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CD4+ T-CELL RECEPTOR TRANSGENIC T CELLS ALONE CAN REJECT VASCULARIZED HEART TRANSPLANTS THROUGH THE INDIRECT PATHWAY OF ALLOANTIGEN RECOGNITION

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The vast array of epitopes presented by allografts and the diversity of T cells responding to them complicates mechanistic studies of rejection. To minimize these problems, we developed a transgenic (Tg) model system limited to a single T-cell receptor (TCR)/peptide/major histocompatibility complex molecule. Two alloantigen-specific CD4 T-cell clones were used to isolate cDNA encoding the TCRα and TCRβ chains that recognize the K^d_{54-68}/I-A^b epitope. Two different TCR Tg lines were produced in C57BL/6 (B6) mice and crossed onto the B6.Rag1$^{-/-}$ background. B6.Rag1$^{-/-}$ recipients of T cells from TCR Tg Rag1$^{-/-}$mice promptly rejected B10.D2, but not irrelevant B10.BR, cardiac grafts. Thus, a single allogeneic epitope presented by self-major histocompatibility complex class II is sufficient to activate TCR Tg T cells and serve as a target for rejection.

It has long been recognized that the molecules of the major histocompatibility complex (MHC) are particularly potent for the induction of allograft rejection, but multiple other "minor" histocompatibility antigens are also capable of inducing rejection. Organ rejection is distinct from other immune responses in that both donor and recipient antigen presenting cells (APCs) can stimulate recipient T cells. Stimulation of recipient T cells by donor APCs, which is referred to as the "direct" allorecognition pathway, was initially believed to be the primary type of antigen recognition driving the rejection of organ allografts. Appreciation of the peptide-binding function of the MHC led to the idea that "minor histocompatibility antigens" represent allograft-derived peptides that are presented by self-MHC molecules of recipient APCs. This process is termed "indirect" allorecognition. The relative quantitative contribution of these two pathways and whether they differ in a qualitative way, however, remain unclear.

One problem in determining the relative contribution of the direct and indirect pathways is the inherent complexity of the antigen in organ allografts. To address this problem, we produced transgenic (Tg) mice, which express $\alpha\beta$ T-cell receptor (TCR) from one of two independent CD4+ T-cell clones that react with the K^d_{54-68}/I-A^b epitope (*1*). IKB3/4 uses Vα11.2/Jα25 and Vβ13/Jβ1.6 genes, whereas IKB7/5 uses Vα1.1/Jα33 and Vβ8.3/Jβ1.4 genes as the functional TCR (see supplementary data). Functionally rearranged TCR Vα/Jα and Vβ/Jβ chains were cloned from phage libraries and ligated into TCR Vα and Vβ shuttle vectors (see supplementary data). B6 ova were injected with equimolar amounts of TCR Vα and Vβ DNAs that had been mixed with a 5.0 Kb DNA construct from the 5' end of the human CD2 locus, which contains locus control elements that promote integration-site independent expression of transgenes in T lineage cells (*2*). One founder mouse was identified that expressed each of the TCRα and TCRβ pairs by both polymerase chain reaction analysis and expression of the Tg TCR by CD4 T cells.

T cells expressing non-Tg TCRs were eliminated by crossing the TCR transgenes onto mice with targeted disruption of the Rag1 gene. Flow cytometric analysis shows that virtually all of the peripheral cells with light scatter characteristics of lymphoid cells express the specific Tg TCRs (Fig. 1). In both TCR Tg lines, the majority of the lymphoid cells are CD4+CD8−, like the original T-cell clones. However, the TCR/CD3 complex is not uniformly expressed; some cells of the B6.Rag$^{-/-}$.TCR34 mice have a low expression pattern without a sharp distinction in receptor density. In addition, both Rag1$^{-/-}$ TCR Tg lines have a small fraction (~0.5%) of CD4−CD8+ cells that express the TCR Tg, which increases as the mice age (data not shown). The few remaining cells within the lymphoid cell fraction are natural killer lineage cells (NK1.1+) and erythroid cells (Ter119+, not shown). As expected, no B cells (CD19+) were detected in these mice.

