Thymic Transplantation in Miniature Swine. II. Induction of Tolerance by Transplantation of Composite Thymokidneys to Thymectomized Recipients

Kazuhiko Yamada, Akira Shimizu, Ryu Utsugi, Francesco L. Ierino, Patricio Gargollo, Gary W. Haller, Robert B. Colvin and David H. Sachs

*J Immunol* 2000; 164:3079-3086; doi: 10.4049/jimmunol.164.6.3079
http://www.jimmunol.org/content/164/6/3079

References

This article cites 43 articles, 14 of which you can access for free at:
http://www.jimmunol.org/content/164/6/3079.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Thymic Transplantation in Miniature Swine. II. Induction of Tolerance by Transplantation of Composite Thymokidneys to Thymectomized Recipients

Kazuhiko Yamada,* Akira Shimizu,† Ryu Utsugi,* Francesco L. Ierino,* Patricio Gargollo,* Gary W. Haller,* Robert B. Colvin,† and David H. Sachs2* 

Previous studies in our laboratory have demonstrated that the presence of the thymus is essential for rapid and stable tolerance induction in allotransplant models. We now report an attempt to induce tolerance to kidney allografts by transplanting donor thymic grafts simultaneously with the kidney in thymectomized recipients. Recipients were thymectomized 3 wk before receiving an organ and/or tissues from a class I-mismatched donor. Recipients received 1) a kidney allograft alone, 2) a composite allogeneic thymokidney (kidney with vascularized autologous thymic tissue under its capsule), or 3) separate kidney and thymic grafts from the same donor. All recipients received a 12-day course of cyclosporine. Thymectomized animals receiving a kidney allograft alone or receiving separate thymic and kidney grafts had unstable renal function due to severe rejection with the persistence of anti-donor cytotoxic T cell reactivity. In contrast, recipients of composite thymokidney grafts had stable renal function with no evidence of rejection histologically and donor-specific unresponsiveness. By postoperative day 14, the thymic tissue in the thymokidney contained recipient-type dendritic cells. By postoperative day 60, recipient-type class I positive thymocytes appeared in the thymic medulla, indicating thymopoiesis. T cells were both recipient and donor MHC-restricted. This article must therefore be hereby marked as an advertisement. 

The Journal of Immunology, 2000, 164: 3079–3086.

Materials and Methods

Animals

Animals were selected from our herd of MHC-inbred miniature swine (10, 11) at 6–9 wk of age (juvenile animals) to create composite thymokidney grafts. Except in one case, pigs of 5–9 mo of age were used as recipients of allogeneic kidney or thymokidney transplants. One of the thymokidney transplant recipients was a 4.4-year-old pig that had previously undergone thymectomy 3.7 years before. We have used SLA\(\text{ab}\) animals (swine leukocyte antigen, SLA\(\text{ab}\)) as the recipients and SLA\(\text{gg}\) animals as the donors for class I-mismatched transplant in all cases to provide a class II match and class I mismatch (10).

Experimental groups

Recipients of class I-mismatched grafts were divided into the following three groups: the recipients receiving a kidney alone (group 1), separate thymic and kidney grafts from the same donor (group 2), or a composite thymokidney graft (group 3).

Received for publication September 22, 1999. Accepted for publication January 5, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2 Address correspondence and reprint requests to Dr. David H. Sachs, Transplantation Biology Research Center, Massachusetts General Hospital, MGH-East, Building 149-9019, 13th Street, Boston, MA 02129. E-mail address: sachs@helix.mgh.harvard.edu

3 Abbreviations used in this paper: SLA, swine leukocyte antigen; CyA, cyclosporine; POD, postoperative day; H&E, hematoxylin and eosin; CML, cell-mediated lympholysis; TNP, trinitrophenyl; SP, single positive.
Creation of thymokidney in the donor. A partial thymectomy (approximately three-fourths of the cervical thymus) was performed to provide autologous thymic tissue, which was minced into 2–3 mm³ pieces and grafted under the renal capsule. The thymokidney was allowed to develop for 3 mo before excision and transplantation into the allogeneic recipient.

