The Limits of Linked Suppression for Regulatory T Cells

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Background: We have previously found that CD4⁺CD25⁺ regulatory T cells (Tregs) can adoptively transfer tolerance after its induction with costimulatory blockade in a mouse model of murine cardiac allograft transplantation. In these experiments, we tested an hypothesis with three components: (1) the Tregs that transfer tolerance have the capacity for linked suppression, (2) the determinants that stimulate the Tregs are expressed by the indirect pathway, and (3) the donor peptides contributing to these indirect determinants are derived from donor major histocompatibility complex (MHC) antigens (Ags).

Methods: First heart transplants were performed from the indicated donor strain to B10.D2 recipients along with costimulatory blockade treatment (250 μg i.p. injection of MR1 on day 0 and 250 μg i.p. injection of CTLA-4 Ig on day 2). At least 8 weeks later, a second heart transplant was performed to a new B10.D2 recipient who had been irradiated with 450 cGy. This recipient was given 40 × 10⁶ naive B10.D2 spleen cells + 40 × 10⁶ B10.D2 spleen cells from the first (tolerant) recipient. We performed three different types of heart transplants using various donors.

Results: (1) Tregs suppress the graft rejection in an Ag-specific manner. (2) Tregs generated in the face of MHC disparities suppress the rejection of grafts expressing third party MHC along with tolerant MHC.

Conclusion: The limits of linkage appear to be quantitative and not universally determined by either the indirect pathway or by peptides of donor MHC Ags.

Keywords: costimulation, indirect pathway, MHC class II, tolerance, regulatory T cells

INTRODUCTION

The physiologically unusual stimulation of T cells by donor antigen-presenting cells (APCs) has been called “direct” recognition, whereas stimulation by self-APCs, presenting peptides of donor origin, has been called “indirect” recognition. Direct recognition has been believed to be the major pathway involved in allograft rejection due to three basic observations, namely, (1) direct stimulation is very strong in a primary allogenic mixed lymphocyte reaction, (2) depletion of donor APCs can

Abbreviations: Ags, antigens; APCs, antigen-presenting cells; MHC, major histocompatibility complex; MST, median survival time; Tregs, regulatory T cells.
sometimes prolong allograft survival, and (3) donor major histo-
compatibility complex (MHC) antigens (Ags) are more important
than minor Ags in causing graft rejection (1). Matching for MHC
Ags achieves better allograft survival. Lechler and Batchelor
showed the importance of MHC class II matching compared
to MHC class I matching at least in the long-term survival (2).
However, there are several remarkable reports of consequences
of T cells responding via the indirect pathway. These reports
showed the indirect pathway (a) helps for priming alloreactive
CD8 T cells (3, 4), (b) is essential for tolerance induction in some
models (5, 6), and (c) is involved in chronic transplant rejection
(7, 8). In addition, several papers have shown the importance of
an indirect response in allograft rejection (1, 3, 9, 10). Indirect
allorecognition contributes not only to acute graft rejection
(2, 9) but also possibly to the continuing response to the allograft
in the long term after transplantation (11). Previously, we tested
the role of costimulatory blockade for prolonging allograft sur-
vival with using class II-deficient mice when only one or the other
pathway of graft rejection was available. We found that to achieve
long-term survival after costimulatory blockade requires that
the recipient expresses MHC class II molecules (12). This result
indicated that indefinite cardiac transplant survival could not
be achieved in the absence of an intact indirect pathway. These
results are consistent with the fact that at least a component of
the regulatory T cell (Treg) response must involve recognition
of peptides of donor Ags presented by recipient MHC molecules.

![Diagram](image-url)

**FIGURE 1** | (A) Allograft survival in B10.D2 recipients: (>100 days, n = 5, p = 0.0017 compared to control: 16.2 days, n = 5). (B) Adoptive transfer model: naive splenocytes transfer (n = 6), both naive and Tol. splenocytes transfer (100 days, n = 6, p = 0.0007). (C) Linkage model: B6 hearts (>100 days), B10.BR hearts (~23 days, n = 6, p = 0.0006) [B6 x B10.BR] F1 (>100 days, n = 4/5, p = 0.0044).
METHODS AND RESULTS

First, we made B10.D2 (H-2d) mice tolerant to B6 (H-2b) with costimulatory blockade [250 μg intraperitoneal (i.p.) injection of MR1 on day 0 and 250 μg i.p. injection of CTLA-4 Ig on day 2] (Figure 1A). At least 8 weeks later, a second heart transplant was performed to a new B10.D2 recipient who had been irradiated with 450 cGy. All recipient received intravenous (i.v.) injection of naive 40 × 10⁵ splenocytes + 40 × 10⁵ splenocytes that are taken from the tolerant mice (tolerized splenocytes: Tol.) significantly prolonged graft survival compared to recipient received only naive splenocyte (12 ± 1 days compared to >100, p < 0.001) (Figure 1B). After these results, we considered linkage of Tregs. Next, we performed a second transplant from B6 mice to irradiated B10.D2 mice. The second donores express the same MHC and minor Ags as the first graft or B10.BR heart grafts differ from the first graft in their MHC Ags or (B6 × B10.BR) F1 mice, which express both H-2b and H-2d Ags. After transplant, the mice received i.v. injection of naive and tolerized splenocytes. All B6 hearts survived over 100 days. But B10.BR hearts expressing third party MHC were rejected by 23 days (Figure 1C). (B10.BR × B6) F1 hearts expressing third party MHC with tolerant MHC showed 80% survival of over 100 days; however, CAV was observed in some specimen. The institutional subcommittee on research animal care at Massachusetts General Hospital approved all animal experiments.

REFERENCES


CONCLUSION

The very limited comparison in this experiment will determine whether the patterns of gene expression can reliably distinguish a regulatory population from one that promotes rejection.

AUTHOR CONTRIBUTIONS

TU, TI, and AT participated in the performance of the research, performed the data collection, performed the statistical analysis, and contributed to the writing of the manuscript; IB, MY, and MM participated in the writing of the manuscript and performed review; and MS and TU designed the study and participated in review. AC participated in review.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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