The peptide specificity of T cells from B6.Rag1$^{-/-}$.TCR34 and B6.Rag1$^{-/-}$.TCR75 mice was determined by proliferation after stimulation with synthetic peptide or different populations of APCs (Fig. 2). In the absence of endogenous APCs, these T cells were unresponsive to B10.D2 spleen cells. However, in the presence of I−A^b+ APCs there was a vigorous response to both the synthetic K^d_{54-68} peptide and B10.D2 spleen cells but not to B10.BR (H-2^k) spleen cells. Thus, TCR Tg T cells recognize the I-A^b/K^d_{54-68} epitope, and the amount of K^d released from dead spleen cells in vitro is sufficient for cross-presentation of the functional K^d_{54-68} peptide by endogenous APCs. Thus, these TCR Tg T cells recognize B10.D2 through the indirect pathway of alloantigen recognition.

To determine whether these Tg T cells alone were sufficient to mediate rejection of cardiac allografts through the indirect pathway, heterotopic cardiac allografts were transplanted into B6.Rag1$^{-/-}$ mice (*3*). Transfer of 1×10^5 of B6.Rag1$^{-/-}$.TCR34 or B6.Rag1$^{-/-}$.TCR75 Tg T cells induced uniform rapid rejection of B10.D2 but not allogeneic B10.BR hearts (Fig. 3A). Thus, both of these two TCR Tg T

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cells can mediate rapid rejection of cardiac grafts through the indirect pathway in the complete absence of antibody, CD8 T cells, and CD4 T cells of other specificities. Rejection correlated with cardiac infiltration by $CD4^+$ B6.Rag1$^{-/-}$.TCR Tg T cells (Fig. 3B). Preliminary data indicate that 1×10^5 TCR Tg T cells also caused rejection under more physiologic conditions when transferred into Rag-1 sufficient recipients (not shown).

These observations verify previous reports that CD4 T cells from Rag1$^{-/-}$ mice expressing a Tg TCR with specificity for the H-Y antigen presented by MHC class II independently mediated the rejection of skin allografts (*4*). However, Sayegh et al. (*5*) recently reported that TCR Tg mice with CD4 Bm12 mutant MHC class II specific cells reject skin grafts but not heart allografts. With the use of TCR Tg mice with CD8 T cells specific for an H-Y peptide/H-2^b epitope, Valujskikh et al. (*6*) demonstrated that these mice rejected male H-2^k skin grafts but not heart allografts. Because these TCR Tg T cells cannot directly recognize any cells derived from the donor tissue, these authors suggested that direct recognition of recipient endothelial cells may account for this distinction between skin and heart allograft rejection. However, Kreisel et al. (*7*) demonstrated that TCR Tg mice with positively selected CD8 T cells specific for H-2K^b (direct alloantigen) can reject hearts, even in the absence of any bone marrow-derived cells capable of H-2K^b expression in the heart allograft. Because these studies (*5–7*) did not use TCR Tg mice with endogenous polyclonal TCR expression inhibited by a Rag mutation, it is difficult to clearly determine the specific antigenic requirements for transplant rejection from these apparently conflicting results.

In contrast with the previously described TCR Tg systems, our studies demonstrate that two independent CD4 TCR Tg T cells, when crossed onto the Rag1$^{-/-}$ background to eliminate any other TCR expression, are sufficient to mediate rapid rejection of a cardiac allograft through the indirect pathway. However, the precise mechanism of the