Complete thymectomy in the recipient. Complete thymectomy was performed before allogeneic transplantation as previously described (8). Briefly, the pretracheal muscles were retracted, exposing the cervical thymus and trachea from the cervico-thoracic junction to the mandibular area. The cervical thymus was excised, after which the mediastinal thymus was removed through a sternotomy.

Allogeneic kidney or thymokidney transplantation. The transplant was performed as previously established for allogeneic kidney transplantation (8, 12). In both the kidney and thymokidney transplants, the renal artery was unclamped end-to-end into the recipient’s aorta (Carrel patch, and the renal vein was anastomosed end-to-side to the recipient’s inferior vena cava. Urinary drainage was accomplished via a ureterovesical anastomosis. The kidney or thymokidney was transplanted into class I-disparate recipients. Both native kidneys were excised. One recipient (#11196) had been thymectomized and had received a life-supporting class I-mismatched kidney allograft (with a 12-day course of cyclosporine (CyA) and 37.7 μg/kg CyA and the kidney allograft had been accepted after a short rejection crisis. In this pig, the pig allograft was removed at the time of composite thymokidney allotransplanting from a donor SLA–matched to the original kidney allograft donor.

Separate allogeneic thymic and kidney transplantation from the same donor. The thymic grafts were obtained in the same manner as the autologous thymic grafts (described above) on the day of kidney harvest from a recipient whose native thymus (equivalent to that in a composite thymokidney graft) were transplanted into the sternomastoid muscles of the recipient. Previous studies in our laboratory have demonstrated that autologous thymic tissue engrafts equally well in this site and under the kidney capsule (our unpublished observations).

Biopsy of the composite thymokidney allografts. Biopsies were conducted through a flank abdominal incision on postoperative days (POD) 0, 30, 60, 80 (#11196 only), and >100 (#12750 and #12827). The thymokidney was examined by macroscopic evaluation, and wedge kidney biopsies were taken (which included both thymic and renal tissue) for histological examination and FACS.

Immunosuppressive therapy
CyA (Sandimmune) was generously provided by Novartis Pharmaceuticals (East Hanover, NJ) and was administered as an i.v. suspension according to the manufacturer’s specifications. CyA was given daily as a single infusion at a dose of 10–15 mg/k (adjusted to maintain a blood trough level of 400–800 ng/ml) for 12 consecutive days, beginning on the day of transplantation. CyA levels were determined by a fluorescence polarization immunomassay (Abbott Laboratories, Dallas, Texas), which measured the parent compound but not metabolites.

Histological examination
Formaldehyde-processed specimens were stained using hematoxylin and eosin (H&E) and periodic acid–Schiff, and frozen tissues were used for immunohistochemistry (with the avidin-biotin-alkaline phosphatase technique). Thymocyte development was assessed using the murine anti-pig mAbs 74-12-4 (IgG2b, anti-swine CD4), 76-2-11 (IgG2a, anti-swine CD8), 76-7-4 (IgG2a, anti-swine CD1) (13), MSA4 (IgG2a, anti-swine CD2) (14), MSB7 (IgG2b, anti-swine CD4), 2-27-3a (IgG2a, anti-swine class I), and ISCR-3 (IgG2a, anti-swine DR) (15). Four-micron sections were incubated with 1% normal horse serum and avidin (100 μg/ml in PBS) to inhibit nonspecific binding of horse IgG and endogenous biotin, respectively. After 20 min, the tissue was covered with optimally diluted primary mAb and incubated for 60 min at room temperature. Sections were rinsed in PBS and incubated in a solution of biotin (10 μg/ml in PBS) with 0.3% hydrogen peroxide for 30 min to block endogenous peroxidase (Life Technologies). 50 μg/ml gentamicin (Life Technologies); 10 μM HEPES (Fisher Scientific, Pittsburgh, PA); 2 mM L-glutamine (Life Technologies); 1 mM sodium pyruvate (BioWhittaker, Walkersville, MD); nonessential amino acids (BioWhittaker); and 5 × 10⁻³ M 2-ME (Sigma). The effector phase of the CML assay was performed using Basal Medium Eagle (Life Technologies) supplemented with 6% controlled processed serum replacement (S) and 10 μM HEPES.

