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Combined Inhibition of Apoptosis and Complement Improves Neural Graft Survival of Embryonic Rat and Porcine Mesencephalon in the Rat Brain

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To define potential mechanisms of cell death during neural cell transplantation, we investigated the role of intracellular caspase activation in combination with the activation of serum complement. We demonstrated that ventral mesencephalic (VM) cells are susceptible to complement-mediated cell lysis that can be blocked with an anti-C5 complement inhibitor (18A10). We also determined that incubating freshly isolated allogenic VM cells with the caspase inhibitor 1-3-Boc-aspartyl(Ome)-fluoromethyl ketone (BAF), followed by immediate striatal implantation, led to a 2.5-fold increase in tyrosine hydroxylase (TH) cell survival 12 weeks postimplantation ($P < 0.05$). In contrast, overnight incubation with BAF followed by striatal implantation led to a 2-fold reduction in TH cell survival at 12 weeks ($P < 0.05$). Using the optimal BAF treatment and complement inhibition, we tested the hypothesis that these treatments would lead to increased cell survival in both allogeneic and xenogeneic transplantation models. We transplanted cell suspensions of (a) rat E14 VM or VM treated with (b) BAF alone, (c) anti-C5, or (d) a combination of BAF and anti-C5. There was a significant increase in the relative number of TH-positive cells in the BAF/anti-C5 group versus control at 12 weeks posttransplantation. Similar results were achieved in a pig to rat xenotransplant paradigm. A neuronal xenograft marker (70-kDa neurofilament) also demonstrated relative increases in graft volume in the BAF/anti-C5 treatment group. These studies indicate that more than one mechanism can mediate cell death during neural cell transplantation and that a combined treatment using caspase and complement inhibition can significantly improve cell survival.

Key Words: **caspase; complement; allotransplantation; xenotransplantation.**

INTRODUCTION

Many factors can contribute to posttransplant neuronal cell death including hypoxia-induced apoptosis or programmed cell death (PCD), trauma, insufficient growth factor support, and humoral and cellular immunity (see (5) for review). Serum complement is an integral component of the innate immune response that can be activated by the classical or alternative pathways. Complement activation leads to a cascade of enzymatic reactions leading to the formation of the membrane attack complex that can be induced by trauma, oxidative stress, ischemia–reperfusion injury, hemorrhagic shock, and antibody reactivity on transplanted tissue (2, 7, 10, 27). Membrane attack complex deposition on the surface of a target cell results in membrane damage and eventual cell lysis. Many studies have demonstrated the efficacy of complement inhibition at reducing tissue damage, cell death, and graft survival. A sialyl Lewis X conjugated recombinant soluble complement receptor 1 (sCR1sLex) was shown to reduce cerebral infarct areas in a rodent stroke model (13). Cardiac and intestinal reperfusion injury was also attenuated using an anti-C5 monoclonal antibody (23, 24). In addition, blocking complement during surgical procedures and transplantation results in reduced cellular activation, tissue damage, and increased graft survival (19, 21, 26). Complement activation in a xenogeneic Parkinson's disease (PD) rodent model was demonstrated by Barker *et al.* (3) and was associated with graft rejection.

Recent evidence in neural cell transplantation models also indicated that the activation of inracellular caspases could mediate cell death. Schierle *et al.* (20) demonstrated that treatment of freshly prepared embryonic ventral mesencephalic (VM) cell suspensions with the caspase-1 inhibitor, Ac-YVAD-CMK, reduced apoptosis in transplants and increased survival of DA neurons leading to greater functional recovery in hemiparkinsonian rats. A subsequent study treating VM cells with a broad-spectrum caspase inhibitor, 1-3-Boc-aspartyl(Ome)-fluoromethyl ketone (BAF), in combination with glial-derived neurotrophic factor, resulted in

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greater cell survival and DA fiber density following transplantation (12).

Based on these previous findings, we have undertaken a transplantation study in rodents aimed at increasing cell survival in intrastriatal VM grafts. We hypothesize that both the caspase and the complement cascades can independently mediate cell death. Therefore, blocking both pathways using a caspase inhibitor in combination with a complement inhibitor would enhance cell survival.

METHODS

Serum-Mediated Cell Lysis Assay

Isolated VM neurons from embryonic day 14 (E14) Sprague–Dawley rat embryos were washed with Hanks' balanced salt solution (HBSS), 1% bovine serum albumin, and exposed to calcein AM (Molecular Probes, Inc.) (10 μM) for 20 min. Cells were subsequently incubated at 37°C for 30 min with increasing concentrations of rat serum (from 0 to 50%). The cell lysis experiments were performed essentially as described previously (8, 19). The experiments ($n = 6$) were performed in rat serum alone or with the inclusion of an isotype control antibody (500 μg/ml) and 18A10 (500 μg/ml, an anti-C5 rat-specific complement inhibitor) (23).

