Immunosuppression for neural xenografts: a comparison of cyclosporin and anti-CD25 monoclonal antibody

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Object. The goal of this study was to compare the effects of short- and long-term immunosuppression induced by cyclosporin with those of immunosuppression induced by a monoclonal antibody against the rat interleukin-2 receptor (anti-CD25 mAb) in rats with xenografts.

Methods. The authors compared the in vivo function and final histological characteristics of fetal mouse mesencephalon xenografts in hemiparkinsonian rats in which immunosuppression was induced by: 1) a short course (2 weeks) of cyclosporin; 2) a long course (8 weeks) of cyclosporin; or 3) a short course of treatment with anti-CD25 mAb. Adult Wistar rats were unilaterally lesioned with 6-hydroxydopamine in their medial forebrain bundle, after which their rotational behavior in response to methamphetamine was quantified. Four groups of 20 rats with rotations numbering greater than six per minute received fetal mouse mesencephalon transplants to their dopamine-denervated striatum. Group 1 received no immunosuppression therapy; Group 2 received daily intraperitoneal injections of 10 mg/kg cyclosporin for 2 weeks; Group 3 received daily intraperitoneal injections of 10 mg/kg cyclosporin for 8 weeks; and Group 4 received daily intraperitoneal injections of 1 mg/kg anti-CD25 mAb for 2 weeks. The rats were tested for rotational behavior every 4 weeks and killed after 16 weeks. Surviving xenografts were assessed using immunohistochemical staining for a mouse neuronal marker (Thy-1.2). Sixteen weeks after transplant, there were significantly more surviving xenografts in Groups 3 (p < 0.001) and 4 (p < 0.001) compared with control Group 1 (Fisher’s exact test) and significantly better functioning xenografts in Groups 3 (p < 0.01) and 4 (p < 0.05) compared with control Group 1 (contrasts of groups following analysis of variance with Bonferroni correction).

Conclusions. A short course of anti-CD25 mAb-induced immunosuppression was as effective as a long course of cyclosporin-induced immunosuppression in this model.

Key Words • xenograft • immunosuppression • CD25 • dopamine • neural transplantation

There is a growing interest in the ability of neural grafts to replace the function of degenerated brain cells. Clinical trials in which fetal human and porcine mesencephalon were used as a source of dopaminergic neurons have been conducted in patients with Parkinson’s disease. These patients face the problem of graft rejection. The immunology of neural transplantation is an emerging field. The brain is an “immunologically privileged” site and foreign antigens placed into the brain are not rejected as fast as those placed in peripheral areas. In animal models, however, it has been repeatedly shown that xenografts (tissue from different species) are strongly rejected within the host brain. The most widely used treatment to prevent neural graft rejection in animal models and clinical trials is a regimen of cyclosporin. Unfortunately this drug has well-known nephrotoxic and neurotoxic side effects. In an attempt to find alternative immunosuppressive techniques, investigators have tested other compounds. In this paper we compare the efficacy of short- and long-term immunosuppression therapy using cyclosporin with that of short-term (2-week) treatment using a monoclonal antibody (mAb) against the interleukin (IL)-2 receptor, anti-CD25 mAb, to prolong the duration of neural xenograft survival and function in a rodent model.

Animal Preparation

The dopamine-denervated striatum in adult male Wistar rats (200–250 g each) was unilaterally lesioned by administering injections of 6-hydroxydopamine HCl (8 μg in 4 μl of saline with 0.02% ascorbate over an 8-minute period) into the right median forebrain bundle (~4.4 mm anterior to the bregma, 1 mm right of the midline, and ~7.8 from the dura with the incisor placed 2.3 mm below the interaural line). Lesioned animals exhibit a quantifiable, rotational behavior that correlates to the loss of dopamine neurons and can be exaggerated by administration of amphetamine. Three weeks later, circling behavior in response to d-methamphetamine HCl (2.5 mg/kg administered intraperitoneally) was quantified using automated rotometers. The number of rotations (360°) each rat made during 1 hour was counted and the net ipsiversive rotations were calculated. Only animals rotating more than six turns per minute were used in the study. The animals were divided into four groups of 20 with similar rotational scores. Group 1 received no immunosuppression therapy; Group 2 received daily intraperitoneal injections of 10 mg/kg cyclosporin A for 2 weeks; Group 3 received daily intraperitoneal injections of 10 mg/kg cyclosporin A for 8 weeks; and Group 4 received daily intraperitoneal injections of 1 mg/kg anti-CD25 mAb for 2 weeks. The anti-CD25 mAb was purified from ascites produced when the NDS 63 myeloma cell line was grown as intraperitoneal tumors in (BALB/c × DBA/2) hybrid mice.
Preparation of Xenografts

