washed three times with HBSS. Next, the cells were incubated for 30 min at 4°C with Ig class or subclass-specific fluorescent antibodies. Cells were subsequently washed twice with HBSS and analyzed on a flow cytometer (Coulter Epics Elite). To allow comparison of individual sera, measurements were done in one run with fixed settings of the flow cytometer. Binding of pfVM-specific antibodies was expressed as the percentage of positive cells. Five percent or more positive cells was considered confident evidence for the presence of pfVM-specific antibodies.

Statistics

Statistical analyses were performed using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA). A Spearman test was performed to calculate correlation.

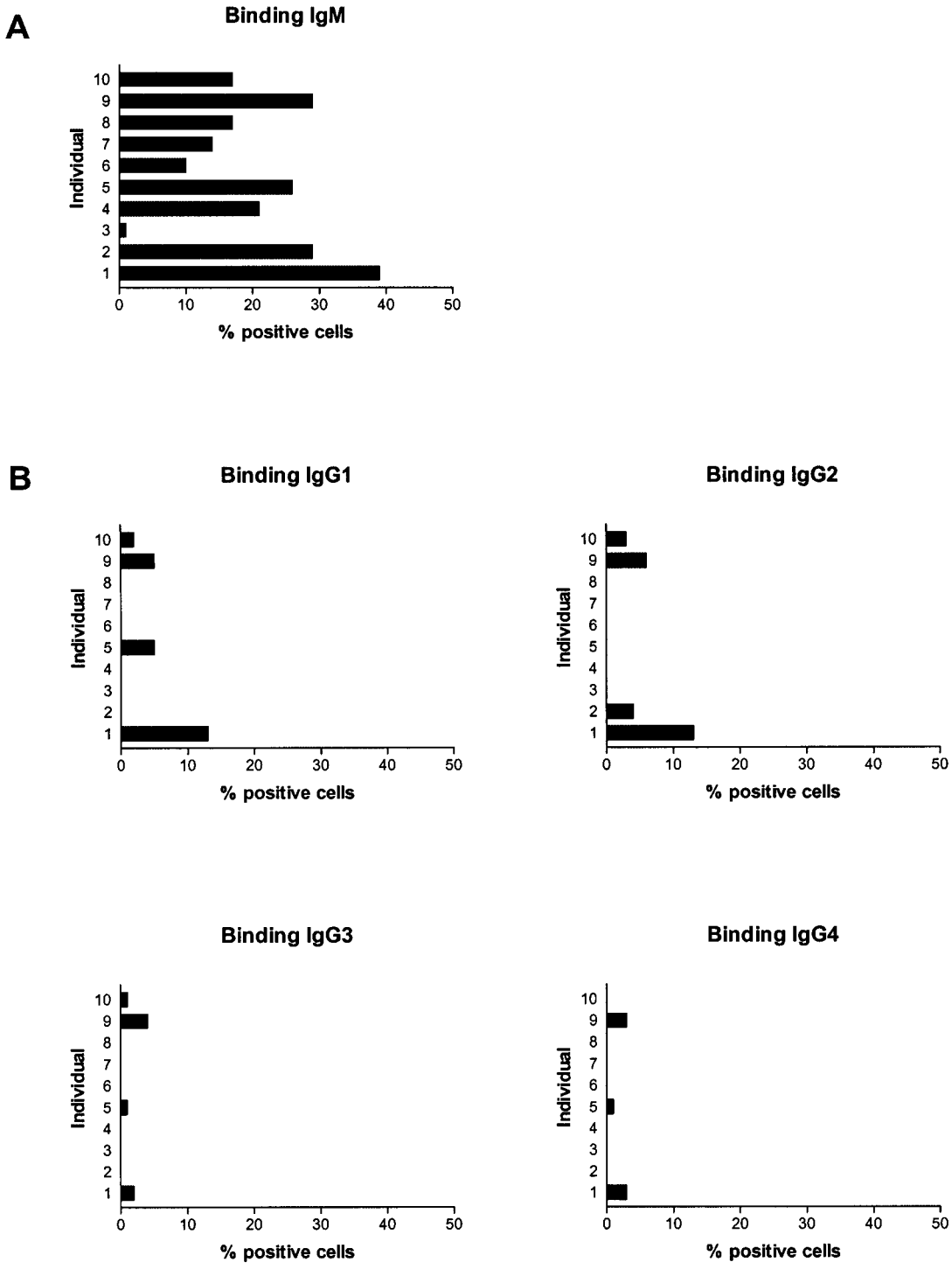

Figure 1. Binding of IgM and IgG subclass antibodies to pfVM as analyzed by flow cytometry and expressed as percentage of positive cells. All measurements for different antibodies were performed in one run with fixed settings of the flow cytometer to allow comparison of 10 individual sera. Five percent or more positive cells was considered confident evidence for the presence of pfVM-specific antibodies.