Transplantation of VM Cells

Donor cells were derived from VM of Sprague–Dawley rats or transgenic pigs expressing human CD59. Rat E14 or E28 pig fetuses were dissected to isolate the VMs and the pieces were incubated in 0.1% trypsin for 20 min at 37°C. BAF (20 μM, diluted in 0.25% DMSO), a general caspase inhibitor (12) (Enzyme Systems Products, Livermore, CA), was added to the cell suspension. We chose to use this compound to inhibit at more than one point in the apoptotic cascade. The VM pieces were then washed with Ca/Mg-free HBSS containing 0.1% DNase (BAF present) and triturated in the same solution through a series of fire-polished pipettes of diminishing diameters until a milky suspension was achieved. The anti-C5 antibody, 18A10 (500 μg/ml), was added to the cell suspension after the trituration step. For fresh cell preparation, cell counts and viability were carried out immediately after dissociation using acridine orange/ethidium bromide (11). The cell counts and viability in the overnight treatment group were assessed immediately after dissociation and following 19 h of incubation prior to transplantation. The overnight treatment groups were incubated in HBSS/glucose $\pm$20 μM BAF 4°C. The suspension was diluted to a concentration of 100,000 cells/μl and 1 μl of the cell suspension was injected into two sites (total of 2 μl per animal) in the host striatum using a 10-μl Hamilton syringe and a 45° beveled needle. The striatal transplantations were performed according to the following coordinates in relation to Bregma: AP: 1.0 mm, L: +3.0 mm, V: −5.0 mm and −4.5 mm (ventral to dura), IB: 0. Animals in the allograft experiment were sacrificed at 12 weeks. For the xenotransplantation model, rats were treated with 30 mg/kg (2×) cyclosporin A (CsA, Sandimmune, Sandoz Pharmaceuticals Corp., NJ) 1 day prior to transplantation. Animals received 15 mg/kg CsA for 5 weeks after surgery until they were sacrificed.

Histology and Immunostaining

Briefly, animals were deeply anesthetized with sodium pentobarbital (60 mg/ml, ip (0.1 ml/100 g)) and perfused intracardially with heparinized saline followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate-buffered saline, pH 7.4. The brains were removed, postfixed in 4% PFA for 6 h, and equilibrated in 20% sucrose in 0.1 M PBS for cryoprotection, and 40-μm sections were cut on a freezing microtome. Brain sections were immunostained with antibodies against tyrosine hydroxylase (TH) (Pel-Freez, Rogers, AK) for dopaminergic cell counts and the pig-specific 70-kDa neurofilament (NF70; Biodesign, Kennebunkport, ME) for pig axons and measurements of graft volumes. The sections were preincubated for 30 min in a solution containing 5% normal goat serum and 0.1% Triton X-100. Sections were incubated overnight at 4°C in a solution containing 5% normal goat serum, 0.1% Triton X-100, and anti-TH (1:500) or anti-NF70 (1:40). As a control for specificity of antibodies, some sections were treated as above except that the primary antibody was omitted from the incubation medium.

After three rinses of 10 min in PBS, the sections were incubated for 1 h at room temperature in secondary antibodies (biotinylated goat IgG) (goat anti-rabbit for TH or goat anti-mouse for anti-NF70) (Vector Laboratories) (dilution 1:200). After three more rinses in PBS, the sections were reincubated for 1 h at room temperature in avidin–biotin complex (Vectastain, ABC kit Elite, Vector Laboratories). All immunohistochemistry was performed on free-floating sections as previously described (8, 11, 15, 16).

TH Cell Count, Volumetric Assessment, and Statistical Analysis

Brains were sliced into a series of six 40-μm-thick sections. The second and fourth series of brain sections were processed for TH immunohistochemistry and every TH cell encountered on every section was counted. Histological analysis was performed using a Zeiss Axioskop microscope with a Real Time Spot camera (Diagnostic Instruments, Inc., MI). All cells present were counted under brightfield illumination at 20× magni-

fication. The total number of TH cells was determined for each animal by applying the Abercrombie (1) correction method. The average TH cell number per group was then calculated and plotted. All cell counts were performed blind by two independent investigators. Quantification of NF70 fiber innervation was derived from NF70-immunopositive striatal graft areas traced using NIH Image 1.41 (NIH).

We used a single factor analysis of variance method to compare TH-positive cell survival and volume measurements between groups. Differences between groups were considered statistically significant at an α level of 0.05. Unpaired *t* tests were individually performed to compare groups (e.g., control vs BAF/C5) and Bonferonni correction was applied ($\propto$0.008). Statistical analysis for the cell lysis assay was performed using a one-tailed paired *t* test at an α level of 0.05.

The data management and statistical analysis work was carried out using the software package JMP (version 3.1.6; SAS Institute, Cary, NC).

The fresh cell suspension versus overnight data (Fig. 1) was derived from the analysis of 4 to 6 animals per group. The effects of BAF/C5 treatment on TH cell survival in allografting (Fig. 3) were derived from the analysis of 6 or 7 animals per group. The effects of BAF/C5 treatment on TH cell survival in xenografting (Figs. 5 and 6) included 8 to 10 animals per group.

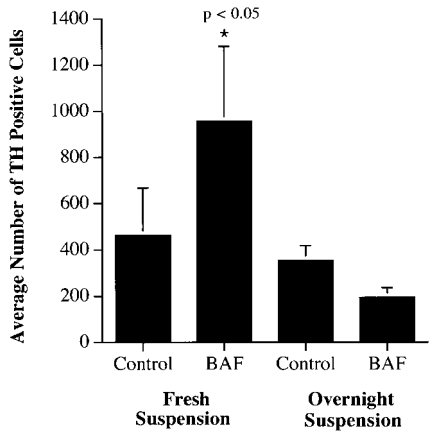


FIG. 1. Graph comparing the average number of TH-positive cells in fresh versus overnight suspension ± BAF treatment 12 weeks posttransplantation into naive rats (mean ± SEM). The comparison between BAF in fresh cell suspension and BAF in overnight cell suspension was statistically significant ($P < 0.05$). Note that the data for fresh suspension groups are derived from the data illustrated in Fig. 3.

