Video Article

Accelerated Type 1 Diabetes Induction in Mice by Adoptive Transfer of Diabetogenic CD4+ T Cells

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Abstract

The nonobese diabetic (NOD) mouse spontaneously develops autoimmune diabetes after 12 weeks of age and is the most extensively studied animal model of human Type 1 diabetes (T1D). Cell transfer studies in irradiated recipient mice have established that T cells are pivotal in T1D pathogenesis in this model. We describe herein a simple method to rapidly induce T1D by adoptive transfer of purified, primary CD4+ T cells from pre-diabetic NOD mice transgenic for the islet-specific T cell receptor (TCR) BDC2.5 into NOD.SCID recipient mice. The major advantages of this technique are that isolation and adoptive transfer of diabetogenic T cells can be completed within the same day, irradiation of the recipients is not required, and a high incidence of T1D is elicited within 2 weeks after T cell transfer. Thus, studies of pathogenesis and therapeutic interventions in T1D can proceed at a faster rate than with methods that rely on heterogenous T cell populations or clones derived from diabetic NOD mice.

Video Link

The video component of this article can be found at https://www.jove.com/video/50389/

Introduction

The NOD mouse develops autoimmune diabetes spontaneously and has been widely used as an animal model for human T1D[1,2]. Pathogenesis of T1D in NOD mice is characterized by infiltration, beginning at 3-4 weeks of age, of the pancreatic islets of Langerhans by dendritic cells and macrophages, followed by T and B cells. This phase of non-destructive peri-insulitis leads to a slow, progressive destruction of insulin-producing pancreatic β cells, resulting in overt diabetes by 4-6 months of age[3]. Transfer of splenocytes[4,5], CD4+[6,7] or CD8+[8,9] T cells from diabetic NOD mice have been shown to mediate diabetes in immunocompromised NOD mice, indicating that islet-reactive T cells play a central role in T1D pathogenesis. Depending on the experimental conditions, diabetes developed in recipient mice slowly, over several weeks in these studies. Similarly, various T cell clones, derived by time-consuming and costly culturing of diabetogenic T cells, have been reported to mediate diabetes several weeks after transfer into recipient mice[7,10]. With the availability of transgenic mice expressing TCRs derived from CD4- or CD8-restricted diabetogenic T cell clones, several laboratories have subsequently shown that splenic T cells from such mice were able to transfer diabetes to recipients[11-13]. Specifically, BDC2.5 NOD mice are transgenic for the BDC2.5 TCR, which is specific for chromogranin A, a protein in pancreatic beta cells[14-16]. Transfer of *in vitro*-activated or un-activated whole or fractionated spleen cells from overtly diabetic or prediabetic BDC2.5 mice transferred diabetes to neonatal or immunodeficient NOD mice at varying efficiencies[11,17-19].

We describe a simple method that utilizes purified transgenic CD4+ T cells from pre-diabetic BDC2.5 mice to induce T1D in recipient mice at high efficiency and consistency. Large numbers of naive, islet antigen-specific CD4+ T cells are isolated from these mice by fluorescence-activated cell sorting (FACS) for CD4+CD62L+ T cells expressing the transgenic TCR Vβ4 chain. Purified transgenic T cells are then transferred without activation into NOD.SCID mice, which lack functional T and B cells and are insulitis- and diabetes-free[20]. The recipient mice are monitored for elevated concentrations of urine glucose indicating T1D, which develops rapidly within two weeks after the T cell transfer.

In contrast to other methods that transfer diabetogenic T cells with heterogenous specificities, our protocol uses FACS-sorted CD4+ T cells that almost exclusively express the diabetogenic BDC2.5 TCR. Due to their homogeneity, only small numbers of transferred T cells (~1x10^6 cells/mouse) are required for rapid T1D development within 2 weeks at 100% incidence. Another advantage of our protocol is that irradiation of recipient mice is not necessary as it is for some other methods. A potential limitation of this method is that it does not allow the investigation into the contribution of both CD4 and CD8 T cell subsets or specifically CD8 T cells in diabetes.

