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Can Thymic Tissue Induce Tolerance to Kidney Allografts?

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ABSTRACT

Introduction: The aim of this study was to investigate the beneficial effect of donor thymic tissue to induce tolerance in *thymokidney* allografts, transplanted to thymectomized cross-bred canines.

Materials and Methods: Seven pairs of transplant donors and recipients were selected from 3- to 4-month-old cross-bred canines with major histocompatibility complex (MHC) mismatches. Recipients underwent partial thymectomy 4 weeks before transplantation and received an autologous thymic graft under the renal capsule, which had been engrafted in the donors 3 months before transplantation (thymokidney). Successful engraftment with evidence of thymocyte development in the donors was determined by gross and histologic examination at the time of transplantation. Biopsy specimens were obtained at the transplant day and 3 months after transplantation and were studied histologically for evidence of hyperacute or acute rejection.

Results: At 90 days after the operation, all 7 juvenile thymic grafts had developed with normal thymic structure under the renal capsule. Hyperacute rejection was not observed in allografts, and all of them were functioning until the end of follow-up; however, all of the allografts showed acute cell-mediated rejection 3 months after transplantation.

Conclusion: No tolerance was induced by vascularized donor thymokidneys in MHC-mismatched canines. The advantages of tolerance over chronic immunosuppression are so great that a potentially tolerogenic approach such as thymic transplantation would seem worthy of further investigations on large animal models. To evaluate the beneficial effects of thymic tissue in tolerance induction, utilizing a short course, low-dose adjuvant immunosuppressant to this regimen and/or application of in-bred MHC-matched canines is suggested.

KEY WORDS: kidney transplantation, thymus, thymokidney, allograft rejection, canine

Introduction

The thymus plays an important role in developing tolerance to alloantigens and is critical for tolerance to self-antigens,[1-5] in which 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]

The definition of *tolerance* in the context of transplantation is challenging. In simple terms, *transplantation tolerance* is the survival and

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function of a graft in the absence of continuing immunosuppression. Although this is only a functional definition that defines any particular mechanism as being responsible for the tolerant state, it may not be appropriate as multiple mechanisms are being increasingly found that can be used to promote the development and maintenance of tolerance to a defined set of antigens in vivo.[10] What is most important, is that the tolerant state be effective in practice, and that it allows the survival and function of a graft in the absence of a destructive immune response against the transplanted tissue.[10]

To transplant a donor's thymus as a composite, the *thymokidney* graft is a recently described method that creates a vascularized thymic graft by implanting autologous thymic tissue under the renal capsule.[11] Such thymokidneys have been able to reconstitute T cells and restore immunocompetency in pigs and mice.[9]

Previous tolerance-induced regimens to kidney allografts by thymic tissue have been found to be effective in rodents and miniature swine.[6-8] Transplanting a donor's thymus as a part of the vascularized organ graft in previously thymectomized major histocompatibility complex (MHC)-matched mice and miniature swine has allowed the thymus to function immediately after transplantation and to induce durable tolerance to the allograft.[6-8] Meanwhile, many of the methods used to induce tolerance in rodents have failed in large animals or human studies.[9] Thus, before testing new approaches clinically, it is necessary to examine these methods in large animal models.

In the present study, we investigate the effectiveness of thymic tissue for inducing tolerance to kidney allografts in fully MHC-mismatched canines.

Materials and Methods

Animals

Seven pairs of transplant donors and recipients were selected from 3- to 4-month-old cross-bred canines. The mismatched status of the pairs was confirmed by positive WBC cross-match test. Therefore, fully MHC-mismatched juvenile animals were used. The experimental protocols used in this study were approved by the committee on animal research at our institution.

Surgical procedure

All of the recipients underwent total thymectomy through cervical incision 4 weeks prior to the transplantation. Creation of the thymokidney in the donors was done 3 months before transplantation. Through a cervical longitudinal incision in the donors, approximately three fourths of the thymus was removed to provide autologous thymic tissue. It was minced into 2-mm3 to 3-mm3 pieces and put under the renal capsule of the left kidney, which was exposed through a flank approach. Thymic tissue was vascularized under the renal capsule for 3 months to create the thymokidney. To confirm successful engraftment, vascularization, and evidence of thymocyte development in the autologous thymic graft, biopsies of the thymokidney were performed before transplantation.

Thereafter, 7 thymokidneys were transplanted into the thymectomized juvenile full MHC-mismatched canines. The renal artery and vein of the donor were harvested with aortic and inferior vena cava patches, respectively; transferred to the recipient; and anastomosed end-to-side to the abdominal aorta and inferior vena cava of the recipient, respectively. Urinary drainage was accomplished via a ureterovesical anastomosis. At the first postoperative day, a biopsy of the allograft was obtained for any evidence of hyperacute rejection. All of the animals were fed appropriately and stored in a well-equipped animal house in a 12-hour light/dark stable with a well-controlled environment.