RESULTS

In Vivo Assessment of Caspase Inhibition: Fresh vs Overnight Treatment Conditions

A comparative analysis of two different BAF treatments was performed in a rat allograft model. Cell preparations were treated with BAF and immediately transplanted or incubated with the caspase inhibitor at 4°C overnight prior to transplantation. Cell viability and concentration were evaluated prior to cell implant. Overnight incubation in the absence of BAF treatment resulted in a lower viable cell concentration due to cell death, whereas inhibiting caspase activity resulted in no apparent loss of viable cell concentration. We observed a 24% difference in viable cell concentration following overnight incubation between the BAF and the control treatments. Rats were transplanted with nontreated (control) or BAF-treated VM cell suspensions from all four conditions and cell implants were evaluated at 12 weeks. Overall, there was no significant difference between overnight incubation versus fresh in the control groups (Fig. 1). Rat recipients receiving VM cell suspensions that were treated with BAF just prior to transplantation resulted in a greater number of TH-positive neurons within the graft and corresponded to a two-fold greater survival compared to control (Fig. 1). In contrast, a two-fold reduction in surviving TH neurons was observed in the BAF overnight treatment group (Fig. 1) compared to the control overnight treatment group. In addition, there was a significant difference in TH cell survival between the fresh BAF treatment group and the overnight BAF treatment group ($P < 0.05$) (Fig. 1).

In Vitro Complement-Mediated VM Cytolysis

Rat VM neurons were assayed in a complement-mediated cell lysis experiment to determine the susceptibility of these cells to serum complement activation and to test the complement inhibitory activity of the anti-rat C5 monoclonal antibody, 18A10, in this model. The fetal VM rat neurons demonstrated susceptibility to rat serum complement although the overall level of cytolysis was low (22%) compared to cross-species serum-mediated cell lysis experiments (i.e., 76% pig cell lysis in 50% rat serum, data not shown) (3, 18). The 18A10 anti-C5 antibody effectively blocked serum-mediated lysis at 500 μg/ml ($P < 0.05$) (Fig. 2). An isotype control antibody had no effect on complement inhibition and resulted in comparable levels of cell lysis as the no treatment control.

Effects of Caspase and Complement Inhibition on Allograft Survival

Long-term (12 weeks) TH cell survival was analyzed *in vivo* in a rat allograft model using VM cell suspensions treated with BAF and anti-C5. The anti-C5 treatment had no effect on enhancing TH cell survival and contained a similar number of TH neurons compared to the control group (Fig. 3). There was a 2.5-fold increase

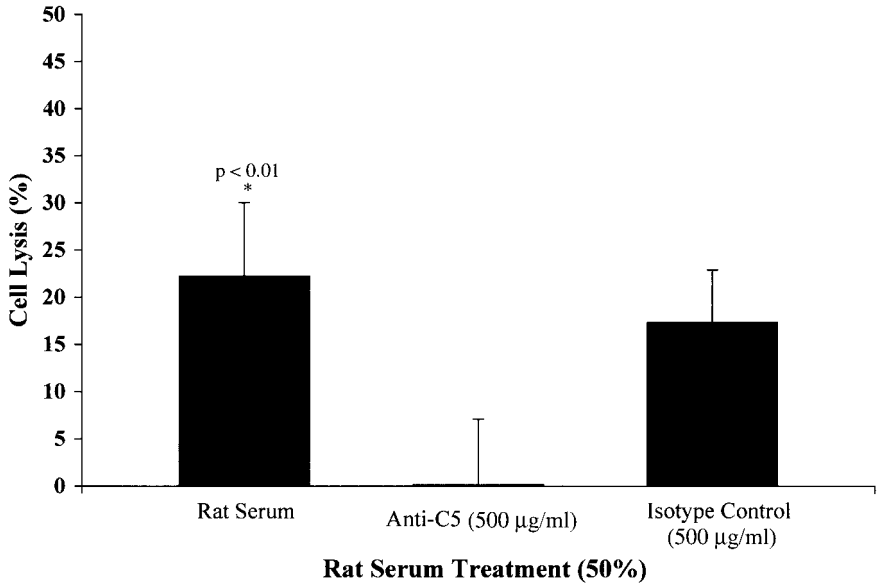


FIG. 2. Fetal rat VM cell lysis experiment. Fetal VM cells were incubated with rat serum (50%) (mean ± SEM). The experiments were performed in rat serum alone or with the inclusion of an isotype control antibody (500 μg/ml) and 18A10 (500 μg/ml, an anti-rat C5 complement inhibitor). Note the complete blockade of rat VM cell lysis with the anti-C5 antibody (500 μm/ml).

in surviving TH neurons in the BAF group versus control that did not reach statistical significance (see Figs. 1 and 3). The average number of surviving TH neurons was enhanced by the addition of the anti-C5 monoclonal antibody to the BAF treatment. The BAF/anti-C5 treatment resulted in a 3-fold increase in TH cells ($P < 0.05$, Fig. 3). The only treatment that reached statistical significance was the BAF/C5 versus control ($P < 0.05$, Fig. 3).

Tissue sections from each of the recipients were analyzed by TH immunohistochemistry which clearly depicted increased TH cell numbers in the combination group (BAF/anti-C5) as well as increased overall graft volume within the striatum (Fig. 4).

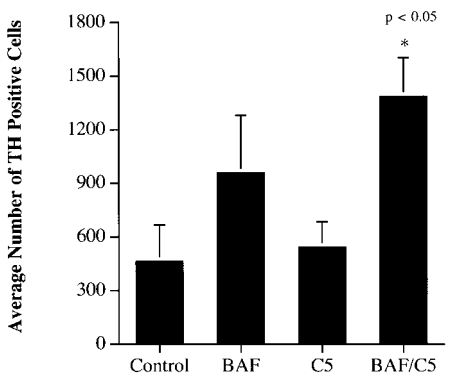


FIG. 3. A comparison of the average number of TH-positive cells after BAF, anti-C5, or BAF/anti-C5 treatment on rat VM cells 12 weeks posttransplantation into naive rats (mean ± SEM). The combination treatment BAF/anti-C5 had a statistically significant effect on the number of TH-positive cells found within the transplant ($P < 0.05$).