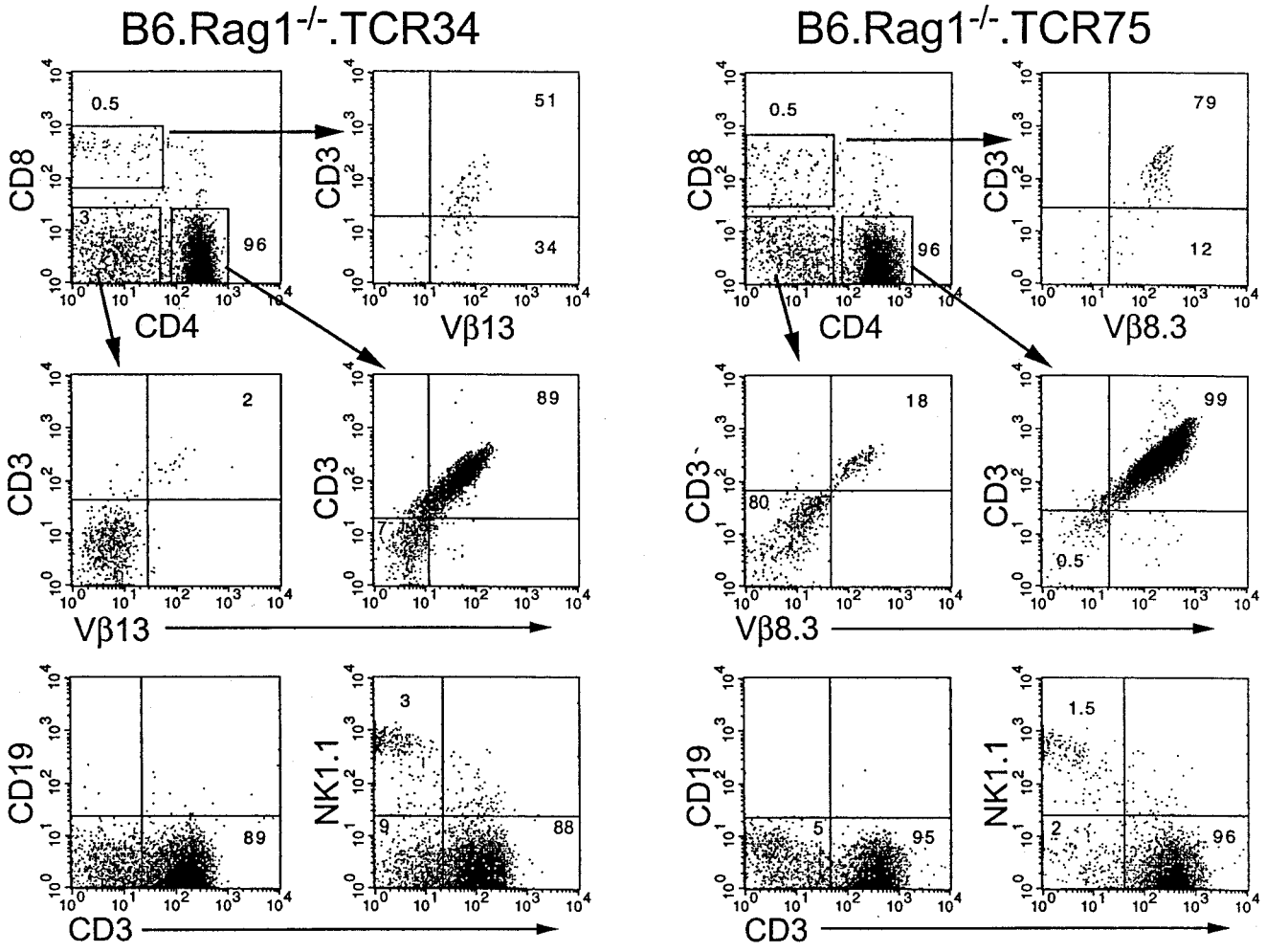

FIGURE 1. Phenotype of lymph node (LN) cells from B6.Rag1$^{-/-}$TCR34 and B6. Rag1$^{-/-}$TCR75 transgenic (Tg) mice. LN cells were stained with fluorescein isothiocyanate-anti-Vβ8.3 (1B3.3, Pharmingen, San Diego, CA), fluorescein isothiocyanate-anti-Vβ13 (MR12–3, Pharmingen), allophycocyanin-anti-CD3 (145–2C11, Pharmingen), phycoerythrin-anti-CD4 (GK1.5, F. Fitch, University of Chicago, Chicago, IL), biotinylated-anti-CD8 (53–6.72, ATCC, Manassas, VA), biotinylated-anti-CD19 (1D3, Pharmingen), and biotinylated-anti-NK1.1 (PK136, Pharmingen). Streptavidin-RED670 (Invitrogen Corporation, Carlsbad, CA) was used as a secondary reagent to detect biotinylated antibodies. Analysis was performed using a FACScan (Becton Dickinson, Mountain View, CA) or FACSCalibur (Becton Dickinson) flow cytometer.

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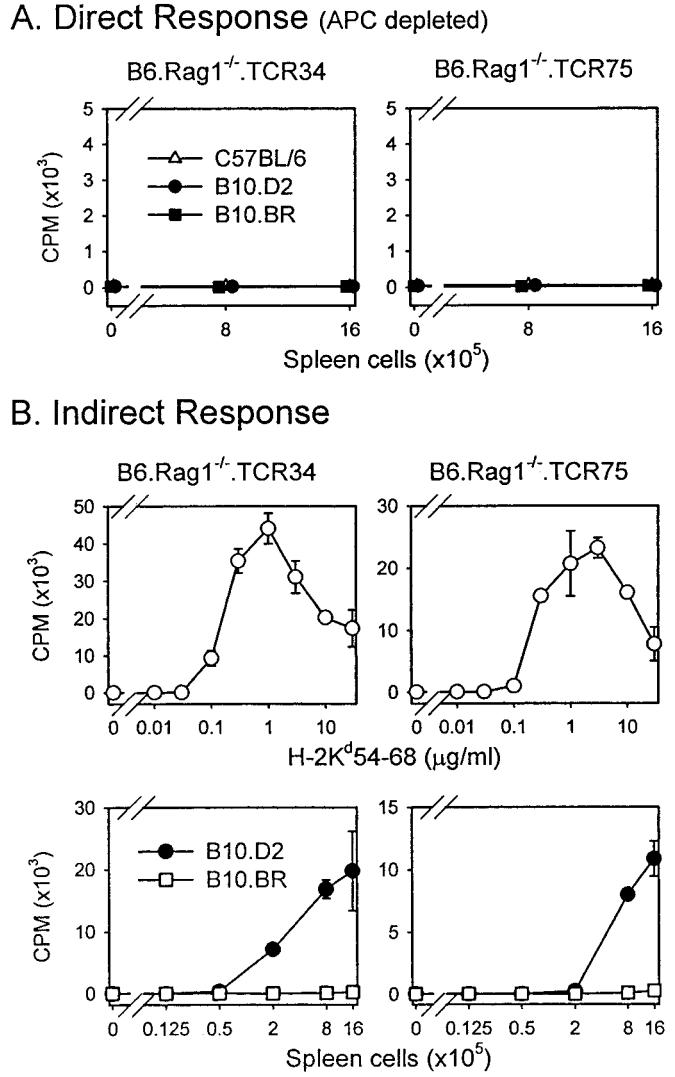
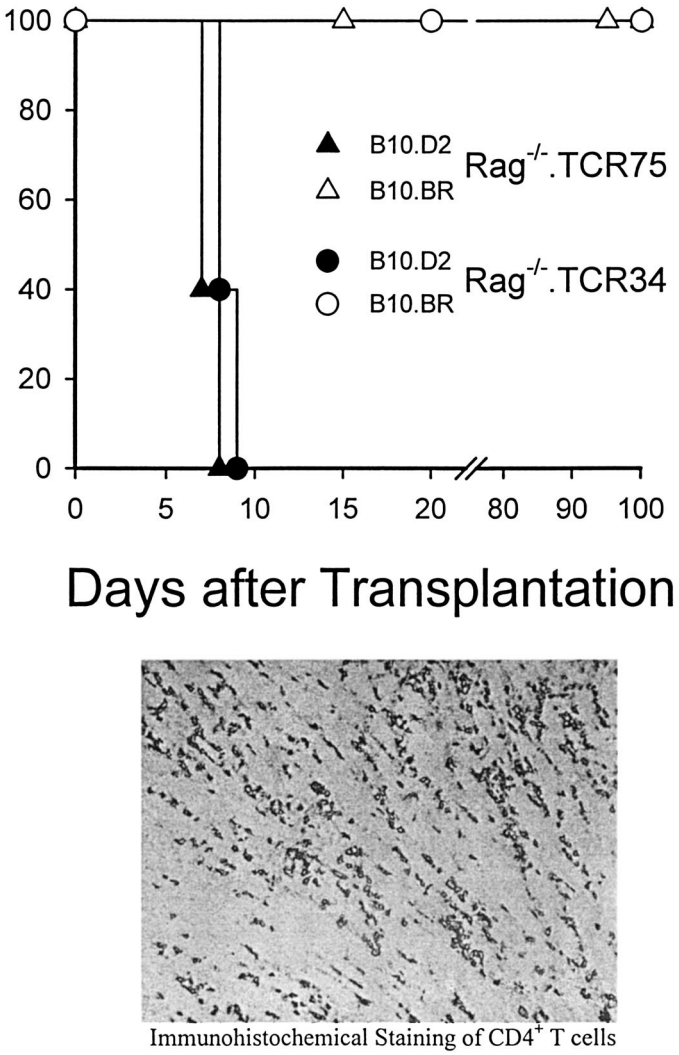