Three months after the transplantation, recipients were killed, and the thymokidney allograft was removed, fixed in formalin, and studied histopathologically for evidence of acute rejection. Before killing, multiple biopsies were taken from the cervical area and anterior mediastinum for any residual thymic tissue. No immunosuppression was administered to the recipients.

Results

Gross and histologic examinations of the thymokidneys showed that the thymic grafts were well vascularized by vessels from both the renal capsule and the renal parenchyma in all canines. They had normal thymic structures and thymocytes, 90 days postoperatively (Figure 1).

Hyperacute rejection was not detected in any of the allografts after transplantation, and gross and histologic examinations of the transplanted thymokidneys a few hours after transplantation

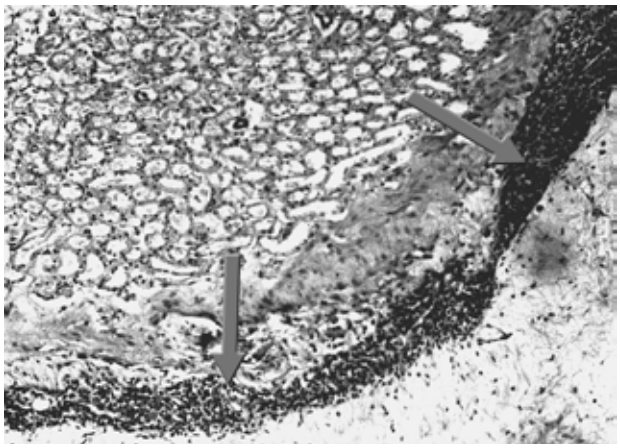

Fig. 1. Histopathology of the thymokidney. Note the normal thymic tissue (arrows) adjacent to the normal kidney tissue (hematoxylin-eosin × 40).

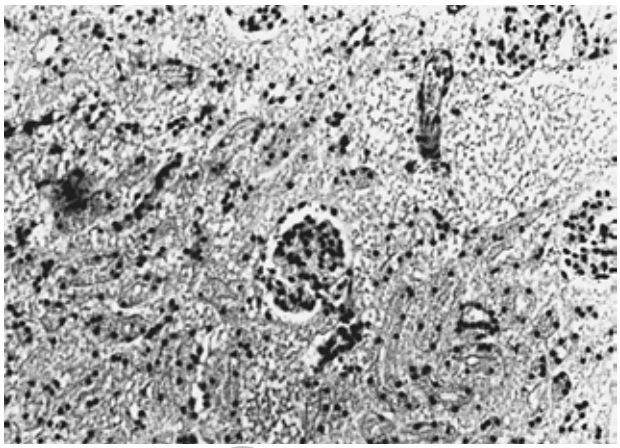

Fig. 2. Acute cell-mediated rejection in the thymokidney allograft. Leukocyte infiltration with evidence of tubular and glomerular destruction is noted (hematoxylin-eosin × 40).

showed normal kidney tissue.

Single native kidney was preserved in all recipients, and overall kidney function was normal at 3 months' follow-up, with acceptable plasma creatinine levels and urine output. However, all 7 allografts demonstrated evidence of acute cell-mediated rejection, including tubulitis, microvascular thrombosis, and vasculitis with destruction of glomerulus on histopathologic examination of the allograft (Figure 2).

Necropsy of the recipients and multiple biopsies from the cervical area and anterior mediastinum showed no residual thymic tissue.

Discussion

The phenomena of rejection and transplantation tolerance are related to each other, although they follow different immunologic mechanisms. One of the most important organs for inducing tolerance to allografts is the thymus. The most essential mechanism for inducing tolerance is clonal T-cell deletion, through which compatible T cells are developed by the thymus during the embryonic and infancy periods. After production of different T cells in the bone marrow, T cells are transferred to the thymus, where incompatible ones are removed by apoptosis. Therefore, thymic tissue seems to be the main physiologic organ for tolerance induction.

Lee and coworkers have used thymic tissue in thymectomized cell-depleted mice to induce tolerance to renal allografts. The same strategy has been applied for tolerance to skin grafts.[12,13] Placing the thymic tissue under the renal capsule and transferring the resultant composite organ are not only technically simpler than transferring the thymus and kidney as separate organs, but they also are effective in inducing tolerance to renal allografts.[6]

The following potential mechanisms have been suggested as inducing tolerance in swine:

1. T-cell progenitors may be positively or negatively selected by donor thymic stroma and/or dendritic cells by a mechanism similar to that of self tolerance.[2] Immunohistochemistry studies have demonstrated that donor-type dendritic cells remain for more that 3 months after 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 shown that thymic epithelial cells are capable of participating in both positive and negative selection of thymocytes[14,15] and of inducing anergy.[16] Thus, the long-term presence of donor stromal cells (donor epithelial cells and dendriticlike 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. Consistent with this hypothesis, it has been shown that new T cells, generated in thymectomized recipients receiving thymokidneys, are taught by both host and donor elements in the thymic graft.[6]
2. After thymectomy, recipients were not T-cell depleted, and mature T cells were present in the recipients of composite thymokidneys. However, mature peripheral T cells also have

been shown to become unresponsive to donor antigens by recirculation to the thymus.[17] Alloreactive T cells could enter the donor thymic graft and could be taught.