Effects of Caspase and Complement Inhibition on Xenograft Survival

The BAF/anti-C5 combination treatment was also tested in a xenotransplantation paradigm. Transplanted pig VM neurons were analyzed at 5 weeks postsurgery. Comparable TH cell numbers were observed in the control, BAF, and anti-C5 treatment groups. There was no statistically significant effect on TH cell survival in either of the single treatment groups compared to control. However, there was a significant difference between the total number of TH positive cells within the grafts in the BAF/anti-C5 treatment versus control ($P < 0.05$, Fig. 5). In addition, the BAF/anti-C5 treatment resulted in significantly greater TH cell survival than either the BAF or the anti-C5 treatments alone ($P < 0.05$, Fig. 5). In the BAF, anti-C5, and control groups, TH immunoreactivity was confined to a small region of the striatum. TH neuronal clusters were in early stages of development (5 weeks) as observed by the lack of fiber outgrowth to the surrounding host tissue. This is consistent with previous work describing the maturation of pig grafts in rodents (11). The largest grafts, observed in the BAF/anti-C5 treatment group, were characterized by a considerable amount of non-TH-positive tissue with TH cells located primarily at the periphery of the graft (Fig. 6). Volumetric analysis of graft size was determined using NF70 staining, which clearly delineated

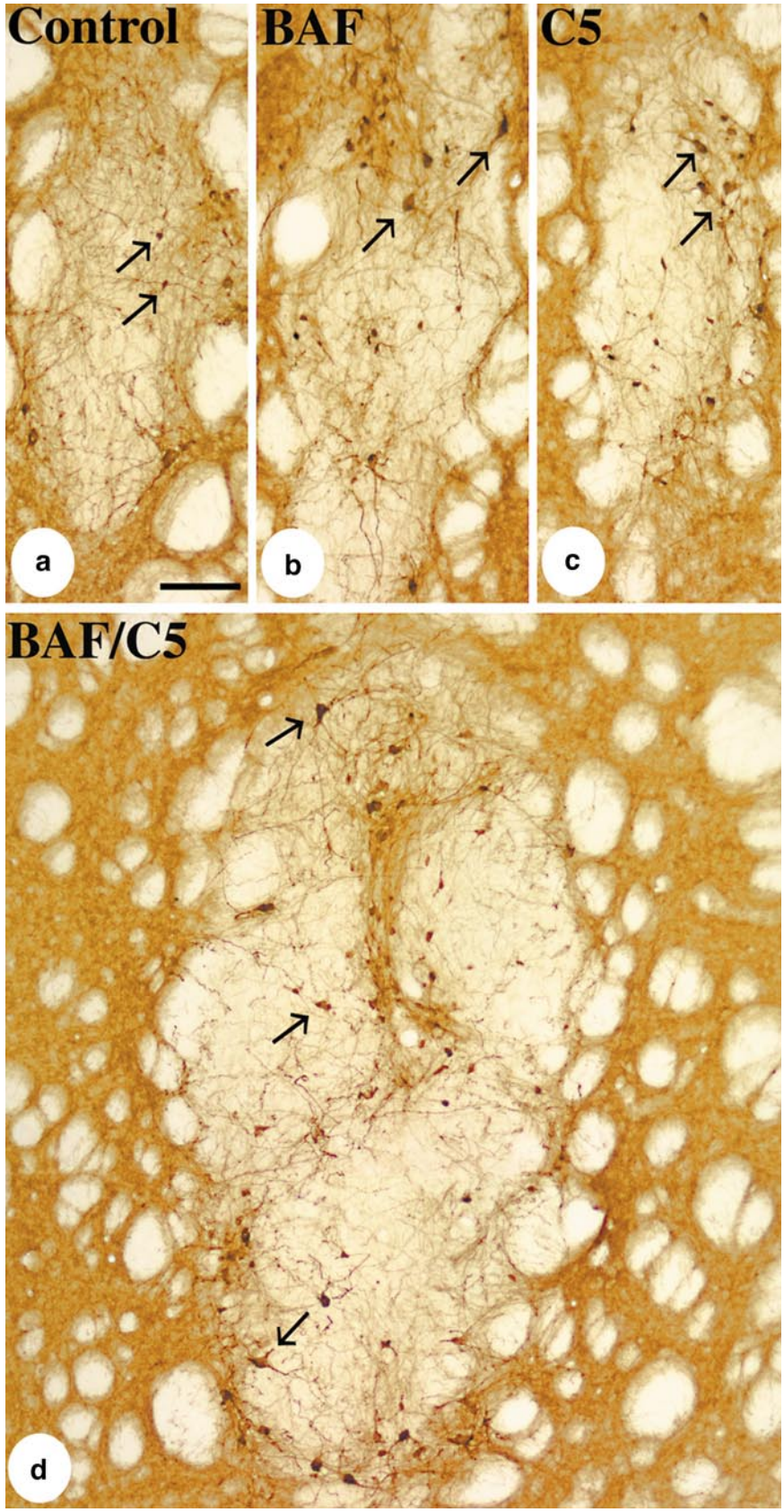


FIG. 4. Immunohistochemical analysis of intrastriatal E14 rat VM grafts in naive animals using anti-TH antibody in (a) control, (b) BAF, (c) anti-C5, and (d) BAF/anti-C5 cohorts. The BAF/anti-C5 VM transplant clearly depicts a greater number of TH cells as well as a greater volume within the striatum. Black arrows point to examples of TH-immunopositive cells. All photomicrographs were taken at the same magnification; scale bar in a (b, c, d) = 200 μm.