Cell-mediated lymphocyte toxicity (CML) assay
Tissue culture media used for CML assays consisted of RPMI 1640 (Life Technologies) supplemented with 6% FCS (Sigma, St. Louis, MO), 100 IU/ml penicillin, and 100 μg/ml streptomycin (Life Technologies); 10 μM HEPES (Fisher Scientific, Pittsburgh, PA); 2 mM L-glutamine (Life Technologies); 1 mM sodium pyruvate (BioWhittaker, Walkersville, MD); nonessential amino acids (BioWhittaker); and 5 × 10⁻³ M 2-ME (Sigma). The effector phase of the CML assay was performed using Basal Medium Eagle (Life Technologies) supplemented with 6% controlled processed serum replacement (S) and 10 μM HEPES.

CML assays were performed as previously described (12, 18, 19). Briefly, lymphocyte cultures containing 4 × 10⁶ responder and 4 × 10⁶ stimulator PBL (irradiated with 2500 cGy) per ml were incubated for 6 days at 37°C in 7.5% CO₂ and 100% humidity. Bulk cultures were harvested and effectors were tested for cytotoxic activity on 51 Cr-labeled (American Type Culture Collection, Manassas, VA) lymphoblast targets. Effector cells were incubated for 5.5 h with target cells at E:T ratios of 100:1, 50:1, 25:1, and 12.5:1. The following three target cells were tested in each assay: SLA–matched PBL to the effectors (negative control), donor-matched PBL (SLAα, class I+ and class II+), and third-party PBL. Supernatants were then harvested using the Skatron (Sterling, VA) collection system and isotopic release was determined on a gamma counter (Canberra Packard Instruments). The results were expressed as percent specific lysis (PSL) and were calculated as: PSL = [{[experimental release (cpm) – spontaneous release (cpm)]/maximum release (cpm) – spontaneous release (cpm)}] × 100%.

Sensitization cultures (Trinitrophenyl (TNP) CML assay)
MHC restriction of T cells was examined by in vitro TNP CML assay as modified from previously published studies in rodents (20). PBL for in vitro sensitization (stimulators and targets) were incubated with 10 μM trinitrobenzenesulfonic acid (Sigma) in CML medium for 10 min at 37°C. After three washes in medium supplemented with 5% FBS, the in vitro-sensitized stimulator cells were irradiated with 2500 cGy, 4 × 10⁶ cells
were added to an equal number of responding cells per well and cultured for 6 days, and the in vitro-sensitized target cells were incubated with $^{51}$Cr for 1.5 h.

**Results**

Clinical course, histological findings, and immunologic status after class I-disparate kidney transplantation

**Thymectomy before kidney transplant (day −21) interferes with the induction of tolerance.** We have previously demonstrated that thymectomy performed 3 wk before kidney transplantation interferes with the induction of tolerance (group 1: historic controls) (8). Four of five thymectomized animals developed severe rejection crises (Fig. 1a) manifested by markedly elevated creatinine levels, the development of anti-donor CML (Fig. 2, filled bars), histologic evidence during the second to fourth postoperative weeks (Fig. 3a), and chronic rejection (Fig. 3b) at later time periods. One animal eventually accepted its kidney allograft but showed a prolonged period of unstable creatinine levels with persistence of anti-donor CTL throughout the experimental period; this has never been observed in euthymic recipients of a class I-mismatched kidney transplant.

**A nonvascularized thymic graft did not induce tolerance (group 2).** Two thymectomized animals (#12834 and #12858) received sep-

---

**FIGURE 1.** Clinical course, as indicated by plasma creatinine level, of class I-mismatched kidney transplants in thymectomized recipients of kidney alone (a), kidney and thymus separately (b), and composite thymokidney transplants (c), all treated by standard CyA protocol (a 12-day course of CyA i.v. to maintain blood levels at 400–800 ng/ml).

**FIGURE 2.** Anti-donor CTL responses on POD 30 in thymectomized recipients of kidney alone (■), kidney and thymus separately (□), and the composite thymokidney (□). Percent specific lysis at E:T ratio of 100:1 is shown.