The xenografts were prepared by dissecting out the ventral mesencephalon from fetal CD-1 mice (crown–rump length 10–12 mm) and making a cell suspension. Fragments of mesencephalon were incubated in 0.1% trypsin for 20 minutes at 37°C and then washed four times in medium (RPMI, 0.004% DNAse, 0.0125% soybean antitrypsin, and 15 mM MgSO4). Fragments were resuspended in 100 μl medium and triturated through a Pasteur pipette to make a cell suspension. Each animal received two 3-μl injections of xenograft cell suspension into the dopamine-denervated striatum (coordinates with the incisor bar set at −2.3 mm were: 1 mm anterior to the bregma, 2.8 mm lateral to the midline, and 3 and 4.1 mm deep with respect to the dura). Following transplantation, the cell viability of the suspension was estimated by using the trypan blue dye exclusion technique and found to be greater than 10⁷/ml (clumps of cells made exact calculation difficult).

Statistical Analysis

Rotational behavior was quantified every 4 weeks for 16 weeks. Net ipsiversive rotations for each animal were counted and recorded as a percentage of their pretransplant values (Fig. 1). The mean scores for each group at each time point were compared using analysis of variance (ANOVA). Groups 2, 3, and 4 were compared with the control animals (Group 1) at 16 weeks by contrast within the ANOVA. A Bonferroni correction was introduced to correct for multiple comparisons.

At 16 weeks, all animals were deeply anesthetized, killed, and perfused with intracardiac heparinized saline; and their brains were removed and quick-frozen in optimum-cutting-temperature embedment compound. Twelve-micron cryostat sections were prepared for histological analysis. The secondary antibody, AAC07P, was conjugated to horseradish peroxidase, revealed by using diaminobenzidine, and counterstained with cresyl violet. The histological characteristics were compared in 2 tables by using Fisher’s exact test with a Bonferroni correction introduced for multiple comparisons. Statistics were calculated using commercially available software.

Sources of Supplies and Equipment

The xenografts were obtained from CD-1 mice, available from Charles River Laboratories (Montreal, Quebec, Canada). The cyclosporin A (Sandimmune) was provided by Sandoz (now Novartis Canada Inc., Mississauga, Ontario, Canada). The automated rotometers used to quantify the rats’ rotational behaviors were obtained from Columbus Instruments (Columbus, OH). The optimum-cutting-temperature compound was provided by Sakura Finetek USA Inc. (Torrance, CA). Both the primary and secondary antibodies, MCA02 and AAC07P, were obtained from Serotec Inc. (Raleigh, NC). Systat software (version 8.0), available from SPSS Inc. (Chicago, IL), was used to perform the statistical analysis.

Results

The rotational scores are shown in Fig. 1. A significant difference between groups (p < 0.001) was found using ANOVA. Individual comparisons at 16 weeks after transplant showed no significant reduction in rotational behavior for Group 2 (cyclosporin A for 2 weeks) compared with Group 1 (control animals with no immunosuppression). Group 3 (cyclosporin A for 8 weeks) had significantly reduced rotational behavior compared with Group 1 (p < 0.01). Group 4 (anti-CD25 mAb for 2 weeks) had significantly reduced rotational behavior compared with Group 1 (p < 0.05).

Histological examples of the graft site 16 weeks after transplant are shown in Fig. 2. These examples show “positive” or “negative” staining for the xenograft neuronal marker, Thy-1.2. We did not attempt to quantify cellular immune rejection. None of the animals in Group 1 (controls) had surviving xenografts. Surviving xenografts were found in 5% of the animals in Group 2, 56% of the animals in Group 3, and 53% of the animals in Group 4. Most surviving grafts, however, showed evidence of ongoing rejection, with abundant lymphocytic infiltration. Xenograft survival at 16 weeks was significantly better in the long-term cyclosporin therapy group (p < 0.001) and those treated with anti-CD25 mAb (p < 0.001) compared with control animals that did not receive immunosuppression therapy.

During the experiment, two animals that received a long-term course of cyclosporin developed infections, re-
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quiring that they be killed prematurely. None of the animals treated with anti-CD25 mAb developed a recognized infection.

Discussion

Human fetal neural grafts have been reported to restore some motor functions in patients with Parkinson’s disease.1,3,11 Two National Institutes of Health–funded, prospective clinical trials are currently underway to evaluate this technique further. If neural grafting moves from experimental research to clinical practice, the immunology of neural graft rejection will become of paramount importance to the neurosurgeon.