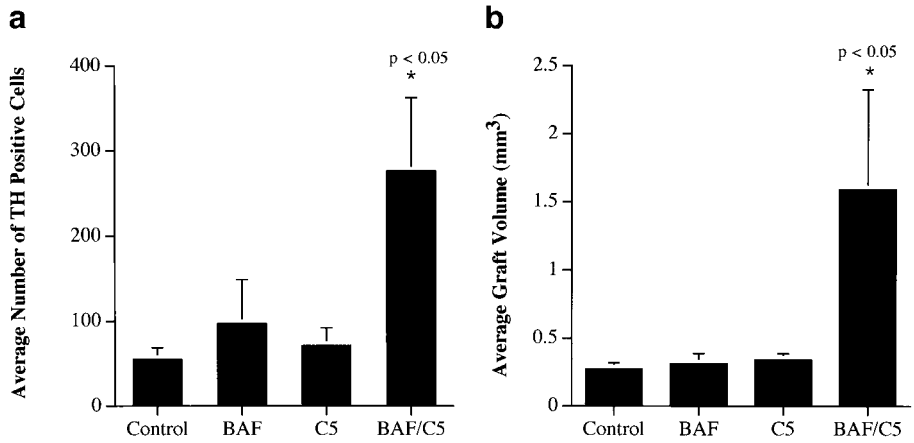


FIG. 5. A comparison of the average number of TH-positive pig cells and graft volume (NF70 staining) after BAF, anti-C5, or BAF/anti-C5 treatment on pig VM cells 5 weeks posttransplantation into naive rats (mean ± SEM). The combination treatment BAF/anti-C5 had a statistically significant effect on the number of surviving TH-positive cells found within the transplant ($P < 0.05$) (a) and the graft volume as assessed by NF70 immunostaining ($P < 0.05$) (b).

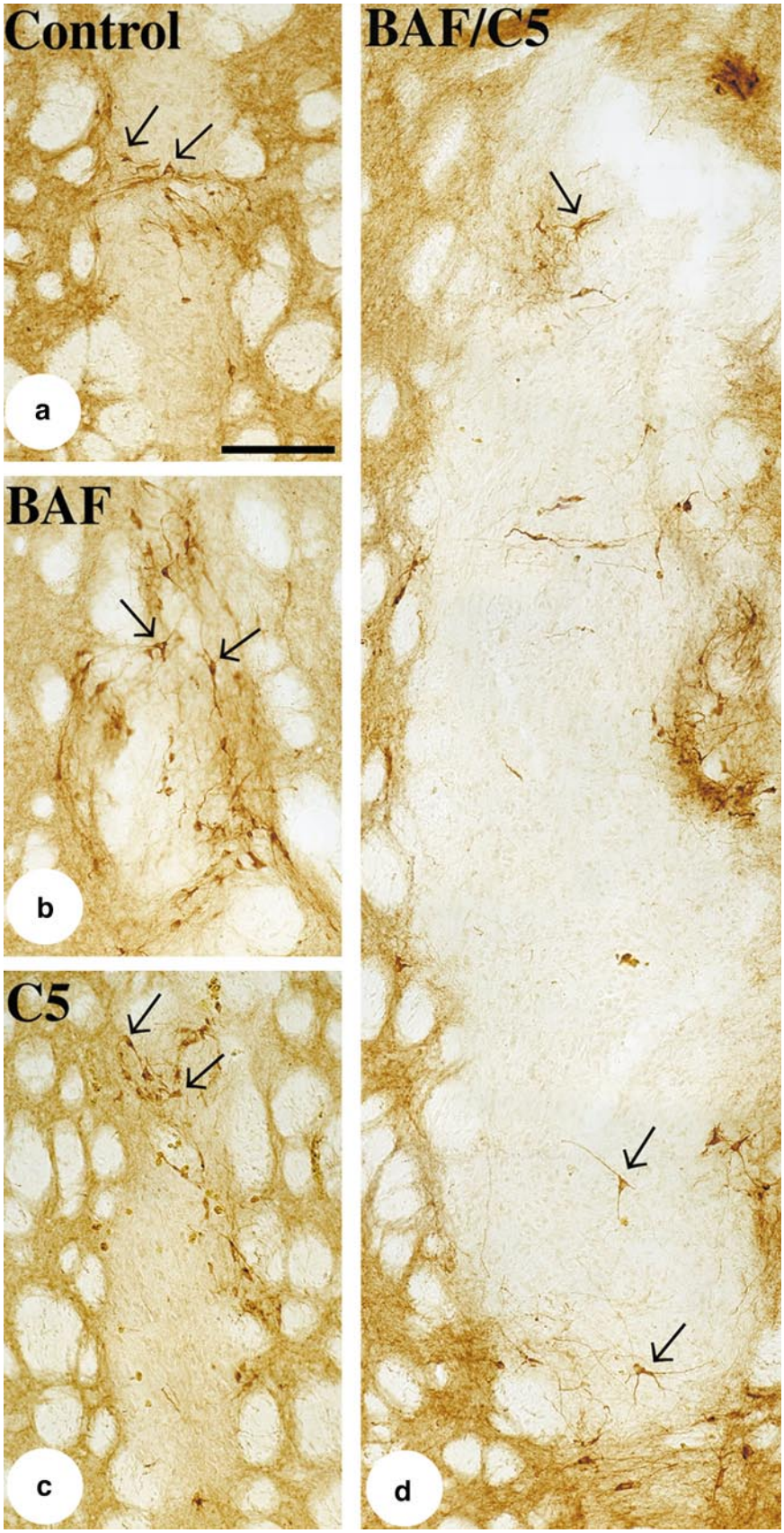
the graft area. The intense NF70 reactivity corresponded to a dense network of pig VM axons (Fig. 7). The pig-specific neurofilament immunohistochemistry, as shown in Fig. 6, clearly illustrates the significant difference in graft size between the BAF/anti-C5 cohort and the other groups ($P < 0.05$). This demonstrated a direct correlation between the graft volume and the TH cell number. A greater than five-fold increase in graft size correlated with a five-fold increase in TH cell number (Fig. 5).

