Whole Eye Allograft Transplantation And Immunosuppression: Survival Of The Transplanted Rodent Eye Using Tacrolimus Monotherapy

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PURPOSE: Visual impairment and blindness present significant social and economic burdens around the world.

Whole eye transplantation (WET) is a potential solution. Our lab has established a viable rodent model and demonstrated promising results in syngeneic transplants. To establish allograft transplantation, a successful immunosuppressive pharmacotherapy regimen is required. Tacrolimus monotherapy is successful in rodent vascularized composite allotransplantation (VCA), but its efficacy in WET is unknown. Here, we present survival of allograft WET treated with Tacrolimus mono therapy.

METHODS: Brown-Norway to Lewis rat whole eye transplantations were performed (n=6), followed by daily intraperitoneal 1mg/kg Tacrolimus (FK-506) monotherapy injection. Animals were examined at post-operative week 1, 3, 5, and 6. Ocular structure and retinal blood flow were studied using Optical Coherence Tomography (OCT). A retina specialist ophthalmologist performed anterior segment examination, fundoscopy, indirect ophthalmoscopy, and tonometry for intraocular pressures. Animals were sacrificed at 6 weeks. Specimens of the transplanted globe, external ear, eyelid, bone and vessel anastomoses were stained with H&E and interpreted by an ocular pathologist.

RESULTS: All composite grafts survived without signs of infection or necrosis. OCT demonstrated generally normal cornea, relative structural maintenance of the retina, and preserved blood flow in the central retinal artery and vein. At one week after transplantation, most (n=3 out of 5 examined) of transplanted allograft eyes exhibited normal perfusion with pink optic nerve heads. At three weeks, the eyes trended toward increased ischemia, with atrophic fundi or 3+ ischemia (n=3 out of 5 examined). By five weeks, there was some improvement in retinal ischemia, but optic nerve heads remained pale and moderate fundus ischemia was sustained to the study endpoint of 6 wks. In previous syngeneic transplants, fundus ischemia and pale optic nerves were present but uncommon. Intraocular pressures (IOPs) in the transplanted eye were within normal range, with a single exception. Average IOPs in the transplanted eyes were 10.9 mmHg (SD 2.2), 15.5 mmHg (SD 7.7), 9.9 mmHg (SD 2.7), and 10.7 mmHg (SD 2.3) at week 1, 3, 5, and 6, respectively. Histologically, inflammation and neovascularization were seen in the cornea. There were no inflammatory cells in the anterior chamber. Central retinal degeneration was noted. Chronic inflammation was observed at the optic nerve. By comparison, the unoperated eye was normal.

CONCLUSION: With Tacrolimus monotherapy, transplanted globes survived for 6 weeks, with grossly intact cornea and retinal structures and normal IOPs. On ophthalmologic examination, some degree of fundus ischemia...
was present despite the presence of retinal blood flow as confirmed by OCT. Allografts may be less vascularized than syngeneic whole eye transplantations. In addition, histopathology reveals some inflammation and neovascularization to the cornea and central degeneration of the retina. These findings serve as targets for future, therapies, improvements and investigation. Future studies of systemic and local immunosuppressive therapies are necessary to support the structural and functional success of whole-eye allotransplantation.

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Ex Vivo Expanded Regulatory T Cells Combined With Short-term Costimulation Blockade To Prevent Rejection Of Vascularized Composite Allografts

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PURPOSE: Reconstructive transplantation represents a valid therapeutic option after devastating tissue loss. Routine clinical application, however, is hampered by the toxicity of long-term maintenance immunosuppression. The current study investigated a novel approach to immunosuppression therapy using ex vivo expanded regulatory T cells combined with a short-term immunomodulatory strategy in a murine hind limb transplantation model.

METHODS: Fully MHC-mismatched orthotopic hind limb transplants were performed from Balb/C into C57BL/6 mice. Recipients in the experimental groups received a combination regimen consisting of 0.5mg CTLA4 Ig on day 0, 2, 4 and 6 post-transplant, 20mg/kg anti-Thy 1.2 mAb on POD-1, and 1mg/kg Rapamycin (POD 0–9), and in one group, 1 week expanded CD4+CD25+ Treg cells in combination with the above therapies. Allograft survival was monitored and flow cytometric analysis was performed to evaluate mixed chimerism and clonal deletion of alloreactive T cells. Treg activity was assessed in vitro using suppression assays in order to support and supplement in vivo data.

RESULTS: Combination of T cell depletion and CTLA4-Ig plus short-course of Rapamycin increased VCA survival significantly compared to untreated controls (MST 105 days with combination therapy vs MST 9 days without; p<0.01). Mixed chimerism was detected in recipients receiving this combined treatment protocol with 5.013±1.23 % of CD11b+ cells being donor-derived on POD 55. Vβ - TCR staining profiles in recipients after full treatment showed 1.570±0.3700 % of Vβ5+CD4+ T cells, while naïve C57BL/6 express 3.567±0.3690 % of Vβ5+CD4+ T cells, suggesting the actuation of central deletion of developing donor-reactive T cells. In order to further prolong allograft survival, one week expanded Tregs were then included in the combination therapy. The suppressive activity of the CD4+CD25+ Tregs was confirmed in vitro suppression assays. The addition of ex vivo expanded regulatory T cells further increased VCA survival to greater than 200 days and induced long-term stable mixed chimerism with approximately 15% of CD11b cells being donor-derived on POD 55 after administration of expanded donor Tregs.

CONCLUSION: The combination of T cell depletion, costimulation blockade, and a short-course of Rapamycin prevents VCA rejection and significantly prolongs graft survival without the need for myeloablative conditioning or maintenance therapy. Moreover, regulatory T cells added in the early post transplant period further optimize immune regulation by inducing sustained mixed chimerism.

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