Mechanisms of tolerance to parental parathyroid tissue when combined with human allogeneic thymus transplantation

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Background: The induction of tolerance toward third-party solid organ grafts with allogeneic thymus tissue transplantation has not been previously demonstrated in human subjects.
Objective: Infants with complete DiGeorge anomaly (having neither thymus nor parathyroid function) were studied for conditions and mechanisms required for the development of tolerance to third-party solid organ tissues.
Methods: Four infants who met the criteria received parental parathyroid with allogeneic thymus transplantation and were studied.
Results: Two of 3 survivors showed function of both grafts but subsequently lost parathyroid function. They demonstrated alloreactivity against the parathyroid donor in mixed lymphocyte cultures. For these 2 recipients, parathyroid donor HLA class II alleles were mismatched with the recipient and thymus. MHC class II tetramers confirmed the presence of recipient $CD4^+$ T cells with specificity toward a mismatched parathyroid donor class II allele. The third survivor has persistent graft function and lacks alloreactivity toward the parathyroid donor. All parathyroid donor class II alleles were shared with either the recipient or the thymus graft, with minor differences between the parathyroid (HLA-DRB1*1104) and thymus (HLA-DRB1*1101). Tetramer analyses detected recipient T cells specific for the parathyroid HLA-DRB1*1104 allele. Alloreactivity toward the parathyroid donor was restored with low doses of IL-2.
Conclusion: Tolerance toward parathyroid grafts in combined parental parathyroid and allogeneic thymus transplantation requires matching of thymus tissue to parathyroid HLA class II alleles to promote negative selection and suppression of recipient T cells that have alloreactivity toward the parathyroid grafts. This matching strategy may be applied toward tolerance induction in future combined thymus and solid organ transplantation efforts. (J Allergy Clin Immunol 2010;126:814-20.)

Key words: *Allorecognition, anergy, class II, DiGeorge, parathyroid, tetramers, thymus, tolerance, transplantation*

Solid organ transplantation offers hope for the treatment of many diseases but continues to face significant challenges in preventing rejection of the graft by the recipient.[1] Alloreactivity by T cells toward foreign HLA molecules presents one of the most significant mechanisms for rejection of transplanted allogeneic tissues.[2,3] Recipient alloreactivity toward donor tissues can be modulated by positive and negative selection processes within the thymus.

Infants with complete DiGeorge anomaly (cDGA) offer an opportunity to study the role of the thymus in controlling allorecognition responses. DiGeorge anomaly results from abnormal embryonic development, leading to possible defects extending from the first to sixth pharyngeal arches.[4] Affected children present at birth with a spectrum of malformations involving the heart, parathyroid glands, and thymus.[5-10] Infants with cDGA lack naive ($CD45RA^+CD62L^+$)[11] T cells because of athymia, resulting in a severe primary immunodeficiency that is usually fatal as a result of infection by 2 years of age. Allogeneic thymus transplantation leads to immunoreconstitution and increased survival.[12,13] The thymus grafts provide an environment in which recipient thymocyte precursors undergo positive and negative selection and emerge in the circulation as functional naive T cells.

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Abbreviations used
APC: Allophycocyanin
cDGA: Complete DiGeorge anomaly
MLC: Mixed lymphocyte culture
PTH: Parathyroid hormone

Although the transplanted thymus tissues are not HLA matched to the subjects, the recipients demonstrate tolerance to the grafts.[14] Although thymus transplantation has shown success in correcting the immune defects in subjects with cDGA,[12] hypocalcemia caused by hypoparathyroidism remains an important cause of morbidity and mortality.[15]

Because of the importance of the thymus in the development of tolerance, we postulated that congenital athymia would provide a suitable model to assess the induction of tolerance to solid organ grafts when combined with thymus transplantation. We hypothesized that in subjects with cDGA, we could achieve tolerance toward parental parathyroid grafts in transplant protocols using cotransplanted allogeneic thymus tissue.

To assess tolerance in the recipients, we used a combination of traditional and novel methods. Traditionally, mixed lymphocyte cultures (MLCs) have assessed alloreactive T-cell proliferation toward donor cells caused by HLA class II differences,[16-18] which appear to contribute more to rejection than HLA class I mismatches.[19] However, these and other immune assays that show a lack of alloreactivity toward the donor have at times been questioned as markers for tolerance because of perceived insufficient specificity.[1,20] Newer technologies now offer the potential to directly visualize the presence of specific alloreactive T cells.[1,21,22] MHC tetramers consist of fluorescently labeled, multimeric MHC molecules of a defined specificity that can be loaded with oligopeptides.[23] As a result, tetramers of recipient MHC molecules containing donor HLA oligopeptides could identify the presence of recipient donor-specific alloreactive T cells.

Here we discuss these efforts to characterize tolerance and the factors associated with tolerance induction in recipients of allogeneic thymus tissue with solid organ transplantation.

