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**CD4⁺ CD45RB Low-Density Cells from Untreated Mice Prevent Acute Allograft Rejection**

Joanna D. Davies, Eric O’Connor, DeShon Hall, Troy Krahl, Joseph Trotter, and Nora Sarvetnick

In the absence of therapy that suppresses the action of the immune system, the immune response to transplantation Ags results in rapid rejection of the transplant. The most successful mechanism so far described that achieves organ-specific immunological tolerance is that which controls peripheral tolerance to self-tissue. Until now, no similarities have been documented between the peripheral response to self-Ags and the response to transplantation Ags. CD4⁺ cells that express a high density of CD45RB (in the mouse) and CD45RC (in the rat) on their surface have been shown to cause a number of autoimmune disorders. In contrast, autoimmunity caused by the CD45RB high-density cells is inhibited by CD4⁺ CD45RB cells that express a low density of CD45RB (CD45RC in the rat). In this paper we show that CD4⁺ CD45RB high-density cells are sufficient to cause rejection of a MHC-mismatched pancreas allograft, whereas CD4⁺ CD45RB low-density cells are not. Unexpectedly, the CD45RB low-density cells prevent the CD45RB⁹⁺ expressing cells from causing rejection. These data suggest that the response to foreign tissue can be controlled in the same way as the response to self-tissue. The Journal of Immunology, 1999, 163: 5353–5357.

CD45RB is a transmembrane protein tyrosine phosphatase expressed on leukocytes and involved in T lymphocyte activation (23). Its density on T lymphocytes has been used to separate T cells into functionally distinct subsets that have either pathological or nonpathological activity (10–16). In addition, although not an absolute marker for the distinction of T cells into Ag-experienced (memory, CD45RBlow) cells and Ag-inexperienced (naive, CD45RB⁹⁺) cells, in combination with two other cell surface molecules, CD62L and CD44, it has been used to identify such subsets (24–28). We have investigated the possibility that CD4⁺ CD45RBlow cells are sufficient to cause allograft rejection and that CD4⁺ C57BL/6 mice are capable of inhibiting that rejection process.

**Materials and Methods**

**Mice**

C57BL/6/J (C57BL/J, H-2b) adult mice and BALB/cByJ (BALB/c, H-2b) neonates were obtained from the Scripps Breeding Colony (La Jolla, CA). C57BL/6-SCID/SJl (C57BL/6-SCID, H-2b) mice were purchased from The Jackson Laboratory (Bar Harbor, ME).

**Grafting of neonatal pancreata**

Pancreata of BALB/c (H-2b) neonates were grafted under the kidney capsules of C57BL/6-SCID males (H-2b).

**Histological assessment**

Grafts were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections (4 μm) were stained with hematoxylin and eosin for histological examination or were tested for the presence of insulin by immunohistochemistry as described (29). The area encompassed by islets and cellular infiltration was measured using a Leica Image Processing and Analyzing System (Q500 MC; Leica, Cambridge, U.K.). Statistical analysis was performed using Instat version 2.0 for Macintosh. Data were analyzed using ANOVA, then the Bonferroni Multiple Comparison Test (30). A p value ≤0.05 is considered significant.

**Cellular infiltration**

In some experiments mice will receive two grafts. The first is given at the time of the cell infusion; the second is given 6 wk later. For first grafts, those that were scored positive for cellular infiltrates were infiltrated in 75–100% of the tissue. Those grafts that were scored negative for cellular infiltrates showed 0% cellular infiltration. For second grafts, those that
were scored positive for cellular infiltrates showed infiltration in 12–100% of the graft. Grafts which scored negative for cellular infiltrates showed 0% infiltration.