FIGURE 2. Specificity of T cells in B6.Rag1$^{-/-}$TCR34 and B6.Rag1$^{-/-}$TCR75 Tg mice. (A) Direct response of LN cells to allogeneic and third-party antigens. LN cells, depleted of endogenous antigen presenting cells (APCs) by adherence to plastic and magnetic bead removal of major histocompatibility complex (MHC) class II+ and CD11c+ cells, were incubated at 1×10^4 cells per well with irradiated spleen cells from normal syngeneic B6, allogeneic B10.D2, or third-party B10.BR mice. (B) LN cells from TCR Tg mice Rag1$^{-/-}$ mice (containing endogenous APCs) were stimulated with different doses of K^d_{54-68} peptide or irradiated spleen cells.



Immunohistochemical Staining of CD4$^+$ T cells

FIGURE 3. Survival of heterotopic vascularized heart transplants in B6.Rag1$^{-/-}$ mice that had received 1×10^5 of the indicated TCR Tg spleen cells 1 day before transplant. (A) Donor hearts from B10.D2 (*solid symbols*) or B10.BR (*open symbols*) mice were transplanted using the technique of Ono and Lindsey (*3*). Briefly, the donor aorta was anastomosed in end-to-side fashion to the recipient abdominal aorta, and the pulmonary artery was similarly anastomosed to the vena cava. Hearts were palpated daily; no detectable beating was presumed to be rejection, which was confirmed by laparotomy. (B) CD4$^+$ T cells detected in the interstitium and perivascular areas of the B10.D2 heart 7 days after transplantation into a Rag1$^{-/-}$ mouse given 1×10^5 Rag$^{-/-}$.TCR75 T cells.

effector response within the allograft is unclear. Infiltration of the allograft with host-derived APCs followed by stimulation of cytokine production from these TCR Tg T cells is most likely because these cells are not capable of direct TCR/peptide MHC-mediated lysis of any donor-derived cells. Experiments are currently in progress to quantify the relative role of rejection mechanisms initiated by the indirect pathway versus those mediated by the hybrid pathway in which donor-derived APCs syngeneic with the recipient MHC class II can also present allopeptides.

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