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 status or suppressive mechanisms.[18,19] However, thymic tissue transplants did not facilitate the induction of tolerance in canines.

There are several ways to look at these observations. One of the most important factors in graft rejection is the existence or lack of compatibility between the MHC antigens of donors and those of recipients. Whenever the donor and recipient are matched in class I MHC and class II MHC, or at least in class II MHC, tolerance will be induced spontaneously at the level of CD4+ helper T cells. Therefore, the main cells involved in rejection will be suppressed. Since CD4+ cells control the activity of CD8+ cytotoxic T cells through cytokines, tolerance at the level of CD4+ cells will suppress the function of CD8+ cells. In nearly all previous reports indicating the beneficial effect of thymic tissue in tolerance induction, the donor-recipient pairs were MHC-matched or at least matched in class II MHC antigens.[6-8] However, the most recent studies on tolerance have revealed that this phenomenon can be effective in MHC-mismatched pairs. Yamada and colleagues have shown that tolerance can be induced in miniature swine using composite thymokidney across fully MHC-mismatched barriers.[20] Also, Li and colleagues have indicated that donor bone marrow can induce tolerance to lung allografts in MHC-mismatched rats.[21] It seems that although tolerance-inducing protocols work better in MHC-matched pairs, they also can be effective in MHC-mismatched ones.

We used a canine model to evaluate the beneficial effect of thymic tissue in this species. The rejection phenomenon and recognition of self- from nonself-antigens varies among different species. A review of veterinary literature revealed that the rejection phenomenon is very severe in canines, since it is reported that despite applying matched donor-recipients and administering immunosuppressive therapy after transplantation, up to 60% of allografts will be lost owing to acute rejection. Given the different tolerance-inducing protocols in different animal models, we can conclude that to induce tolerance to a specific allograft, that these methods are more effective if the animal models are smaller (eg, rat, mice, and miniature swine), since tolerance has been achieved using more-simple protocols. Nevertheless, when we use large animal models with more complex immunological systems (as in the present study), adjuvant modalities (eg, use of biologic agents or immunosuppressive drugs) will be beneficial.

Despite the difficulty in inducing tolerance to allografts in larger animal models, it seems likely that investigations using these protocols in humans will be appropriate for humans in the future.

The interaction of immunosuppressive drugs on tolerance induction is one of the main challenges to allograft tolerance. In many of the previous studies in which thymic tissues have caused the induction of tolerance, immunosuppressive drugs had been used.[5-8] Cyclosporin A can effectively inhibit both the CD4 helper pathway and the direct CD8 helper pathway.[22] Immunosuppressives, even in low doses and/or for a short course, might block or inhibit development of rejection in the presence of thymic tolerance. On the other hand, contrary to the above-mentioned findings, recent studies have shown that calcineurin inhibitors (such as cyclosporin A), administered along with tolerance-inducing regimens, might block or inhibit the induction of tolerance. Experiments have revealed that tolerance to the alloantigens could not be induced in interleukin-2 knockout mice. With the administration of calcineurin inhibitors, interleukin-2 gene transcription would be inhibited. Therefore, tolerance might not be induced.[23] It also has been established that in the presence of calcineurin inhibitors, apoptosis and, in turn, clonal T-cell deletion (which is the major mechanism for tolerance induction) will be blocked.[24]

Something else must be taken into consideration: As discussed before, different immunosuppressive drugs in various animal models may have different effects on tolerance induction, considering the complexity of the immunologic system in these animals.

At present, the advantages or disadvantages of immunosuppressive drugs on tolerance remain to be proved.

Conclusion

Although the capability of thymic tissue to induce tolerance, with and without immunosuppression, is acceptable in small animal models such as mice, the thymus could not induce tolerance to kidney allografts in fully MHC-mismatched canines. This may be related to parameters such as which animal model is used, the MHC-mismatched status of donor and recipients, and the abandonment of immunosuppressive drugs. Owing to the strength of the immune system in large animal models and the difficulty in suppressing it, introduction of any novel strategy for tolerance induction into clinical practice will necessitate more investigations. To prove the potential effects of thymic tissue for tolerance induction, combining this approach with one or more immunosuppressive drugs at the time of transplantation and/or applying MHC-matched pairs is suggested. How clinically successful this approach would be remains unclear.

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