RESULTS

Individual Differences in Human pfVM-Specific Antibodies

Interestingly, strong differences were found between individuals in the levels of mainly IgM class pfVM cell-specific antibodies as was indicated by flow cytometry analysis of binding of human antibodies to pfVM: antibody levels differed up to 40-fold. All but one sera contained pfVM cell-specific IgM antibodies, while two sera additionally contained small amounts of pfVM cell-specific IgG2 subclass antibodies (sera 1 and 9) and three sera contained pfVM-specific IgG1 subclass antibodies (sera 1, 5, and 9) (Fig. 1).

Individual Serum Levels of pfVM-Specific IgM Correlates With Ability to Lyse pfVM

The ability to lyse pfVM cells was present in almost all sera, but large interindividual differences were found. The ability to lyse pfVM cells ranged from negligible lysis up to 66.5% specific lysis (1:4 dilution of the serum) (Fig. 2).

There was a strong correlation between individual serum levels of pfVM-specific IgM antibodies and the ability to lyse pfVM cells in vitro ($r = 0.93$, $p = 0.0002$, Spearman correlation) (Fig. 3).

Rabbit Serum as a Complement Source Toxic to pfVM Cells

In our experiments testing isolated IgM and IgG from different donors for cytotoxicity using rabbit serum as a complement source, we found that rabbit serum from different sources is toxic to pfVM cells. Up to 63% specific lysis (1:4 dilution) was found (Fig. 4). Heat-inactivated rabbit serum did not kill pfVM cells (Fig. 4). As an alternative to rabbit complement we used serum from donor 3 as a complement source (Fig. 4).

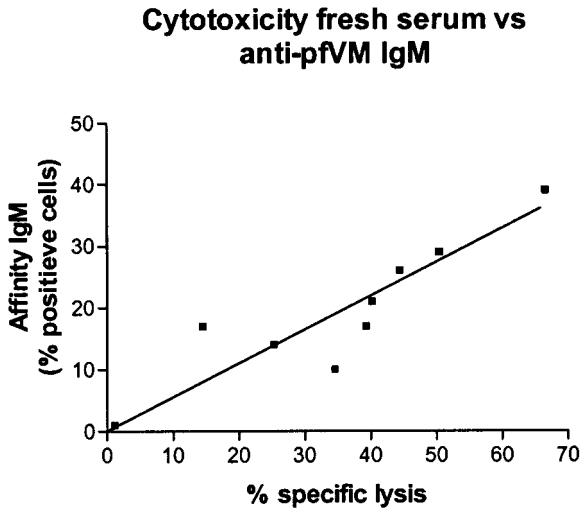

Figure 3. Correlation between the amount of pfVM-specific IgM and cytotoxicity of 10 respective human sera (data from Figs. 1 and 2). Using Spearman correlation: $r = 0.93$ and $p = 0.0002$, indicating a strong correlation between individual serum levels of pfVM-specific IgM antibodies and the ability to lyse pfVM cells in vitro.

Isolated IgM and Not IgG Toxic to pfVM Cells in the Presence of Complement

The ability of isolated IgM from different individuals to lyse pfVM cells showed marked individual differ-