**FIGURE 3.** Histology (H&E) of kidney allografts on POD 30 in a recipient of kidney alone (#11809) (a), a recipient of kidney and thymus separately (#12858) (c), and a recipient of the composite thymokidney (#12750) (e). Histology (H&E) of a kidney allograft on POD 60 in a recipient of kidney alone (#11809) (b), a kidney allograft on POD 100 in a recipient of kidney and thymus separately (#12858) (d), and a kidney allograft on POD 150 in a recipient of the composite thymokidney (#12750) (f).
artere thymic and kidney grafts on the same day from the same class I-mismatched donor. Both recipients had a marked rejection crisis early (before POD 30), and both grafts developed chronic changes similar to the clinical course in thymectomized recipients of a kidney alone (Fig. 1b). Histologically, diffuse mononuclear cell infiltration as well as acute glomerulitis and vasculitis were seen in the kidney graft on POD 30 (Fig. 3c), and chronic glomeropathy developed later (Fig. 3d). The thymic grafts were rejected by POD 30 with evidence of mononuclear cell infiltration (Fig. 4a). Immunologically, both animals maintained the same level of anti-donor CTL reactivity as a thymectomized recipient of a kidney alone (Fig. 2, open bars) and developed anti-donor alloantibodies (IgG and IgM). The clinical courses and histological findings as well as immunological statuses of these animals were similar to those of thymectomized control animals.

A vascularized thymic graft (composite thymokidney) induced rapid and stable tolerance (group 3). Three thymectomized recipients (#12750, #12827, and #11196) received class I-mismatched composite thymokidney grafts. Fig. 4b shows a transplanted composite thymokidney graft in the recipient. The thymic grafts were vascularized by vessels from both kidney parenchyma and kidney capsule. All recipients accepted their grafts and had stable renal function indefinitely (Fig. 1c). Plasma creatinine levels were 1.1 mg/dl on POD 314 in #12750, 1.5 mg/dl on POD 225 in #12827, and 1.0 mg/dl on POD 165 (#11196). Histologically, there was minimal cell infiltration, and no vascular changes were observed in the kidney allograft on POD 30 (Fig. 3e). No chronic changes developed in later biopsies (Fig. 3f, POD 150 kidney biopsy). The thymic portion of the thymokidney also showed an intact thymic structure without any cell infiltration (Fig. 4c). All recipients had donor-specific unresponsiveness on day 30 and thereafter (Fig. 2, gray bars), and no measurable anti-donor Abs (IgM or IgG) developed.

These results indicated that a vascularized composite thymokidney graft but not a neovascularized thymic graft induced rapid and stable tolerance to a class I-mismatched kidney allograft in a thymectomized recipient in a manner similar to that reported in euthymic recipients receiving kidneys alone (21).

Mechanisms of tolerance in the composite thymokidney transplant

Nonvascularized donor thymic tissue failed to induce tolerance, indicating that donor thymic emigrants are unlikely to be entirely responsible for this process. To further elucidate the mechanisms underlying this phenomenon, we examined 1) changes in thymic stroma and thymopoiesis in the transplanted vascularized thymus, and 2) T cell restriction after a composite thymokidney transplant, as well as the presence of peripheral macrochimerism.

Thymopoiesis in vascularized thymic grafts. Fig. 5 shows immunohistochemistry demonstrating 1) migration of recipient-type dendritic cells and 2) thymopoiesis in the thymic graft. To differentiate donor and recipient cells, class I (donor-type) mAb and class II (recipient-type) mAb were used.

The specimen from POD 14 showed recipient-type class I-positive cells that morphologically appeared to be dendritic cells migrating to the cortico-medullary junction of the thymic graft (Fig. 5a, brown cells). Double-staining demonstrated that the putative dendritic cells were recipient-type class I-negative/blue/class II-positive (brown) (Fig. 5d). The cells were cytokeratine-negative (not stained brown) (Fig. 5e), indicating that these recipient-type cells were likely to be dendritic cells.

Although recipient-type dendritic cells had migrated into the thymic graft by POD 14, there were few recipient-type cells seen in the medulla on POD 14 and 30. However, by POD 60, recipient-type class I-positive cells were observed (Fig. 5b, brown cells), and their numbers increased thereafter (after POD 60) in the medulla but not in the cortical zone, indicating that thymopoiesis was occurring without rejection.