Cell purification

Spleen cells from 2- to 4-mo-old C57BL/6 male mice were prepared for single cell suspensions. RBC were removed with lysing buffer (Sigma, St. Louis, MO), and the remaining spleen cells were resuspended in PBS with 1% FBS (Intergen, Purchase, NY). Splenocytes were labeled with a PE-conjugated CD4-specific mAb, GK1.5 (Becton Dickinson, Mountain View, CA) (31), and a FITC-conjugated CD45RB-specific mAb, MB23G2 (PharMingen, La Jolla, CA) (32), and sorted under high speed on a FACS-Vantage SE with TurboSort (Becton Dickinson Immunocytometry Systems, Mountain View, CA). The CD45RB<sup>high</sup> and CD45RB<sup>low</sup> populations are defined as the brightest-staining 30% and dullest-staining 15% of the CD<sup>+</sup> cells, respectively. All cell populations were sampled and analyzed using FACSCalibur with CELLQuest version 3.2 software (Becton Dickinson Immunocytometry Systems, Mountain View, CA). The CD45RB<sup>high</sup> and CD45RB<sup>low</sup> populations were used to identify CD4<sup>+</sup> cells that express CD45RB, which they express. The figure shows the fluorescence intensity of CD4<sup>+</sup> cells stained with FITC-labeled CD45RB-specific mAb. a shows the fluorescence intensity of CD45RB staining before sorting, and b shows the fluorescence intensity of the purified CD45RB<sup>high</sup> and CD45RB<sup>low</sup> cells.

Results

CD<sup>4</sup><sup>+</sup> CD45RB<sup>high</sup> but not CD<sup>4</sup><sup>+</sup> CD45RB<sup>low</sup> cells reject a pancreas allograft

To determine whether CD45RB<sup>high</sup> cells can cause the rejection of a pancreas allograft, C57BL/6-J-SCID mice were grafted with a pancreas from a 1-day-old BALB/c donor and infused with 0.25 × 10<sup>6</sup> CD4<sup>+</sup> CD45RB<sup>high</sup> cells, 0.25 × 10<sup>6</sup> CD4<sup>+</sup> CD45RB<sup>low</sup> cells, or no cells. The grafts were assessed histologically, and the area encompassed by islets and cellular infiltration was determined. C57BL/6-J-SCID mice that were infused with CD4<sup>+</sup> CD45RB<sup>high</sup> cells rejected 70% of the allografts within 3 wk after transplantation (Fig. 2a). The grafts contained massive cellular infiltration (Fig. 2b) and a reduction in islet area compared with the group that received no cells (Fig. 2c). In contrast, only 1 of 10 of the grafts harvested from mice that received an equal number of CD4<sup>+</sup> CD45RB<sup>high</sup> cells displayed any signs of rejection (Fig. 2d). As expected, none of the grafts (0 of 7) from mice that did not receive a cell infusion showed signs of rejection (Fig. 2e). The infusion of fewer CD<sup>4</sup><sup>+</sup> CD45RB<sup>high</sup> cells (0.125 × 10<sup>6</sup>) resulted in a much lower incidence of allograft rejection (data not shown). In contrast, the infusion of 0.5 × 10<sup>6</sup> CD45RB<sup>high</sup> cells resulted in a similar incidence of rejection as with 0.25 × 10<sup>6</sup> cells (data not shown).

Because we were interested in determining whether rejection could be inhibited by CD4<sup>+</sup> CD45RB<sup>low</sup> cells, we have decided to use the minimum number of cells required to give a consistently high incidence of rejection. This number is 0.25 × 10<sup>6</sup>. Therefore, in subsequent experiments 0.25 × 10<sup>6</sup> CD4<sup>+</sup> CD45RB<sup>high</sup> cells will be used.

CD<sup>4</sup><sup>+</sup> CD45RB<sup>low</sup> cells prevent CD<sup>4</sup><sup>+</sup> CD45RB<sup>high</sup> cells from rejecting a pancreas allograft

Having established that CD4<sup>+</sup> CD45RB<sup>low</sup> cells were substantially less efficient at causing allograft rejection than CD45RB<sup>high</sup>
cells, we determined whether the CD45RB<sup>low</sup> population had the capacity to prevent the CD45RB<sup>high</sup> population from rejecting foreign tissue. The experiment was set up in the same way as above but with an additional group infused with both CD45RB<sup>high</sup> cells and CD45RB<sup>low</sup> cells. A 1:1 ratio of high (0.25 × 10<sup>6</sup>) to low (0.25 × 10<sup>6</sup>), and a 2:1 ratio of high (0.25 × 10<sup>6</sup>) to low (0.125 × 10<sup>6</sup>) were used with similar results. Only the data for the 2:1 ratio are shown. Pancreas allografts in mice that had received a mixture of CD45RB<sup>high</sup> and CD45RB<sup>low</sup> cells (Fig. 2d) were morphologically similar to those grafts in the groups that received CD45RB<sup>low</sup> cells only (Fig. 2b) or no cells (Fig. 2c); that is, no signs of rejection were evident. Pancreas grafts from mice that had received CD45RB<sup>high</sup> and CD45RB<sup>low</sup> cells displayed a significant increase in islet area compared with grafts from mice that had received CD45RB<sup>high</sup> cells only (p < 0.05). Therefore, these data suggest that CD4<sup>+</sup> cells expressing low levels of CD45RB are able to prevent allograft rejection mediated by CD45RB<sup>high</sup> cells.