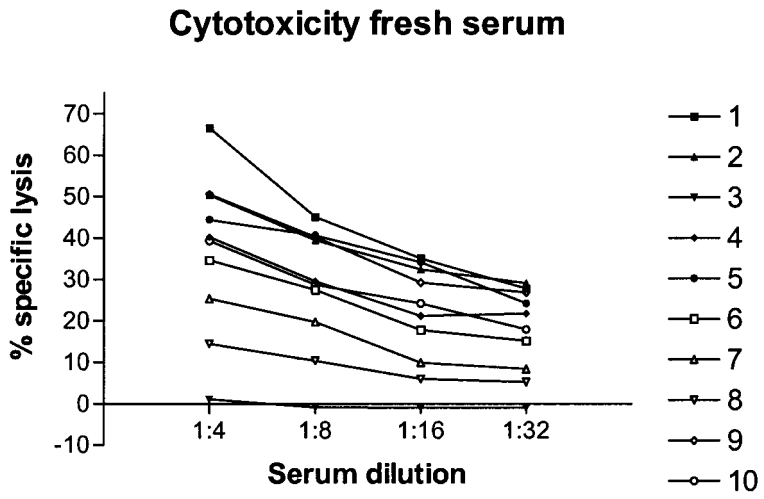

Figure 2. Cytotoxicity of 10 individual fresh sera to pfVM cells analyzed in a ^{51}Cr release assay and expressed as the percentage specific lysis. For each individual serum cytotoxicity of serum (in 4 twofold dilutional steps) was determined. All measurements were performed in triplet. Data are expressed as mean values.

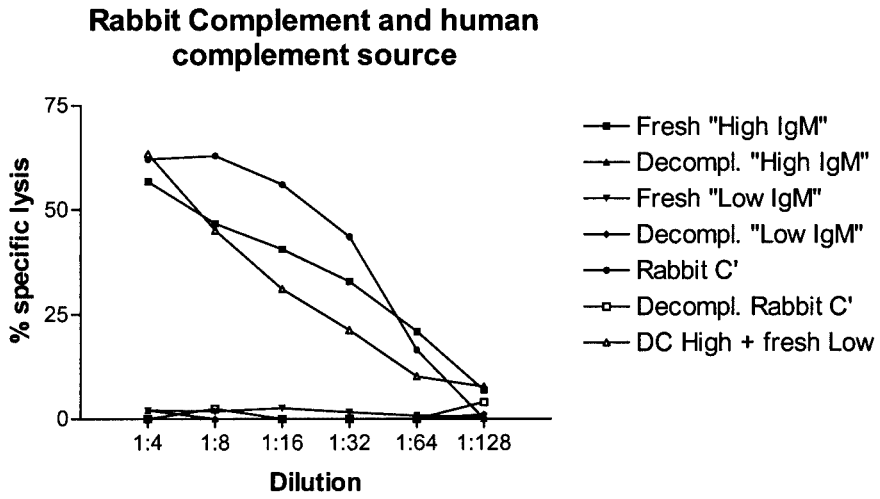

Figure 4. Cytotoxicity of fresh and heat-inactivated rabbit complement as well as fresh and decomplemented serum of individuals 1 and 3. Individual 1 demonstrated high level of pfVM-specific IgM level and toxicity to pfVM, whereas individual 3 demonstrated no detectable level of pfVM-specific IgM and was not toxic to pfVM cells (data from Figs. 1 and 2). To test whether individual 3 could serve as a complement source, decomplemented serum from individual 1 ("high IgM") was mixed with fresh serum from individual 3 ("low IgM"). Data show that the rabbit complement we used is toxic to pfVM cells and that this toxicity is complement dependent because heat-inactivated serum does not kill pfVM cells. Mixing heat-inactivated serum from individual 1 (in itself nontoxic to pfVM) with fresh serum from individual 3 (nontoxic to pfVM cells), 63% specific lysis was measured, decreasing with further dilution of serum from individual 1. Based on these data we used individual 3 as an alternative complement source for further experiments with either isolated IgM or IgG.

ences as observed with fresh serum, ranging between not detectable and 24.1% specific lysis (Fig. 5A). There was a strong correlation between fresh serum and isolated IgM in the ability to lyse pfVM cells in vitro ($r = 0.68$, $p = 0.347$, Spearman correlation). However, isolated IgM showed a decrease in the percentage of specific lysis compared with fresh serum. The ability of isolated IgG to lyse pfVM cells never exceeded 5.8% specific lysis and should be considered negative (Fig. 5B).