METHODS

Subjects

All subjects were enrolled in clinical trials under a research protocol approved by the Duke Institutional Review Board. Informed consent for these studies and procedures was obtained from the parents of all thymus donors and transplant recipients, the parathyroid donors, and healthy adult control subjects. All recipients met the clinical and immunologic criteria for cDGA[4-6,8,10,12] with primary hypoparathyroidism (for more details, see the Methods section in this article's Online Repository at www.jacionline.org). They required the initiation of calcium supplementation shortly after birth and had multiple intact parathyroid hormone (PTH) levels measured near or less than the limit of detection before transplantation.

Donors

All thymus and parental parathyroid donors underwent donor screening, as previously described.[12,24,25] The parathyroid donors had normal intact PTH levels and were as follows: the mothers for subjects 1, 3, and 4 and the father for subject 2. For subject 3, parathyroid transplantation was delayed by 37 days after thymus transplantation because of postponed collection of the donor parathyroid gland.

Thymus and parathyroid transplantation

Thymus tissue was processed as previously described.[12,13,26] All recipients were given rabbit antithymocyte globulin and methylprednisolone before transplantation, as used in protocols for subjects with cDGA with immunosuppression.[12,27]

The cultured allogeneic thymus tissues were transplanted into each recipient's quadriceps muscles.[28] At the same time, the parathyroid donor underwent open exploration of the neck after achievement of general anesthesia in an adjacent operating room. After a parathyroid gland was located, the presence of parathyroid tissue was confirmed by means of histology. For further confirmation, a small amount of tissue was suspended in saline, yielding high levels of PTH on rapid testing (Elecsys PTH STAT Test; Roche, Zurich, Switzerland). The gland was then removed and sectioned. The parathyroid tissues were placed into the quadriceps muscle of the subject adjacent to the area used for thymus transplantation.

Routine immune and clinical assessments

Immune phenotyping by means of flow cytometry, lymphocyte proliferative responses to PHA and tetanus toxoid, and MLCs were performed according to standard protocols.[12,27] All MLCs were performed after 10% of the recipients' circulating T cells had the naive phenotype ($CD45RA^+CD62L^+$). PBMC proliferative responses were assessed by counts per minute of tritiated thymidine incorporation.

Intact PTH levels were measured according to the standard practices of the clinical laboratories at our institution and the referring institutions. The lower limits of normal for intact PTH levels at all facilities ranged from 10 to 15 pg/mL. The limit of detection for samples tested at our institution was 5 pg/mL.

High-resolution typing for HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 alleles was obtained as a part of routine clinical testing. HLA-DQA1 and HLA-DPB1 analyses were performed by the Duke Clinical Transplantation Immunology Laboratory using a Luminex (Austin, Tex) reverse sequence specific oligonucleotide multiplex bead assay and Invitrogen (Carlsbad, Calif) sequence specific primer kit, respectively. Standard panel-reactive HLA antibody screening was conducted by the Duke Clinical Transplantation Immunology Laboratory for subjects 1, 3, and 4 (at 3.3, 2.5, and 2.2 years after transplantation, respectively).

Assessments for alloantigen-specific T cells

MHC class II tetramers were created to assess for recipient allospecific T cells by means of flow cytometry. "Positive" tetramers consisted of recipient HLA-DR molecules loaded with parental parathyroid donor oligopeptides from the HLA-DRB1 allele not shared with the recipient (see example in Fig E1 in this article's Online Repository at www.jacionline.org). Positive tetramers were constructed (Beckman Coulter Tetramer Synthesis Facility, Fullerton, Calif) for subject 1 (amino acids 62-82 of HLA-DRB1*0701 bound within HLA-DRB1*0101 tetramers) and subject 4 (amino acids 71-90 of HLA-DRB1*1104 loaded into HLA-DRB1*0301 tetramers) (see Table E1 in this article's Online Repository at www.jacionline.org). Tetramers could not be successfully generated for subject 3. For "negative" tetramers, which were used to assess the specificities of the HLA oligopeptides, the tetramer molecules contained an antigenically irrelevant oligopeptide from the human class II–associated invariant chain peptide instead.[29] A detailed description of the tetramer synthesis process is provided in the Methods section of this article's Online Repository.

Tetramer staining of subjects' PBMCs was performed according to the manufacturer's (Beckman Coulter) instructions. In brief, 10^6 PBMCs were incubated with either positive or negative phycoerythrin-conjugated tetramers for 90 minutes at 37°C. Two percent murine serum (Jackson ImmunoResearch Laboratories, West Grove, Pa) was added for another 30 minutes at 37°C to block nonspecific staining of surface antibodies. Surface antibodies to CD3 (allophycocyanin [APC]-Cy7 conjugated; BD Biosciences, San Jose, Calif) and CD4 (fluorescein isothiocyanate conjugated, Beckman Coulter) were applied. An additional mix of APC-conjugated antibodies to CD8, CD13, CD14 (all from BD Biosciences), CD16 (BioLegend, San Diego, Calif), CD19, and CD56 (both from Beckman Coulter) was added to label nonrelevant cell populations. The cells were then further incubated for 20 minutes and washed