The brain is an immunologically privileged site.19 Foreign grafts transplanted into the brain are rejected more slowly than when transplanted to other sites within the body. This is likely due to the reduced efficiency of the afferent arc of the immune system within the brain.25,30 This privilege, however, is far from absolute and numerous studies have shown that neural xenografts are eventually rejected by the host.22

To prolong neural xenograft (and allograft) survival, immunosuppression of the host animal has been used. Cyclosporin A is the most common immunosuppressant.46 It binds an intracellular receptor, cyclophilin, and then inhibits calcineurin.19 Inhibition of calcineurin results in the failure to activate the genes for IL-2, IL-4, and the CD40 ligand. Interleukin-2 is responsible for differentiating T-helper and T-cytotoxic precursors into effector cells and driving activated T cells through the G, and S phases into mitosis. Unfortunately, cyclosporin A therapy is accompanied by protean side effects including nephrotoxicity, seizures, and encephalopathy.1,12 In an attempt to find alternate means of effecting immunosuppression, researchers have used a variety of different agents. Other immunosuppressant drugs include SandImmune Neoral, FK 506, mycophenolic acid, rapamycin, brequinar, leflunamide, and deoxyspergualine.5,14 Monoclonal antibodies against specific elements of the immune system, such as the CD4, CD25, CD54, and major histocompatibility complex Class I surface molecules, have also been used for immunosuppression.5,10,17,21 These mAbs selectively impair components of the immune system in an attempt to block graft rejection without impairing all functions of the immune system (such as infection defense).

The interaction between IL-2 and its receptor, CD25, is required for the generation of cytotoxic T cells and for T-cell proliferation. Because T cells are crucial to neural xenograft rejection, these antibodies should selectively interfere with the rejection process.25,30 In this experiment, the anti-CD25 mAb was effective in prolonging neural xenograft survival.

Several points rise out of this work. First, it is clear that short-term immunosuppression achieved with cyclosporin (Group 2) is insufficient for prolonged neural xenograft survival. Although the blood-brain barrier is reconstituted 2 weeks after grafting,7 this did not help the xenografts survive. Rejection was swift and complete. All xenografts were nonfunctioning less than 2 weeks after the cyclosporin therapy ceased.

Second, rejection following long-term immunosuppression achieved with cyclosporin (Group 2) also occurred, but was delayed. Half of the xenografts survived 8 weeks beyond the termination of cyclosporin therapy. This suggests that neural xenografts are susceptible to a prolonged immune attack and that any clinical trial may have to use induced immunosuppression indefinitely. This is different from allograft data from which one can conclude that long-term immunosuppression may not be required.30

Third, the delayed rejection following termination of immunosuppression in Group 3 (8 weeks cyclosporin) compared with the rejection in Group 2 (2 weeks cyclosporin) suggests that the initial postsurgical inflammatory response may accelerate the rejection process within the brain. Inflammatory cytokines such as interferon-γ induce major histocompatibility complex expression of brain cells, making them a target for the immune system. Duan,
et al.,11 showed that daily treatment with high-dose methylprednisolone (30 mg/kg) can prevent murine xenograft rejection in the striatum of adult rats for up to 6 weeks. This suggests that any clinical trial of neural xenografts may benefit from at least a 2-week regimen of antiinflammatory medication.

Fourth, short-term treatment with anti-CD25 mAb prolonged xenograft survival. This treatment would have little effect on the early postsurgical inflammatory response or any preformed antibodies against the xenograft. It is likely, therefore, that these mechanisms are not the major contributor of early graft rejection in this model.

Fifth, although cyclosporin and anti-CD25 mAb both block IL-2 function, the effects following anti-CD25 mAb treatment are significantly more prolonged. This could occur because the antibody is cleared more slowly than the cyclosporin. At 16 weeks, however, there was no immunohistochemical evidence of the anti-CD25 mAb. Sections of the host animals’ xenograft, spleen, and lymph nodes stained for mouse immunoglobulin were negative (data not shown). Tellides and associates30 postulated that the anti-CD25 mAb NDS 63 functioned by target-cell inhibition, but their sampling of T cells was done only 5 days into treatment. Another mechanism of immunosuppression caused by this anti-CD25 mAb could be target-cell deletion. Activated T cells prevented from proliferating may be removed from the circulation. Deletion of these xenograft-specific T cells could potentially lead to a state of tolerance. Wood and colleagues12 demonstrated an indefinite length of survival by using this antibody in neural allografts grafted into the rat brain. Cyclosporin, on the other hand, functions by preventing these xenograft-specific T cells from becoming activated. Nonactivated T cells would not be deleted from the immune system and could be activated once the cyclosporin therapy is discontinued. Their access to the xenograft may be delayed (if all perisurgical inflammation has settled), but they ultimately effect rejection.

Conclusions

Short-term (2 weeks) immunosuppression achieved with a course of anti-CD25 mAb can prolong neural xenograft survival and function in the rodent. Its effects on xenograft survival are better than those produced by a short-term regimen of cyclosporin and similar to those of cyclosporin survival are better than those produced by a long-term (8 weeks) regimen of cyclosporin. Although both therapies interfere with IL-2 pathways, neither resulted in neural xenograft survival in all animals.

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References

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