We also examined changes in donor-type cells. Class I donor-type staining demonstrated that the number of donor-type thymocytes had decreased markedly in the POD 60 specimen and thereafter. However, donor-type class I-positive stromal cells and donor-type cells having dendritic morphology were still present at POD 100 (Fig. 5c).

Thymopoiesis was also examined by three-color FACS analysis to determine whether it involved both CD4 and CD8 T cells. The

**FIGURE 4.** Histology (H&E) of thymic graft in neck muscle on POD 30 in a recipient of kidney and thymus separately (#12858) (a), and of a thymic graft under the renal capsule on POD 30 in a recipient of composite thymokidney (#12750)(c). The thymic graft in a shows fibrosis and a mononuclear cell infiltrate with no residual thymic structure or Hassall’s corpuscles, indicating a rejected thymic graft. In contrast, the thymic graft in the composite thymokidney (c) shows that thymic structure is well-preserved without evidence of rejection. The macroscopic appearance of the composite thymokidney graft (b) demonstrates the autologous thymic graft (arrow) under the kidney capsule 3 mo after thymic transplantation.
phenotypic analysis of donor-type and recipient-type thymocytes is shown in Fig. 6. Recipient-type CD4-positive cells and CD8-positive cells were detected with FACS by POD 30 (Fig. 6a), and the number of recipient-type cells markedly increased by POD 100 (Fig. 6b), indicating that both CD4 and CD8 T cells developed in the donor thymic tissue. In addition, 30% of the total thymocytes expressed both CD3 and class I, indicating that they were mature thymocytes. These levels were similar to those seen in a thymokidney before it was transplanted as well as in a native thymus. Immunohistochemistry and FACS data confirmed that rapid thymopoiesis without any rejection crisis occurred in the thymic graft of the vascularized composite thymokidney.

**Peripheral macrochimerism.** Macrochimerism, defined as chimerism detectable by FACS, was evaluated in the recipients of composite thymokidney grafts and of thymic grafts transplanted simultaneously with kidney allografts but in separate locations. Both animals receiving the thymokidney and animals receiving the kidney and thymus separately showed donor cell macrochimerism (0.07–0.18% in lymphocyte population) by POD 8 (during CyA treatment period), indicating that both CD4 and CD8 T cells developed in the donor thymic tissue. In addition, ~30% of the total thymocytes expressed both CD3 and class I, indicating that they were mature thymocytes. These levels were similar to those seen in a thymokidney before it was transplanted as well as in a native thymus. Immunohistochemistry and FACS data confirmed that rapid thymopoiesis without any rejection crisis occurred in the thymic graft of the vascularized composite thymokidney.

**FIGURE 6.** Thymopoiesis in the thymic graft of a composite thymokidney as assessed by three-color FACS analysis with staining to determine whether thymopoiesis involved both CD4 and CD8 T cells. Recipient-type CD4-positive cells and CD8-positive cells were detected on POD 30 (a), and the number of recipient-type cells markedly increased on POD 100 (b).

Recipients of composite grafts and separate thymus and kidney transplants both demonstrated similar levels of circulating donor leukocytes postoperatively, including recent donor thymic emigrants. These results suggested that comparable populations of donor leukocytes were not sufficient alone to induce tolerance in this model.

**MHC restriction after the composite thymokidney graft.** We recently demonstrated that 1) the number of T cells, particularly of CD4 single positive (SP) cells, decreased after thymectomy over a period of 2 years, and 2) CD45RA+/CD4 SP cells increased after a thymokidney transplant in a long-term thymectomized animal, indicating that T cell development was occurring in the thymokidney (45). We have now studied MHC restriction after transplantation of the composite thymokidney graft in the same long-term animal (#11196), which had been thymectomized and received a life-supporting class I-mismatched kidney allograft 3.7 years earlier. Before the thymokidney transplant, this animal had few CD4 SP cells, and its general unresponsiveness was demonstrated by MLR. The animal accepted the composite thymokidney allograft. CD4 SP cells, including CD45RA+/CD4 SP cells, increased markedly from 30 days after the transplant, indicating that the composite thymokidney was capable of reconstituting T cells (45). Because new T cells developed from the thymic graft and the animal became immunocompetent, as shown by MLR 3 mo after the composite thymokidney transplant, we examined the MHC restriction