The described protocol will be useful for studying rapid T1D development, mediated by naïve, monospecific CD4+ T cells, as well as therapeutic strategies to intervene in homing of islet antigen-specific Th cells to the target organ.

Protocol

1. Isolation of T Cells from Spleen and Lymph Nodes of BDC2.5 Mice

1. Use 6-week-old pre-diabetic female BDC2.5 mice as donors of diabetogenic CD4+ T cells. Mice should be diabetes-free as determined by urine glucose measurement (see below).
2. Euthanize each mouse using CO_2 asphyxiation and remove the spleen, axillary and brachial lymph nodes under sterile conditions. To remove the spleen, soak the fur with 70% ethanol, then cut and retract skin. The spleen will be visible as a dark red organ on the left side of the mouse. Make a 1-inch incision in the peritoneum using the small scissors and gently grasp the spleen in the center with a pair of small tweezers. Carefully trim the connective tissue and attached fat as much as possible and remove the spleen.
3. Collect the spleens and lymph nodes in 10 ml Dulbecco's-Modified Eagle's Medium (DMEM) in a 15 ml conical tube on ice.
4. Prepare a single cell suspension by using the end of a sterile 10 ml syringe plunger to gently press the lymphoid organs through 70 µm cell strainers (one spleen per strainer and 4-6 lymph nodes per strainer) into the same 50 ml conical tube.

During the process, rinse each strainer with 1 ml DMEM several times to maximize the recovery of cells from the strainer.

5. Transfer the collected cells from the 50 ml conical tube into a 15 ml conical tube and centrifuge at 300-400 x g for 7 min at RT.
6. To lyse red blood cells, discard the supernatant, re-suspend the cells in 5 ml Ammonium-Chloride-Potassium (ACK) buffer (pH 7.2), and incubate the ACK-cell suspension at RT for 5 min.
7. Add 10 ml of DMEM to the ACK-cell suspension and centrifuge the tube as above. Wash the cell pellet once in 10 ml DMEM.

2. Fluorescence-activated Cell Sorting of Diabetogenic CD4+ T Cells from BDC2.5 Mice

1. Re-suspend cells in 5 ml FACS staining buffer and count the number of viable cells (using a phase contrast microscope and a hemocytometer) by trypan blue dye exclusion.
2. Using FACS buffer, adjust the cell suspension volume to 5 x 10^7 cells/ml. Remove ~1 x 10^6 cells per staining control (1 no stain, 3 single stains). Stain the sample for non-activated transgenic CD4+ T cells with anti-CD4 (APC), anti-TCR Vβ4 (FITC) and anti-CD62L (PE) monoclonal antibodies (mAb) in a 15 ml tube. Perform cell staining using the mAb concentrations suggested by the manufacturer for 20-30 min at 4 °C in the dark.
3. Wash the sample and single stain controls with FACS buffer at >3X the staining reaction volume. Centrifuge the tube at 300-400 x g, remove the supernatant, and re-suspend the cells in FACS buffer (1-2 x 10^7 cells/ml for sorting sample and 300 µl for single stain controls) and store on ice until the next step.
4. Pass the cell sample through a 35 µm cell strainer cap tube to remove cell clumps. Sort the samples for CD4+TCR Vβ4+CD62L+ cells on a cell sorter with a trained operator. Collect the sorted cells into 3 ml DMEM in a 15 ml tube. Allow 2.5-3 hr, including setup, to sort 1.5 x 10^8 cells (the approximate number of cells obtained from 3 donor mice) at a sort rate of 2 x 10^4 total events/sec. Expect ~2.5 x 10^6 non-activated transgenic CD4+ T cells per mouse after cell sorting.
5. Record the absolute number of sorted cells at the end of the sort and centrifuge them for 7 min at 300-400 x g. Discard the supernatant and re-suspend the cells (2 x 10^6 cells/ml) in sterile phosphate-buffered saline (PBS) (Mg^{2+}/Ca^{2+} free) for the adoptive T cell transfer.