DISCUSSION

We hypothesized that blocking caspase and complement activation would lead to greater TH cell survival in two different transplantation models. The initial experiments were aimed at determining the optimal use of the caspase inhibitor BAF. We showed that incubating fetal VM neurons with BAF during the cell dissociation process and just prior to transplantation increased cell survival in a rodent allograft model. However, overnight incubation of the VM neurons with the caspase inhibitor, BAF, resulted in fewer surviving neurons *in vivo* (Fig. 1). *In vitro*, we demonstrated that cytolysis of fetal VM can be blocked by an anti-C5 antibody, 18A10 (Fig. 2). The initial caspase inhibitor experiments in combination with the *in vitro* complement-mediated cell lysis study confirmed that two mechanisms can lead to neuronal cell death. In the allograft VM transplant model using caspase and complement inhibitors, we observed a slight increase in cell survival with BAF alone that was not statistically significant. This observation is consistent with recent evidence that caspase-1 and -3 inhibitors do not promote DA neuronal survival *in vitro* as well as *in vivo* (14). Treatment with anti-C5 in the cell suspension had no effect on enhancing cell survival. Interestingly, the BAF/anti-C5 combination treatment showed a 3-fold greater number of surviving TH positive cells than the control groups (Figs. 3 and 4). Furthermore, we observed a 1.5- to 2.5-fold increase in TH survival compared to the single treatment groups (Figs. 3 and 4). In the xenograft model, we observed similar results that were further supported by graft volume measurements (Figs. 5–7). Overall, there was a wide variance in all groups and that was not specific to any one cohort. These results indicate that activation of either pathway acutely following transplantation leads to cell death and results in significantly smaller grafts.

In our study, cell concentrations and viability counts were very high following overnight BAF incubation; however, cell survival in the transplant recipients was lower than in the control group. This raises the possibility that overnight incubation with BAF led to an overestimation of viable cells compared to actual posttransplantation *in vivo* survival. This could be due to the fact that the implanted cells are damaged but have been kept alive by blocking apoptosis and that prolonged caspase inhibition in an *in vitro* hibernation environment does not translate into increased TH cell survival *in vivo*. This raises the question of whether it is always beneficial to block PCD in unhealthy or damaged cells (5).

Complement activation can induce cell activation and cell death in allotransplantation as well as in reperfusion injury models (21, 23, 24). In addition, complement activation can occur in the brain following stroke and contusion injury (17, 22). The activation of complement during VM transplantation has been demonstrated in the pig to rat xenogeneic model (3). We demonstrated that rat serum complement can mediate fetal rat VM cell lysis *in vitro* and that acute complement inhibition can protect these cells. The level of activation on the autologous cells is relatively low com-


Control
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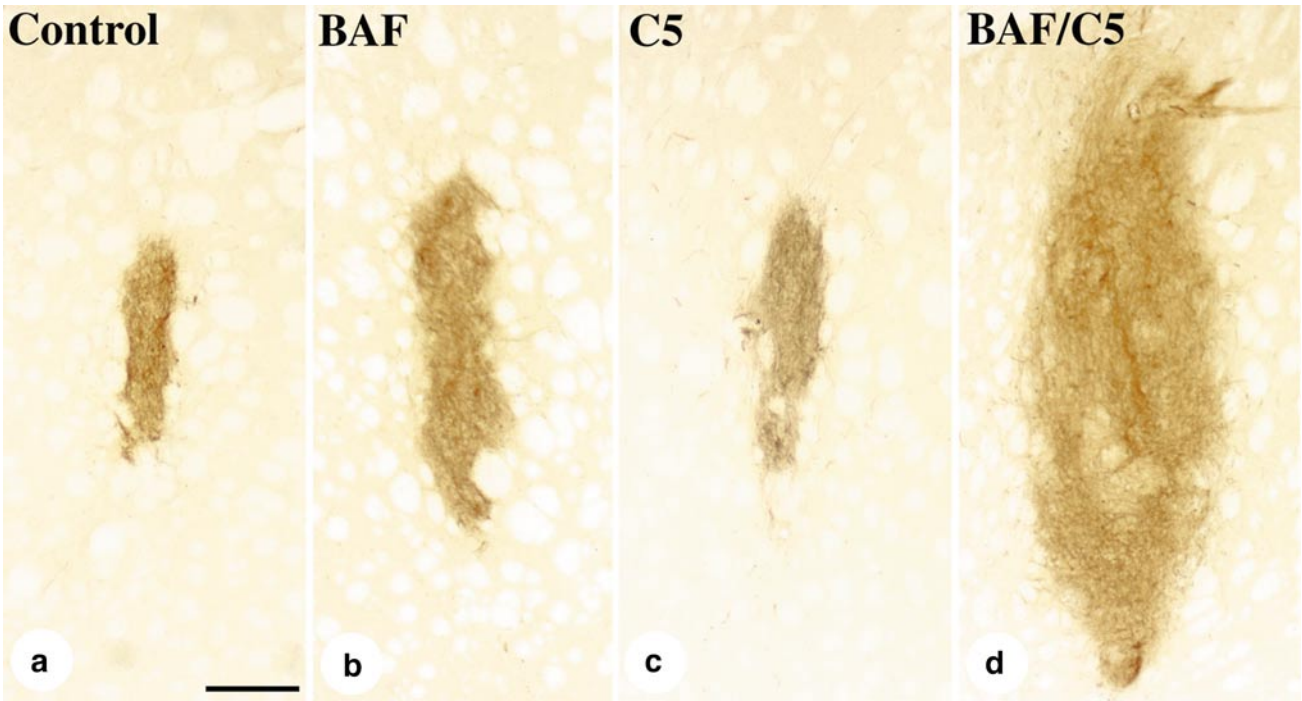