**FIGURE 5.** Immunohistochemical study of thymic grafts in the composite thymokidney grafts. Recipient-type class I-positive cells (brown) are seen at corticomedular junction of thymic graft on POD 14 (a); recipient-type class I-positive cells (brown) in medulla on POD 60 (b); and donor type class I-positive cells (brown) in thymic graft on POD 100 (c). Double staining of thymic graft in the composite thymokidney on POD 14: arrows indicate recipient-type class I-positive (blue)/class II-positive (brown) cells (d) and recipient-type class I-positive (blue)/cytokeratine negative (not stained as brown) cells (e).
of T cells in vitro by TNP CML assays 3 mo after the composite thymokidney transplant in this animal (Fig. 8). Although the animal had no anti-donor response (donor-specific unresponsiveness (Fig. 8, top left, open bar)), a positive response was seen if both anti-donor effector cells and donor target cells were incubated with TNP (Fig. 8, top left, filled bar). This result indicated that donor MHC-restricted CTL were present. In addition, a positive response was seen if anti-self effector cells and self target cells were incubated with TNP, indicating that self MHC-restricted CTL were also present (Fig. 8, bottom left, filled bar). No killing was seen if either stimulator or target cells were incubated without TNP (Fig. 8, bottom left, open or gray bar). These data demonstrated that there were both donor and recipient MHC restrictions of CTL reactivity after composite thymokidney transplantation.

Discussion

It has previously been demonstrated that fetal porcine thymic tissue transplanted under the kidney capsule of thymectomized, T cell-depleted mice can induce tolerance to swine Ags (6, 7). We hope to eventually assess this strategy in a large animal xenogeneic transplant model. In preparation for such studies, the ability of the thymic grafts to induce tolerance across a full allogeneic barrier in miniature swine was evaluated. We have previously demonstrated that the presence of an intact thymus is required for the development of rapid and stable tolerance in miniature swine (8, 23, 46). Thus, if the recipient thymus was removed 3 wk before a class I-disparate kidney or heart transplant, tolerance was not obtained (8, 24). In the present study, thymectomized recipients were used to determine whether a thymic graft is capable of replacing the effect of the host thymus in permitting the induction of tolerance to a class I-mismatched kidney transplant. We have found that a vascularized thymic graft induces tolerance, whereas the transplantation of nonvascularized thymic tissue does not.

It is generally accepted that vascularized organs are more tolerogenic than nonvascularized tissues. Thus, vascularized grafts are relatively tolerogenic (21, 25, 26), whereas skin and tissue grafts are relatively immunogenic, probably because they release cells or Ags from cells into the lymphatic system where they sensitize the host (27). Even in the case of autografts, direct thymic grafts require a revascularization period after transplantation. The

FIGURE 7. a, Flow cytometric analysis of lymphopoietic donor macrochimerism (percent of CD3-positive donor cells) in peripheral blood in recipients of composite thymokidney (#12750 and #12827) and in recipients of kidney and thymus separately (#12834 and #12858). b, Immunohistochemical study of macrochimerism in a mesenteric lymph node in a recipient of composite thymokidney (#12750) on POD 30. Donor class I-positive cells (brown) are seen in T cell area of a mesenteric lymph node.

FIGURE 8. TNP-modified CTL responses of a thymus-grafted animal (#11196) and a naive SLA-matched animal. Percent specific lysis at E:T ratio of 100:1 is shown.
ischemic period in the first 1–3 wk leads to temporary loss of the thymic graft structure, which is reconstituted over the next 5–7 wk (45). Thus, during the period of revascularization, there is increased susceptibility to nonspecific graft loss and an increased potential for sensitization of the recipient. In contrast to direct thymic grafts, transplantation of thymic tissue as a composite thymokidney graft avoids the necessity for revascularization in the recipient, and the thymic graft functions immediately.

