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Graft 2001; 4; 105

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The thymus plays a pivotal role in the development of systemic central tolerance to self antigens. Potentially autoreactive T cells are deleted or anergized by exposure to the appropriate self antigens presented by either bone marrow-derived cells or thymic stromal cells.[1-5] Similar intrathymic mechanisms are also important in the induction of donor-specific T cell tolerance to alloantigen. Studies in rodent models have demonstrated that inoculation of alloantigen into the thymus induces donor-specific tolerance in vivo and in vitro.[6-8] Our laboratory has demonstrated in a large animal model of partially-inbred miniature swine that the thymus is essential for rapid and stable tolerance induction to renal allografts by a short course of cyclosporine (CyA).[9-11]

Elucidation of the mechanisms of xenograft hyperacute rejection have made it possible to develop protective strategies, including adsorption of natural antibodies (nAbs) and the generation of transgenic pigs expressing human complement-regulatory proteins-mediated responses involved in xenograft rejection.[12,13] Several groups have confirmed that human T cells recognize xenogeneic cells directly and that reactivity between human and pig T cells is at least equal to allogeneic responses.[14,15] Thus, thymic-dependent mechanisms might induce T cell tolerance to xeno-antigens in a similar manner to that which occurs for both auto- and alloantigens.

Thymic Transplantation in Rodent Models

Studies in a rodent model have confirmed the ability of thymic tissue to facilitate the development of xenogeneic cellular tolerance. Sykes and colleagues have demonstrated the capacity of xenogeneic thymic tissue to reconstitute functional host T cells in thymus-deficient or thymectomized mice.[16-18] Fetal swine thymic and liver fragments were implanted under the kidney capsule of irradiated SCID mice,[16] growing and surviving for at least 4 months post-transplant. Histology was consistent with normal thymus and immunohistochemistry revealed mouse T cells and immature thymocytes. The mouse thymocyte populations that developed in pig thymic grafts were phenotypically indistinguishable from normal mouse thymocytes. In addition, pig thymic grafts were responsible for reconstitution of normal numbers of mouse CD4 cells in the periphery. These cells were functional, as evidenced by normal immune responses, including allo-responses, in vitro and in vivo.[18] Importantly, these thymic grafts induced tolerance to donor pig skin grafts in normal mice treated with a nonmyeloablative preparative regimen including thymectomy, T and NK cell depletion and low-dose whole body irradiation.[17] After reconstitution, these animals demonstrated long-term acceptance of donor-pig skin grafts while rejecting both allogeneic and xenogeneic third-party skin grafts. Additional studies have demonstrated that mouse lymphocytes selected on these xenogeneic thymic grafts are restricted by both host and donor MHC.[19] These findings suggest a promising strategy for the clinical induction of xenogeneic tolerance by transplanting porcine thymus.

The advantages of thymic transplantation from fetal swine donors are:

1. the growth potential of fetal tissues,
2. the lesser immunogenicity of fetal tissue, and
3. host vascularization of thymic tissue, which would avoid rejection by xenogeneic nAbs antibodies.

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The limited supply of donor fetuses, however, poses a significant barrier to fetal swine thymic tissue transplants. Either adult or juvenile donors would provide a greater quantity of thymic tissue. However, direct thymic tissue transplants from adult and juvenile donors have not succeeded long-term in either the rodent[20,21] or miniature swine models.[22] More mature thymic tissue grafts might be more susceptible to significant ischemic damage during revascularization, whereas neovascularization might occur more rapidly in fetal tissues.