DISCUSSION

Our results show that most human serum is able to lyse pig fetal ventral mesencephalic cells by way of complement activation. There are however large differences in the extent to which these sera are able to lyse pfVM cells. In the sera used for this study, specific lysis of pfVM ranged from negligible lysis up to 66.5% at 1:4 serum dilutions. There was a strong correlation between the amount of IgM class antibodies with affinity for pfVM and the ability to lyse pfVM in the respective sera. We found only very low levels of IgG subclass antibodies with affinity for pfVM in a minority of donors, and were not able to show IgG-induced lysis of pfVM by complement activation.

Xenoreactive antibodies have been shown to cause lysis of donor cells by complement activation. Sumitran et al. demonstrated that pooled human AB serum contains IgG and IgM antibodies directed against galα1,3-gal as well as non-gal epitopes on pfVM tissue, and that human serum as well as human serum depleted of α-gal antibodies is able to lyse pfVM cells by complement activation (22). In their studies they showed that both isolated IgG and IgM are cytotoxic to pfVM cells in the presence of complement (22). In contrast, we could not demonstrate cytotoxicity of isolated IgG to pfVM. Pooled serum as was used by Sumitran et al. perhaps contains serum from an individual with high IgG levels with affinity for pfVM. We do not think that our results are influenced by the use of fresh serum from a donor without IgM with affinity for pfVM instead of rabbit complement, although it cannot be excluded.

Differences between human sera in their toxicity to pfVM are significant. In our study, lysis of pfVM cells by human serum differed from 0 to 66.5% specific lysis. Schaapherder et al. tested a large series of different human donors and found substantial interindividual differences in both toxicity against nonneural tissue [porcine endothelial cells (PEC) and porcine islet cells (PIC)] (61% specific lysis average, range 38–81%; $n = 30$) and the amount of antibodies directed against nonneural por-

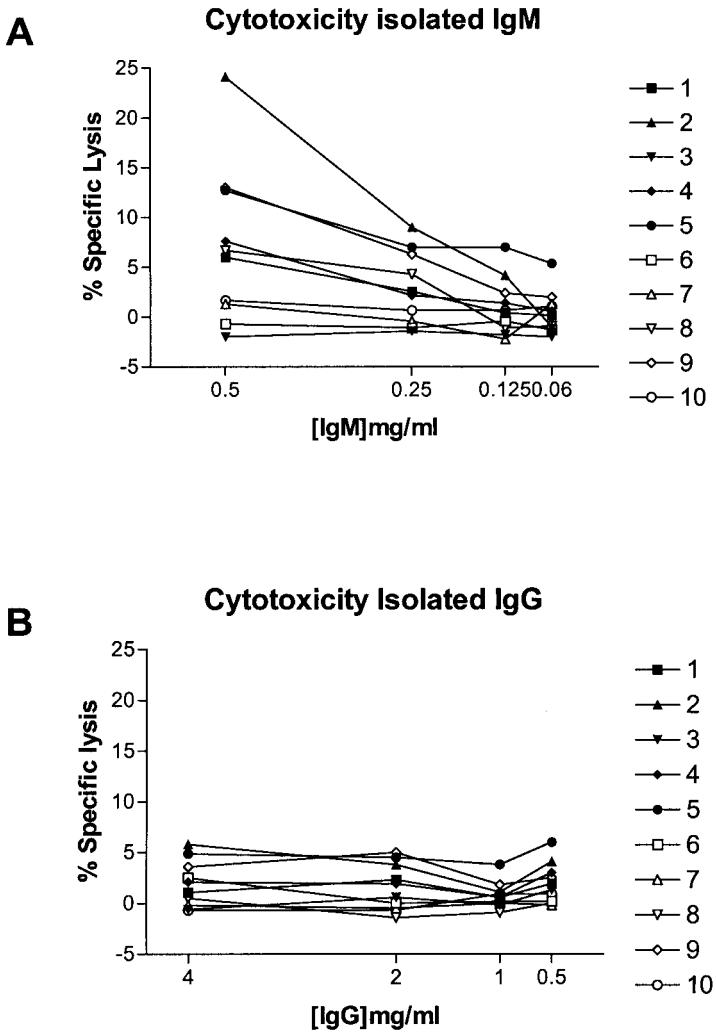

Figure 5. Cytotoxicity of isolated IgM (concentration 0.5, 0.25, 0.125 and 0.06 mg/ml) (A) and isolated IgG (concentration 4, 2, 1, and 0.5 mg/ml) (B) from 10 individuals, expressed as the percentage specific lysis of pfVM cells, using fresh serum from individual 3 as a complement source. All measurements were performed in triplet and data expressed as mean values. Isolated IgM, like fresh serum, shows large individual differences in the ability to lyse pfVM cells whereas isolated IgG shows no detectable lysis.