Pancreas allografts in recipients of a CD4<sup>+</sup> CD45RB<sup>high</sup> and CD45RB<sup>low</sup> cells are tolerant of graft Ags at 10 wk after grafting

The data shown thus far are taken at 3 wk after grafting. Therefore, it was important to determine whether allograft protection mediated by CD45RB<sup>low</sup> cells was maintained for longer periods and whether the recipient was then rendered tolerant of graft Ags. Tolerance was tested using the classical method of placing a second graft into the recipient. Specifically, C57BL/6-SCID recipients were infused with a 1:1 ratio of CD45RB<sup>low</sup> and CD45RB<sup>high</sup> cells, low-density cells only, or no cells. The first graft was placed 1 day before the cell infusion. The second graft, also a pancreas graft from a BALB/c donor, was placed under the contralateral kidney 6 wk later, and subsequently all grafts were removed for histologic examination after an additional 4 wk (see Fig. 3 for time course of treatment).

The first pancreas allografts from mice that received no cells showed no signs of rejection 10 wk after grafting (Fig. 4a). This was also true of the grafts taken from mice that received CD45RB<sup>low</sup> cells only (Fig. 4b) or CD45RB<sup>low</sup> and CD45RB<sup>high</sup> cells (Fig. 4c). Specifically, cellular infiltration was absent in all grafts from those three groups. In addition, islet area was not significantly different between groups and was comparable to the islet area seen in the single grafts described in Fig. 2. These data suggest that allograft survival was maintained long term.

The islet area in the second grafts from mice that received no cells (Fig. 4d) or CD45RB<sup>low</sup> cells only (Fig. 4e) were similar to grafts in mice in the groups that received both CD45RB<sup>low</sup> and CD45RB<sup>high</sup> cells (Fig. 4f). Therefore, the protective effect of the CD45RB<sup>low</sup> cells on the response of the CD45RB<sup>high</sup> cells seen in the long term appears to be mediated by inducing functional tolerance at the whole animal level. Despite the fact that the islet area

![Diagram of protocol](http://www.jimmunol.org/Download)
in the second grafts was maintained, cellular infiltration was observed in 100% of second grafts (Fig. 4f) but not first grafts (Fig. 4c) from the mice that had received the mixture of CD45RB<sup>high</sup> and CD45RB<sup>low</sup> cells.

Discussion
We have shown that the CD<sup>+</sup> CD45RB<sup>high</sup> cell subset is sufficient for pancreas allograft rejection. In contrast, those CD<sup>+</sup> cells that express a low density of the CD45RB molecule do not reject a pancreas allograft and protect such allografts from rejection by CD<sup>+</sup> CD45RB<sup>high</sup> cells. This protection lasts for at least 10 wk and functional tolerance is shown in the protected recipients by the finding that second grafts in these recipients are also not rejected.

Our data confirm the finding that the CD<sup>+</sup> CD45RB<sup>low</sup> cell subset is a regulatory cell subset capable of preventing pathological responses. It also extends these findings to show that the regulatory cells do not distinguish between self-Ag and foreign MHC Ags. It also extends these findings to show that the regulatory subset is a regulatory cell subset capable of preventing pathogenic mediators (16, 34–35).

Effects might occur through cell-mediated events or through soluble mediators (16) that might affect the effector capacity of the graft-Ag-specific CD45RB<sup>high</sup> cells. This protection lasts for at least 10 wk and functional tolerance is shown in the protected recipients by the finding that second grafts in these recipients are also not rejected.

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