3. Adoptive Transfer of Diabetogenic CD4+ T Cells from BDC2.5 Mice

1. Using a 1 ml syringe and an 18-1½ gauge needle, gently re-suspend the FACS-purified CD4+ T cells and load the 1 ml syringe. In preparation for injection, replace the 18-1½ gauge needle with a 27½ gauge needle.
2. Expose 6-8 week-old female NOD.SCID recipient mice to a heating lamp until they exhibit grooming behavior.
3. Restrain recipient mice in a restrainer and wipe their tail with 70% (v/v) ethanol to disinfect the injection site.
4. Inject 1-2 x 10^6 FACS sorted cells/mouse (in up to 500 µl PBS) into either of the lateral tail veins.

Do not force the plunger. If the needle is located appropriately in the vein, the injection will take place with almost no resistance.

4. Monitoring Recipient Mice for Hyperglycemia and T1D

1. Beginning five days after the T cell transfer, NOD.SCID recipient mice are monitored daily for elevated urine glucose using reagent strips (Bayer Diastix) according to the manufacturer's instructions. Mice with two consecutive urine glucose readings >250 mg/dl are considered diabetic.

Representative Results

Our results show the isolation of transgenic BDC2.5 cells expressing CD62L, which is critical for T cells to home to secondary lymphoid organs such as pancreatic lymph nodes. Our findings further demonstrate the potent ability of this monospecific T cell population to transfer rapidly and efficiently T1D to NOD.SCID recipient mice.

Isolation of diabetogenic CD4+ T cells from BDC2.5 mice is shown in **Figure 2**. Approximately 5 x 10^7 cells from pooled spleen and lymph nodes were obtained per donor mouse before cell sorting. Following the flow cytometric sort, ~2.5 x 10^6 naive transgenic CD4+ T cells (CD4+TCR Vβ4+ CD62L+) were obtained from each BDC2.5 donor mouse by using the indicated gating strategy.

As expected, adoptive transfer of small numbers of CD4+TCR Vβ4+CD62L+ cells (~1 x 10^6 cells/mouse) from BDC2.5 donor mice induced T1D in all NOD.SCID recipients (100% incidence) by day 11, as determined by elevated levels of urine glucose (**Figure 3**). In comparison, transfer of heterogeneous CD3+ BDC2.5 T cells required 3-5 fold more cells to induce T1D in 80% NOD.SCID recipients by 3 weeks [21].

These data demonstrate that adoptive transfer of small numbers of FACS-purified BDC2.5 transgenic CD4+CD62L+ T cells into NOD.SCID mice induced T1D more rapidly and efficiently.