cine cells (20,21). Importantly, they demonstrated that agammaglobulinemic sera killed approximately 42% of PIC (21), suggesting complement activation via the alternative pathway. In contrast, we found no indication of the latter, as serum without IgM directed against pfVM gave no detectable lysis of pfVM. Further, there was a strong correlation between the individual serum levels of pfVM-specific IgM antibodies and the ability to lyse pfVM cells in vitro, suggesting complement activation via the classical pathway. This may indicate that transplantation of neural tissue is safe with respect to activation via the alternative complement activation pathway.

In vivo studies have shown that there may be an important role for antibodies and complement in the rejection of an intracerebral xenograft, both indirectly by facilitating a cellular response, and directly via complement activation and subsequent cell death (1,2,15,16). Acknowledging these findings, our data, showing considerable differences between different human individuals in levels of (primarily) IgM class antibodies directed against pfVM cells and their ability to lyse pfVM cells via complement activation, can in our opinion have clinical significance in terms of patient selection for porcine transplants and chances of graft survival in individual patients.

ACKNOWLEDGMENTS: The authors would like to thank Jane Briggs for her help with the manuscript. The work presented in this article was supported by the Netherlands Organization for Health Research and Development (ZonMW).

REFERENCES

1. Barker, R. A.; Dunnett, S. B.; Richards, A. The rejection of neural xenotransplants: A role for antibodies. Transplantation 68:1091–1092; 1999.
2. Barker, R. A.; Ratcliffe, E.; McLaughlin, M.; Richards, A.; Dunnett, S. B. A role for complement in the rejection of porcine ventral mesencephalic xenografts in a rat model of Parkinson's disease. J. Neurosci. 20:3415–3424; 2000.
3. Björklund A.; Stenevi, U. Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. Brain Res. 177:555–560; 1979.
4. Deacon, T.; Schumacher, J.; Dinsmore, J.; Thomas, C.; Palmer, P.; Kott, S.; Edge, A.; Penney, D.; Kassissieh, S.; Dempsey, P.; Isacson, O. Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. Nat. Med. 3:350–353; 1997.
5. Deacon, T. W.; Pakzaban, P.; Burns, L. H.; Dinsmore, J.; Isacson, O. Cytoarchitectonic development, axon-glia relationships, and long distance axon growth of porcine striatal xenografts in rats. Exp. Neurol. 130:151–167; 1994.
6. Fink, J. S.; Schumacher, J. M.; Ellias, S. L.; Palmer, E. P.; Saint-Hilaire, M.; Shannon, K.; Penn, R.; Starr, P.; VanHorne, C.; Kott, H. S.; Dempsey, P. K.; Fischman, A. J.; Raineri, R.; Manhart, C.; Dinsmore, J.; Isacson, O. Porcine xenografts in Parkinson's disease and Huntington's disease patients: Preliminary results. Cell Transplant. 9:273–278; 2000.
7. Freed, C. R.; Breeze, R. E.; Fahn, S. Placebo surgery in trials of therapy for Parkinson's disease. N. Engl. J. Med. 342:353–354; 2000.
8. Freed, C. R.; Breeze, R. E.; Rosenberg, N. L.; Schneck, S. A.; Kriek, E.; Qi, J. X.; Lone, T.; Zhang, Y. B.; Snyder, J. A.; Wells, T. H.; et al. Survival of implanted fetal dopamine cells and neurologic improvement 12 to 46 months after transplantation for Parkinson's disease. N. Engl. J. Med. 327:1549–1555; 1992.
9. Freeman, T. B.; Olanow, C. W.; Hauser, R. A.; Nauert, G. M.; Smith, D. A.; Borlongan, C. V.; Sanberg, P. R.; Holt, D. A.; Kordower, J. H.; Vingerhoets, F. J. Bilateral fetal nigral transplantation into the postcommissural putamen in Parkinson's disease. Ann. Neurol. 38:379–388; 1995.