Based upon our results, we would suggest the following potential mechanisms through which the thymic grafting may induce tolerance in this model: 1) T cell progenitors may be positively and negatively selected by donor thymic stroma and/or dendritic cells by a mechanism similar to that of self-tolerance (2). Immunohistochemistry demonstrated that donor-type dendritic cells remained for >3 mo after the transplant. Negative selection of potentially autoreactive thymocytes occurs mainly in the thymus and is thought to be induced primarily by interaction with bone marrow-derived cells (2). Other reports have demonstrated that thymic epithelial cells are capable of taking part in both positive and negative selection of thymocytes (28, 29) and of inducing anergy (30). Thus, the long-term presence of donor stromal cells (donor epithelial cells, dendritic-like cells, and vascular endothelial cells) in the donor thymic graft may play an important role in the induction of tolerance by deletion, anergy, or a combination of the two. Immunohistochemistry also demonstrated that 1) recipient dendritic-like cells had migrated into the thymic graft within 2 wk. The migrant recipient-type dendritic cells are cytokeratin-7/class II+ cells, strongly suggesting that they are bone marrow-derived dendritic cells and thus may delete self-reactive cells. Consistent with this hypothesis, we evaluated T cell restriction in an animal (#11196) whose T cells had progressively decreased in number during the 3.7 years since thymectomy but were reconstituted after the composite thymokidney transplant. T cells were restricted by both the recipient and donor MHC, indicating that newly developed T cells were educated by both host and donor elements in the thymic graft.

2) All recipients in this study were not T cell depleted. Thus, mature T cells were present in recipients of composite thymokidney grafts. However, mature peripheral T cells have also been shown to become unresponsive to donor Ag by recirculation to the thymus (31), and a similar mechanism could be operative for the thymic graft. Re-entry of activated T cells into the thymus has been reported previously (31); thus, alloreactive T cells could enter the donor thymic graft and could be educated. In addition, because the recipient and donor are class II- matched in this model, processed class I Ags presented by class II Ags in the thymus would be expected to be identical regardless of whether the APC were of donor or recipient origin. Tolerance at the level of CD4 helper cells recognizing class I peptides through the indirect pathway may be possible in both cases. CyA can effectively inhibit both the CD4 helper pathway and the direct CD8 helper pathway (32–34). Thus, T cell depletion was not required to induce rapid and stable tolerance in this model.

3) Another possibility is that thymic emigrants from the thymic graft, which may include regulatory cells, facilitate tolerance induction peripherally. Such peripheral tolerance could be mediated by a changed cytokine milieu or by suppressive mechanisms. Suppression of this type has been reported previously in rodent models. One group has identified CD4-positive cells as the regulatory cell population (35–37). However, direct thymic tissue transplants did not facilitate the induction of tolerance in our model and, therefore, donor thymic emigrants may not be sufficient to induce tolerance.

Our strategy eventually may be clinically applicable for the induction of transplantation tolerance to xenografts, which is the ultimate goal of these experiments. We have chosen to begin the studies in an allogeneic system rather than in a discordant xenogeneic system to assess the cellular immune response without the complications provided by natural Abs, which are a formidable barrier to vascularized xenografts (38–41). The present data indicate that transplantation of a vascularized thymic graft is a potential strategy to induce tolerance across a class I-mismatch barrier. Therefore, we are evaluating the effect of composite thymokidney grafts on the induction of tolerance across a two-haploidentity MHC mismatch barrier. Preliminary data demonstrate that vascularized thymokidney grafts can induce tolerance across fully MHC-mismatched barriers in thymectomized recipients after a T cell deple- tion regimen. Because xenogeneic T cell reactivity between human and pig has been demonstrated to be at least as strong as that seen in allogeneic responses (42–44), the induction of T cell tolerance by this strategy may also prove to be of importance to the eventual success of xenotransplantation.

Acknowledgements
We thank Drs. David K. C. Cooper and Isabel McMorrow for their helpful review of the manuscript, Scott Arn for herd management and quality control typing, Joseph Ambroz for technical assistance, and Lisa A. Bernard for help in manuscript preparation. We would also like to thank Novartis for generously providing CyA and Schering-Plough Animal Health for providing Flunixamine.

References