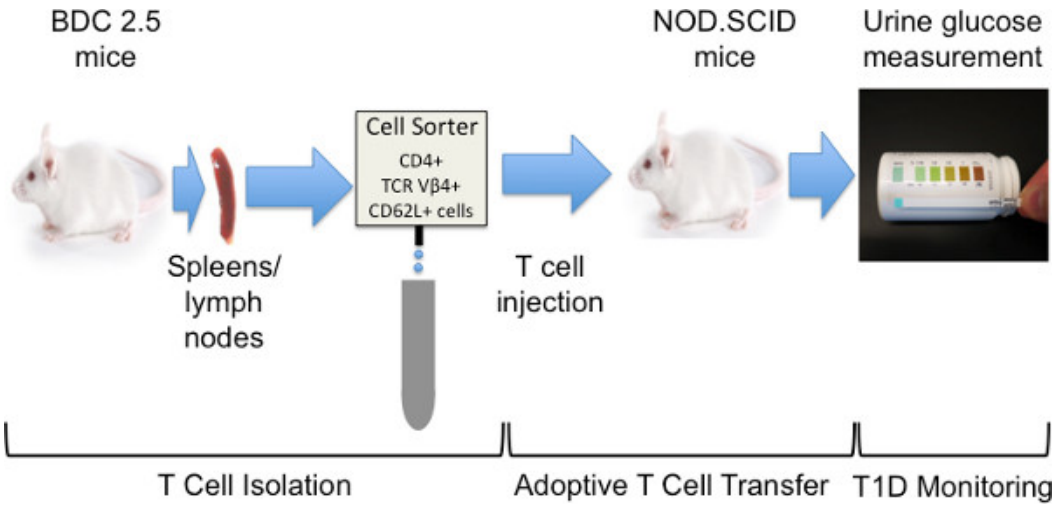

Figure 1. Sequence of experimental events. Spleen and lymph nodes were collected from BDC2.5 mice. Naive transgenic CD4+T cells (CD4+TCR Vβ4+CD62L+) were isolated by FACS. T cells were re-suspended in PBS and injected intravenously into NOD.SCID recipients, which were subsequently monitored for elevated urine glucose indicating T1D.

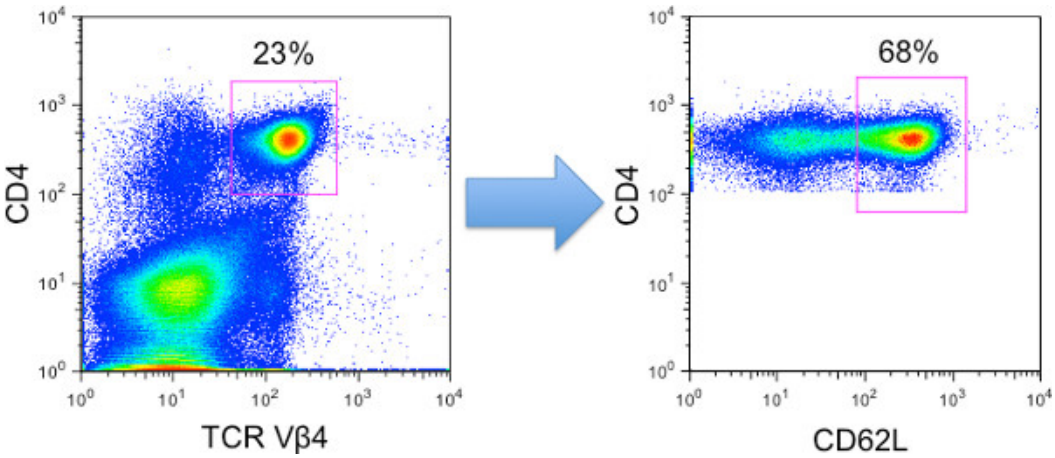

Figure 2. Gating strategy to sort CD4+TCR Vβ4+CD62L+ spleen and lymph node-derived cells from BDC2.5 mice by FACS. A single-cell suspension of pooled spleens and lymph nodes was stained with fluorescently-labeled anti-CD4 (APC), anti-TCR Vβ4 (FITC), and anti-CD62L (PE) mAb. The dot plot on the left shows the CD4+TCR Vβ4+ cell population that was used to gate the CD62L+ cells, shown in the right dot plot. Boxed cells represent percentages of gated cell populations.