FIG. 7. High-power photomicrographs depicting graft volume of one (a) control, (b) BAF, (c) anti-C5, and (d) BAF/anti-C5 animal using pig-specific NF70 immunohistochemistry. All photomicrographs were taken at the same magnification; scale bar in a (b, c, d) = 100 μm.

pared to discordant xenogeneic cells (18) and could account for the absence of a noticeable effect *in vivo* (Fig. 3). The anti-C5 treatment also had no effect on enhancing cell survival in the xenograft model (Figs. 5 and 6). This could be due to the lack of prolonged inhibition (acute treatment in the cell preparation only). Barker *et al.* (3) observed peak complement deposition at 10 days following transplantation in a pig to rat xenogeneic model. However, it is likely that the lack of any effect with anti-C5 alone in either transplant paradigm is due to apoptotic events, and in the absence of caspase inhibition the effect of complement inhibition is masked. This observation is in agreement with the synergy observed with the combined treatment in the xenograft paradigm.

As our data illustrate, combined caspase and complement inhibition plays a critical role in TH cell survival and could be beneficial for clinical PD cell therapy applications. The utility of caspase and complement inhibition could be extended into other cell-based therapies. For example, it is well established that islet isolation induces β cell apoptosis prior to transplantation (6). In addition, isolated human islets trigger an instant blood-mediated inflammatory reaction characterized by platelet activation, coagulation, and complement activation. The use of a complement inhibitor and an anti-coagulant was shown to reduce the inflammatory response and islet damage (4). Individual clinical applications of anti-complement modalities as well as caspase inhibitor therapies are currently being pursued for various disease indications (9, 12, 13, 19–21, 24, 26). It is therefore feasible to combine these treatments and our data demonstrate that simultaneously inhibiting both pathways enhances cell engraftment.

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FIG. 6. Intrastriatal E28 pig VM transplant into naive animals immunostained for TH in (a) control (b) BAF, (c) anti-C5, and (d) BAF/anti-C5 cohorts. All photomicrographs were taken at the same magnification. The BAF/anti-C5 VM transplant comprised a significantly greater number of TH cells and clearly occupied a greater volume within the striatum. Note that a great proportion of the cell implant in the BAF/anti-C5 group is non-TH positive and that most TH-positive cells lie in the periphery of the transplant. Black arrows point to examples of TH-immunopositive cells. Scale bar in a (b, c, d) = 200 μm.