Thymic Transplantation in Swine

We have recently described a successful strategy for the transplantation of vascularized thymic grafts as a composite thymo-kidney[23-25] or thymo-heart[26] transplants using juvenile porcine thymus. Autologous thymic tissue was harvested and implanted under either the renal capsule or pericardium. After a period of 30-60 days these grafts have developed a vascular supply from the associated organ and were transplanted as vascularized allogeneic tissue as a part of the composite organ (Fig. 1). We have published our data demonstrating that these vascularized composite thymic grafts

1. reconstitute T cells in immunodeficient, thymectomized recipients,[23]
2. induce tolerance across class I allogeneic barriers with a 12-day course of cyclosporine, and[24]
3. induce tolerance and reconstitute T cells across a two-haplotype, fully MHC-mismatched barrier when the recipient was treated with a regimen of thymectomy, T cell depletion, and a 12-day course of cyclosporine (K. Yamada, manuscript in preparation).[27]

In order to function effectively in tolerance induction, thymic grafts needed to be vascularized prior to transplantation. Thymic tissue co-transplanted at the same time as kidney transplants (non-vascularized thymic grafts) failed to permit the rapid and stable induction of tolerance, and kidney grafts demonstrated both acute and chronic rejection.[23] Additionally, initial experience suggests that both adequate T cell depletion and recipient thymectomy are necessary for the function of composite thymic allografts (K. Yamada, manuscript in preparation).

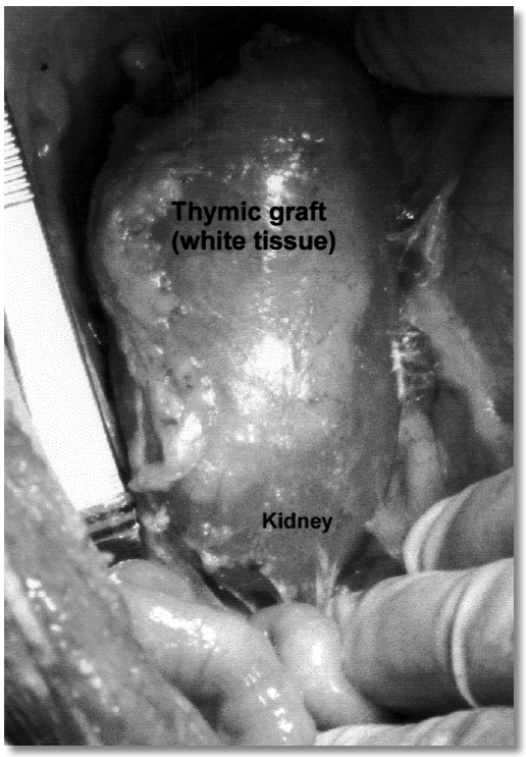

Figure 1. Macroscopic appearance of a porcine composite thymokidney graft after transplantation into an allogeneic pig. The composite thymo-kidney resided in the donor pig for 3 months after implantation of autologous thymic tissue under the renal capsule.

Thymic Transplantation in the Pig-to-Primate Model

Based upon our promising results from vascularized thymic allotransplantation,[23,27] we have begun to apply this strategy to the induction of xenogeneic tolerance in a large animal model. Although transplanting non-vascularized fetal porcine thymic grafts into baboons receiving a regimen of thymectomy and T cell depletion achieved a transient return of thymic-derived T cells, these thymic grafts showed little other evidence of engraftment (A. Wu et al, manuscript in preparation).[28]

The most recent studies in our laboratory have investigated transplanting vascularized, composite porcine thymokidney grafts to baboons. This strategy has permitted the survival of donor thymic epithelium and generated donor-specific unresponsiveness for longer than 2 months after withdrawal of immunosuppression (R. Barth, manuscript in preparation). Although these grafts were eventually rejected by

humoral mechanisms, there is evidence that tolerance may have been achieved at the cellular level in these animals. These data thus support the potential application of vascularized thymic tissue transplants to the induction of xenogeneic T cell tolerance in the pig-to-primate model. Additional strategies aimed at reducing humoral mechanisms of rejection may permit the long-term survival of vascularized thymic grafts and could induce tolerance to a xenogeneic donor. The approach of composite swine thymo-organ transplantation could then represent a strategy applicable to clinical xenotransplantation.

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