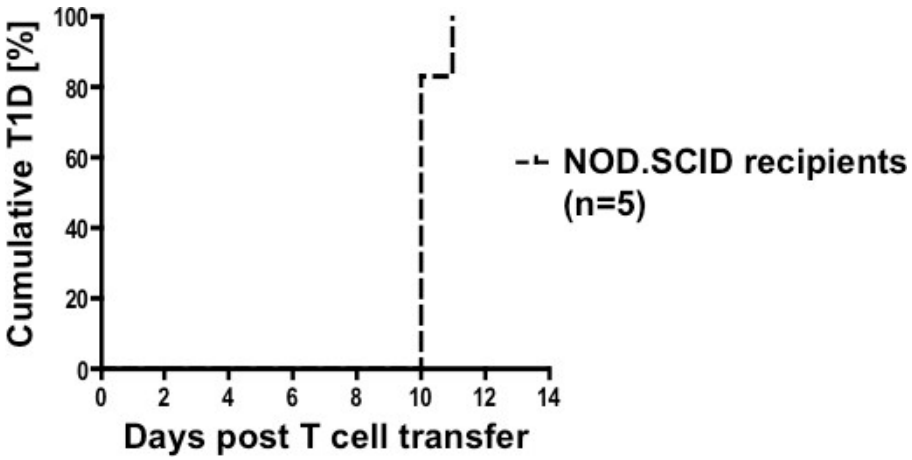

Figure 3. Adoptive transfer of diabetogenic CD4+ T cells. FACS-isolated CD4+TCR Vβ4+CD62L+ cells from BDC2.5 mice were intravenously injected (1.3 x 10^6 cells/mouse) into NOD.SCID recipient mice (n=5). Mice were monitored for T1D by measuring urine glucose concentrations in the recipients. Mice with two consecutive readings of >250 mg/dl were considered diabetic.

Discussion

T1D can be induced in recipient mice at varying efficiencies by adoptive transfer of whole spleen cells or T cell subsets from diabetic NOD mice or mice transgenic for TCRs derived from diabetogenic T cell clones. We report herein a reproducible method to induce T1D in recipient mice within two weeks at 100% incidence by transferring FACS-purified CD62L+ BDC2.5 transgenic CD4+ T cells into NOD.SCID mice.

Specific advantages of the BDC2.5 T cell transfer model described here include the very short induction time of T1D compared to months for spontaneous diabetes and up to several weeks for diabetes transfer by diabetogenic T cell subsets or T cell clones. In addition, due to their homogeneity, only small numbers of purified BDC2.5 transgenic T cells are required to transfer T1D with high efficiency and consistency. In contrast to some other T cell transfer methods, *in vitro* activation of transgenic BDC2.5 T cells prior to transfer is not necessary in our protocol. Another advantage of our monospecific T cell transfer method is that novel therapeutic strategies to intervene in the homing of islet antigen-specific T cell to the target organ can be investigated more selectively than with other protocols that transfer diabetes with heterogenous T cell populations.

A potential limitation of our method is that it is not suited to determine the contribution of both CD4+ and CD8+ T cell subsets in T1D pathogenesis because monospecific, diabetogenic CD4+ T cells are used to transfer diabetes.

In the absence of a FACS instrument, BDC2.5 transgenic T cells may be isolated by column-purification using PE-labeled anti-TCR Vβ4 mAb and anti-PE magnetic beads followed by CD4+CD62L+ magnetic beads (Miltenyi).

It should be noted that FACS-purified, as well as column-purified T cells, will include a minor population of CD4+ T cells that do not express the transgenic TCR due to incomplete allelic exclusion of endogenous TCR genes[15]. The purified T cell fraction may also include CD4+CD25+ Treg cells that may suppress T1D development in recipients. Since both T cell populations have been reported to increase in BDC2.5 mice with age, we recommend using BDC2.5 mice that are not older than 6 weeks[22,23]. CD4+CD25+ Treg cells can alternatively be excluded from BDC2.5 T cells by FACS-sorting or separation with CD4+CD25+ magnetic beads (Miltenyi) in older BDC2.5 mice.

Alternatively, or in addition to T1D monitoring by urine glucose measurements, blood glucose levels can be determined more accurately in recipient mice using a handheld glucometer.

Critical steps within this protocol include the age of BDC2.5 donor and NOD.SCID recipient mice. Using young BDC2.5 mice (6 weeks) will ensure that they are diabetes-free and that the frequency of both endogenous non-transgenic T cells and Treg cells are low, as pointed out above. It is also important to use young (<12 weeks) NOD.SCID mice as recipient mice because this strain is prone to developing thymomas that manifest themselves after 20 weeks of age[24] and may confound T1D development following adoptive T cell transfer.

Future applications of the described method include investigation in CD4 T cell-mediated mechanisms of T1D pathogenesis and novel strategies to intervene selectively in disease induction, which is not feasible in humans.

Disclosures

All mice were housed at the Penn State College of Medicine specific pathogen-free (SPF) facility in accordance with the guidelines of the Penn State Institutional Animal Care and Use Committee.

The authors declare that they have no competing financial interests.

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