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Thymectomy Does Not Abrogate Long-Term Acceptance of MHC Class I-Disparate Lung Allografts

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Abstract

Background— We have previously reported that tolerance to class I disparate lung allografts in miniature swine could be induced using an intensive 12-day course of tacrolimus and that pre-transplant sensitization with immunogenic MHC class I alloptides failed to block the induction of tolerance. We also have previously reported the importance of the presence of the thymus in the induction of tolerance to isolated heart, kidney, and combined heart-kidney transplants. In this study, we examined the impact of thymectomy on tolerance induction.

Methods— Orthotopic left lung transplantation was performed using MHC class I-disparate donors. The recipients received a 12-day course of high-dose tacrolimus ($n = 6$). Total thymectomies were performed in three of the swine, 21 days prior to transplantation. Lung grafts were monitored by chest radiography and serial open lung biopsy.

Results— All euthymic recipients maintained their grafts over one year. None of the thymectomized recipients has shown graft loss in the six to ten months following transplantation. Although isolated lesions of obliterative bronchiolitis (OB) were occasionally seen in one thymectomized animal on biopsy, donor-specific unresponsiveness has been observed on assays of cell-mediated lymphocytotoxicity (CML) in all recipients. Moreover, co-culture CML assays have shown that recipient lymphocytes can strongly inhibit the normally robust response of naïve recipient-matched lymphocytes to donor antigen. This inhibition was not seen when using stimulators primed with third-party antigens against appropriate targets.

Conclusions— These data suggest that thymus-independent peripheral regulatory mechanisms may be sufficient to induce and maintain long-term acceptance of the lung allografts.

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INTRODUCTION

We have previously reported that tolerance to class I disparate lung allograft could be induced using an intensive 12-day course of tacrolimus in miniature swine¹ and that pre-transplant sensitization with immunogenic MHC class I allopeptides failed to block the induction of tolerance². These long-term acceptors developed donor-specific cellular and humoral unresponsiveness. However, this *in vitro* cellular unresponsiveness could be reversed by donor class-I matched, class-II mismatched skin grafting without concomitant pulmonary graft rejection². These data suggest that clonal deletion was not the main immunologic mechanism of long-term graft acceptance, and that anergy or peripheral regulation might be the principal mechanism of the tolerance observed in this model. Previous studies in miniature swine in our laboratory have shown that a functioning thymus is required for the induction of tolerance to isolated kidney³, heart⁴, and combined heart-kidney⁵ transplants. In this study, we examine the impact of pre-transplant thymectomy on tolerance induction in a preclinical, large-animal model model.

METHODS

Animals

Transplant donors and recipients were selected from our herd of partially inbred miniature swine at 4–7 months of age. The immunogenetic characteristics of this herd have been described previously⁶. Swine lymphocyte antigen (SLA)^{gg} (class I^c/II^d) donors and class I disparate homozygous SLA^{dd} (class I^d/II^d) recipients were used to achieve a 2-haplotype class I mismatch. Genotyping has been controlled by strict pedigree breeding and confirmed by microcytotoxicity testing using allospecific antisera. All animal care and procedures were in compliance with the "Principles of Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (revised 1996).

Experimental Groups

Twelve swine were used in this study to conduct 6 class I-disparate orthotopic left lung allografts. Tacrolimus (Fujisawa, Deerfield, IL) was administered to all recipients for 12 consecutive days, starting on the day of transplantation (0.15 mg/kg/day, as a continuous IV infusion; target level = 35 to 50 ng/ml). Three recipients underwent complete thymectomy, 21 days prior to lung transplantation.

Complete Thymectomy in the Recipient

Complete thymectomies were performed as previously described³. Briefly, the pretracheal muscles were retracted, exposing the cervical thymus and trachea from the cervicothoracic junction to the mandibular area. The cervical thymus was excised, and then the mediastinal thymus was removed through a sternotomy.

Lung Transplantation

Orthotopic left lung transplantation was performed as previously described⁷. Briefly, under general anesthesia, two indwelling silastic central venous catheters were placed into external jugular veins in order to facilitate tacrolimus administration and frequent blood sampling for *in vitro* assays and for monitoring of whole-blood tacrolimus levels. The recipient's chest was entered through a left thoracotomy, and the hilar structures of the native lung were isolated. After heparinization (300 U/kg), the native lung was removed. The donor lung was approached through a median sternotomy. After heparinization (300 U/kg), the heart–lung block was

topically cooled with iced saline and flushed in situ with 4 L of cold Euro-Collins solution (Fresenius Medical Care AG, Bad Homburg, Germany) containing prostaglandin E₁ (500 µg/L). Immediately after harvesting, the left lung was surgically prepared and transplanted.

Rejection Monitoring

In all experiments, lung allografts were monitored for rejection by a combination of daily physical examination, serial chest radiography and open lung biopsies. The timing of the open lung biopsies was protocol-driven. Immune responses were monitored with cell-mediated lympholysis (CML). Graft loss (our principal endpoint) is defined as high-grade histologic rejection in association with loss of graft aeration as observed on chest radiograph, and/or loss of graft perfusion or compliance as observed intra-operatively at the time of open lung biopsy.

CML Assays

Primary CML assay was performed as described previously⁸. Secondary co-culture CML assay was described in detail previously⁸. Briefly, this assay was performed in two sequential phases, consisting of a priming phase (day 0–6), and a co-culture phase (day 7–13). In the priming phase, 4×10^6 peripheral blood lymphocytes (PBLs) drawn from experimental or control animals were first primed for 6 days with 4×10^6 stimulator cells (irradiated with 2500 rads) at 37 °C in 7.5% CO₂. After 6 days, the cells were harvested and rested overnight at 4×10^6 cells/mL in CML media at 37 °C in 7.5% CO₂. These cells were then used as the experimental or control population in a co-culture CML assay.

RESULTS

As previously reported¹, all euthymic animals accepted their lung grafts for over one year. Likewise, none of the thymectomized recipients have yet to reject their grafts (>307, >272, >168 days; experiment in progress). In the thymectomized recipients, only minimal mononuclear cell infiltration has been seen (Figure 1) although one thymectomized recipient showed isolated lesions of obliterative bronchiolitis (OB) on the post-operative day (POD) 150 biopsy. However, similar lesions have not been observed on subsequent biopsies. All thymectomized recipients demonstrated significant *in vitro* anti-donor cytotoxic activity (>30% percent specific lysis) prior to transplantation and exhibited donor-specific hyporesponsiveness, after transplantation. In co-culture CML assays, PBLs from long-term lung acceptors that were primed with donor antigen completely suppressed the CTL response of naïve recipient-matched PBL to donor antigen. This inhibition was not seen when using third party-primed stimulators with appropriate targets.

CONCLUSIONS

In conclusion, this study demonstrates that thymus-independent mechanisms may be sufficient to induce and maintain long-term acceptance of the lung allograft in tacrolimus-treated MHC class I mismatched pulmonary allografts. Moreover, a state of *in vitro* donor-specific hyporesponsiveness and the generation of thymus-independent regulatory T cells, as seen in the co-culture CML assays, suggest that the differs from the heart and kidney in its ability to induce strong peripheral regulation. Further experiments are required to determine the nature of these putative regulatory cells, and to test whether these findings can be extended to a fully allogeneic model.

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Figure 1.
Histology of lung allografts from thymectomized swine #16639 on POD 250; no mononuclear cell infiltration (H&E staining, $\times 100$).