10. Freeman, T. B.; Wojak, J. C.; Brandeis, L.; Michel, J. P.; Pearson, J.; Flamm, E. S. Cross-species intracerebral grafting of embryonic swine dopaminergic neurons. Prog. Brain Res. 78:473–477; 1988.
11. HogenEsch, R. I.; Koopmans, J.; Copray, J. C.; van Roon, W. M.; Kema, I.; Molenaar, G.; Go, K. G.; Staal, M. J. Fetal porcine ventral mesencephalon graft. Determination of the optimal gestational age for implantation in parkinsonian patients. Exp. Brain Res. 132:345–350; 2000.
12. HogenEsch, R. I.; Staal, M. J.; Kema, I. P.; Buys, C. H.; Go, K. G. Utility of fragmented human fetal tissue as a potential dopaminergic brain graft in Parkinson's disease. Stereotact. Funct. Neurosurg. 61:1–11; 1993.
13. Huffaker, T. K.; Boss, B. D.; Morgan, A. S.; Neff, N. T.; Strecker, R. E.; Spence, M. S.; Miao, R. Xenografting of fetal pig ventral mesencephalon corrects motor asymmetry in the rat model of Parkinson's disease. Exp. Brain Res. 77:329–336; 1989.
14. Koopmans, J.; HogenEsch, I.; Copray, S.; Middel, B.; van Dijk, H.; Go, K. G.; Staal, M. Cryopreservation of porcine fetal ventral mesencephalic tissue for intrastriatal transplantation in Parkinson's disease. Cell Transplant. 10: 573–581; 2001.
15. Larsson, L. C.; Czech, K. A.; Widner, H.; Korsgren, O. Discordant neural tissue xenografts survive longer in immunoglobulin deficient mice. Transplantation 68:1153–1160; 1999.
16. Larsson, L. C.; Duan, W. M.; Widner, H. Discordant xenografts: Different outcome after mouse and rat neural tissue transplantation to guinea-pigs. Brain Res. Bull. 49: 367–376; 1999.
17. Molenaar, G. J.; HogenEsch, R. I.; Sprengers, M. E.; Staal, M. J. Ontogenesis of embryonic porcine ventral mesencephalon in the perspective of its potential use as a xenograft in Parkinson's disease. J. Comp. Neurol. 382: 19–28; 1997.
18. Pakzaban, P.; Isacson, O. Neural xenotransplantation: Reconstruction of neuronal circuitry across species barriers. Neuroscience 62:989–1001; 1994.
19. Peschanski, M.; Defer, G.; N'Guyen, J. P.; Ricolfi, F.; Monfort, J. C.; Remy, P.; Geny, C.; Samson, Y.; Hantraye, P.; Jeny, R. Bilateral motor improvement and alteration of L-dopa effect in two patients with Parkinson's disease following intrastriatal transplantation of foetal ventral mesencephalon. Brain 117:487–499; 1994.
20. Schaapherder, A. F.; Daha, M. R.; te, B. M.; van-der-Woude, F. J.; Gooszen, H. G. Antibody-dependent cell-mediated cytotoxicity against porcine endothelium induced by a majority of human sera. Transplantation 57: 1376–1382; 1994.
21. Schaapherder, A. F.; Wolvekamp, M. C.; te, B. M.; Bouwman, E.; Gooszen, H. G.; Daha, M. R. Porcine islet cells of Langerhans are destroyed by human complement and not by antibody-dependent cell-mediated mechanisms. Transplantation 62:29–33; 1996.
22. Sumitran, S.; Liu, J.; Czech, K. A.; Christensson, B.; Widner, H.; Holgersson, J. Human natural antibodies cytotoxic to pig embryonic brain cells recognize novel non-Galalpha1,3Gal-based xenoantigens. Exp. Neurol. 159:347–361; 1999.
23. van Roon, W. M. C.; Copray, J. C. V. M.; HogenEsch, R. I.; Staal, M. J.; Go, K. G. Fetal porcine mesencephalon grafts: Dissection procedure and cellular characterization in culture. Rest. Neurol. Neurosci. 7:199–205; 1995.
24. Widner, H.; Tetrud, J.; Rehncrona, S.; Snow, B.; Brundin, P.; Gustavii, B.; Björklund, A.; Lindvall, O.; Langston, J. W. Bilateral fetal mesencephalic grafting in two patients with parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). N. Engl. J. Med. 327:1556–1563; 1992.