REFERENCES

1. Abercrombie, M. 1946. Estimation of nuclear population from microtome sections. *Anat. Rec.* **94:** 239–247.
2. Baldwin, W. M., 3rd., M. Samaniego-Picota, E. K. Kasper, A. M. Clark, M. Czader, C. Rohde, A. A. Zachary, F. Sanfilippo, and R. H. Hruban. 1999. Complement deposition in early cardiac transplant biopsies is associated with ischemic injury and subsequent rejection episodes. *Transplantation* **68:** 894–900.
3. Barker, R. A., E. Ratcliffe, M. Mclaughlin, A. Richards, and S. B. Dunnett 2000. 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.
4. Bennet, W., C. G. Groth, R. Larsson, B. Nilsson, and O. Korsgren. 2000. Isolated human islets trigger an instant blood mediated inflammatory reaction: Implications for intraportal islet transplantation as a treatment for patients with type 1 diabetes. *Ups. J. Med. Sci.* **105:** 125–133.
5. Boonman, Z., and O. Isacson. 1999. Apoptosis in neural development and transplantation: Role of caspases and trophic factors. *Exp. Neurol.* **156:** 1–15.
6. Cattan, P., T. Berney, S. Schena, R. D. Molano, A. Pileggi, C. Vizzardelli, C. Ricordi, and L. Inverardi. 2001. Early assessment of apoptosis in isolated islets of Langerhans. *Tranplantation* **71:** 857–862.
7. Collard, C. D., R. Lekowski, J. E. Jordan, A. Agah, and Stahl, G. L. 1999. Complement activation following oxidative stress. *Mol. Immunol.* **36:** 941–948.
8. Deacon, T. W., J. Schumacher, J. Dinsmore, C. Thomas, P. Palmer, S. Kott, A. Edge, D. Penney, S. Kassissieh, P. Dempsey, and O. Isacson. 1997. Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. *Nat. Med.* **3:** 350–353.
9. Fitch, J. C., S. Rollins, L. Matis, B. Alford, S. Aranki, C. D. Collard, M. Dewar, J. Elefteriades, R. Hines, G. Kopf, P. Kraker, L. Li, R. O'Hara, C. Rinder, H. Rinder, R. Shaw, B. Smith, G. Stahl, and S. K. Shernan. 1999. Pharmacology and biological efficacy of a recombinant, humanized, single-chain antibody C5 complement inhibitor in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass. *Circulation* **100:** 2499–2506.
10. Fosse, E., J. Pillgram-Larsen, J. L. Svennevig, C. Nordby, A. Skulberg, T. E. Mollnes, and M. Abdelnoor. 1998. Complement activation in injured patients occurs immediately and is dependent on the severity of the trauma. *Injury* **29:** 509–514.
11. Galpern, W. G., L. H. Burns, T. W. Deacon, J. Dinsmore, and O. Isacson. 1996. Xenotransplantation of porcine fetal ventral mesencephalon in a rat model of Parkinson's disease: Functional recovery and graft morphology. *Exp. Neurol.* **140:** 1–13.
12. Helt, C. E., G. R. Hoernig, D. S. Albeck, G. A. Gerhardt, B. Ickes, M. E. Reyland, D. O. Quissell, I. Stromberg, and A. C. Granholm. 2001. Neuroprotection of grafted neurons with a gdnf/caspase inhibitor cocktail. *Exp. Neurol.* **170:** 258–269.
13. Huang, J., L. J. Kim, R. Mealey, H. C. Marsh, Jr., Y. Zhang, A. J. Tenner, E. S. Connolly, Jr., and D. J. Pinsky. 1999. Neuronal protection in stroke by an sLex-glycosylated complement inhibitory protein. *Science* **285:** 595–599.
14. Hurelbrink, C. B., R. J. E. Armstrong, L. M. Luheshi, S. B. Dunnett, A. E. Rosser, and R. A. Barker. 2001. Death of dopaminergic neurons in vitro and in nigral grafts: Reevaluating the role of caspase activation. *Exp. Neurol.* **171:** 46–58.
15. Isacson, O., and T. W. Deacon. 1996. Specific axon guidance factors persist in the adult brain as demonstrated by pig neuroblasts transplanted to the rat. *Neuroscience* **75:** 827–837.
16. Isacson, O., T. W. Deacon, P. Pakzaban, W. R. Galpern, J. Dinsmore, and L. H. Burns. 1995. Transplanted xenogeneic neural cells in neurodegenerative disease models exhibit remarkable axonal target specificity and distinct growth patterns of glial and axonal fibres. *Nat. Med.* **1:** 1189–1194.
17. Keeling, K. L., R. R. Hicks, J. Mahesh, B. B. Billings, and G. J. Kotwal. 2000. Local neurotrophil influx following lateral fluid-percussion brain injury in rats is associated with accumulation of complement activation fragments of the third component (C3) of the complement system. *J. Neuroimmunol.* **105:** 20–30.
18. Kennedy, S. P., S. A. Rollins, W. V. Burton, P. J. Sims, A. L. M. Bothwell, S. P. Squinto, and G. B. Zavoico. 1994. Protection of porcine aortic endothelial cells from complement-mediated cell lysis and activation by recombinant human CD59. *Transplantation* **57:** 1494–1501.
19. Rinder, C. S., H. M. Rinder, M. J. Smith, J. B. Tracey, J. Fitch, L. Li, S. A .Rollins, and B. R. Smith. 1999. Selective blockade of membrane attack complex formation during simulated extracorporeal circulation inhibits platelet but not leukocyte activation. *J. Thorac. Cardiovasc. Surg.* **118:** 460–466.
20. Schierle, G. S., O. Hansson, M. Leist, P. Nicotera, H. Widner, and P. Brundin. 1999. Caspase inhibition reduces apoptosis and increases survival of nigral transplants. *Nat. Med.* **5:** 97–100.
21. Schmid, R. A., A. Zollinger, T. Singer, S. Hillinger, J. R. Leon-Wyss, O. M. Schob, K. Hogasen, G. Zund, G. A. Patterson, and W. Weder. 1998. Effect of soluble complement receptor type 1 on reperfusion edema and neutrophil migration after lung allotransplantation in swine. *J. Thorac. Cardiovasc. Surg.* **116:** 90–97.
22. Stahel, P. F., M. C. Morganti-Kossmann, and T. Kossmann. 1998. The role of the complement system in traumatic brain injury. *Brain Res. Rev.* **27:** 243–256.
23. Vakeva, A. P., A. Agah, S. A. Rollins, L. A. Matis, L. Li, and G. L. Stahl. 1998. Myocardial infarction and apoptosis after myocardial ischemia and reperfusion: Role of the terminal complement components and inhibition by anti-C5 therapy. *Circulation* **97:** 2259–2267.
24. Wada, K., M. C. Montalto, and G. L. Stahl. 2001. Inhibition of complement C5 reduces local and remote organ injury after intestinal ischemia/reperfusion in the rat. *Gastroenterology* **120:** 126–133.
25. Wang, H., S. A. Rollins, Z. Gao, B. Garcia, Z. Zhang, J. Xing, L. Li, R. Kellersmann, L. A. Matis, and R. Zhong. 1999. Complement inhibition with an anti-C5 monoclonal antibody prevents hyperacute rejection in a xenograft heart transplantation model. *Transplantation* **68:** 1643–1651.
26. Wang, X., J. O. Karlsson, C. Zhu, B. A. Bahr, H. Hagberg, and K. Blomgren. 2001. Caspase-3 activation after neonatal rat cerebral hypoxia-ischemia. *Biol. Neonate* **79:** 172–179.
27. Younger, J. G., N. Sasaki, M. D. Waite, H. N. Murray, E. F. Saleh, Z. A. Ravage, R. B. Hirschl, P. A. Ward, and G. O. Till. 2001. Detrimental effects of complement activation in hemorrhagic shock. *J. Appl. Physiol.* **90:** 441–446.