

Adoptive Transfer of Autoimmune Diabetes Using Immunodeficient Nonobese Diabetic (NOD) Mice

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Abstract

Studying Type 1 Diabetes (T1D) in the nonobese diabetic (NOD) mouse model can be cumbersome as onset of disease does not usually occur naturally prior to the age of 12–14 weeks and is often restricted to female mice. Furthermore, the onset of disease occurs at random, which makes studying T1D in statistically meaningful cohorts of NOD mice a challenge. Transfer models of T1D into immunodeficient mice, such as NOD SCID mice, allows the study of potential therapeutic interventions in larger cohorts of animals, over shorter periods of time. In this chapter we discuss the adoptive transfer of diabetes into immunodeficient mice on the NOD genetic background that are generally available to the research community.

Keywords: Intravenous cell transfer, CD8 T cells, Adoptive transfer models of type 1 diabetes

1 Introduction

The nonobese diabetic (NOD) mouse is a good model for human type 1 diabetes (T1D), as it develops autoimmune diabetes with some features that are very similar to human type 1 diabetes. CD4 and CD8 T cells, as well as B cells, are important in the development of diabetes. These cells infiltrate the islets of Langerhans and the insulin-producing β cells are damaged and destroyed, with diabetes occurring between 12 and 35 weeks of age [1]. One of the hallmarks of the autoimmune disease is that diabetes can be adoptively transferred by splenocytes from diabetic NOD mice into immunodeficient NOD.SCID mice [2]. Islet antigen-specific CD4 T cells such as BDC2.5 cells recognizing chromogranin A [3–5], CD8 T cells such as G9 cells recognizing insulin B chain 15–23 (B_{15–23}) [6, 7] or NY8.3 cells recognizing islet-specific glucose-6-phosphatase catalytic-subunit-related protein (IGRP) [8–10] can also transfer diabetes. Whereas splenocytes ($1\text{--}2 \times 10^7$ cells) extracted and purified from diabetic NOD mice are easily transferred directly *ex vivo*, in our experience, antigen-specific CD8 T cells taken from T cell receptor (TCR) transgenic mice generated using the TCR from highly diabetogenic CD8 T cell clones require prior activation for reliable adoptive transfer of diabetes. We discuss

the protocol of adoptive transfer of autoreactive islet antigen-specific CD8 T cells.

2 Materials

All mice are housed in specific pathogen-free conditions on a 12 h light–dark cycle, were fed autoclaved food and filtered water and kept in cages either individually ventilated or in isolator cages, ventilated with filtered air. All experimental procedures should be performed in accordance with ethically approved institutional protocols for animal research.

2.1 Mice

1. NOD mice are used as donors of bone marrow to generate dendritic cells
2. NOD SCID mice are used for intravenous (IV) cell transfers between the ages of 4 and 6 weeks.
3. CD8 T cell receptor (TCR) G9C α –/– transgenic mice were generated expressing a specific TCR recognizing insulin B_{15–23}, which was previously cloned from highly diabetogenic G9C8 cells, and are referred to as G9 mice [6].
4. CD8 TCR transgenic mice, recognizing IGRP_{206–214} [10], are available from the Jackson Laboratories and are referred to as 8.3 mice.
5. Donor spleen cells for adoptive transfer are harvested between the ages of 4 and 12 weeks. 8.3 mice are tested for glycosuria and excluded for in vitro stimulation if they are found to be diabetic (blood glucose >13.9 mmol/l).

2.2 Dendritic Cells

1. Bone marrow from 6- to 12-week-old NOD mice are collected and cultured with 1.5 ng/ml GM-CSF (in house supernatant from X63-GM-CSF cells) followed by 1 μ g/ml LPS 026:B6 (Sigma) activation to generate mature dendritic cells (DCs).

2.3 Peptides and Reagents

1. G9 CD8 cells are activated with 1 μ g/ml insulin B_{15–23} (LYLVCGERG) peptide, supplied as a lyophilized powder from GL Biochem Ltd, Shanghai. The peptide is reconstituted at 5 mg/ml in DMSO (*see Note 1*) (10 % of total volume) followed by saline (90 % of total volume). Aliquots are stored at –20 °C.
2. 8.3 CD8 cells are activated with 10 ng/ml IGRP_{206–214} (VYLKTNVFL) peptide, supplied as a lyophilized powder from GL Biochem Ltd, Shanghai. The peptide is reconstituted at 5 mg/ml in DMSO (*see Note 1*) (10 % of total volume) followed by saline (90 % of total volume). Aliquots are stored at –20 °C.

3. Complete RPMI medium: RPMI1640 containing 2 mM L-glutamine, 100 U/ml penicillin/streptomycin, 50 μ M 2-mercaptoethanol, and 5 % FBS.
4. Magnetic activated cell sorting (MACS) buffer: PBS (phosphate buffered saline) pH 7.2 containing 0.5 % BSA and 2 mM ethylenediaminetetraacetic acid (EDTA).
5. Mouse CD8a-microbeads for magnetic sorting (Miltenyi).
6. Normal saline, sterile and stored at room temperature.

2.4 Equipment

1. Sterile scissors and forceps.
2. Small petri dishes.
3. Glass Dounce homogenizer, sterilized with alcohol or autoclaved prior to use.
4. 25G and 27G needles.
5. 1–10 ml syringes.
6. Mouse restrainer.
7. MACS columns and magnetic separators.

3 Methods

3.1 Generation of Mature Dendritic Cells (DCs)

1. Humanely cull 6–12-week-old female or male NOD mouse according to institutional guidelines.
2. Remove both tibiae and femora using scissors and forceps, ensuring all fur and as much muscle as possible is removed; ensure work takes place in a sterile environment.
3. Spray bones with 70 % ethanol and quickly add to 20 ml complete RPMI medium.
4. Transfer bones to sterile petri dish containing complete RPMI medium, remove both ends of each bone with sterile scissors and flush bone marrow out of bones with a 10 ml syringe fitted with a 25G needle.
5. Withdraw bone marrow suspension using syringe without the needle and expel cells through needle into a fresh 50 ml tube fitted with a 40 μ m cell strainer.
6. Spin cell suspension at $400 \times g$ for 5 min at room temperature (RT).
7. Pour off the supernatant and resuspend cells in 20 ml of warm complete RPMI medium.
8. Transfer cells into a 75 cm² flask (maximum cells from six sets of tibiae and femora per flask).
9. Incubate cells at 37 °C, 5 % CO₂ for 2 h (to remove adherent cells).

10. After incubation remove the supernatant into a new 50 ml tube.
11. Gently wash the flask with 20 ml of complete RPMI medium and add to cell suspension.
12. Using a hemocytometer, count all large cells.
13. Spin cell suspension at $400 \times g$ for 5 min at RT.
14. Resuspend cells at 1×10^6 cells/ml in complete RPMI medium containing 1.5 ng/ml GM-CSF.
15. Add 5 ml cell suspension per well of a 6-well plate.
16. Incubate cells at 37 °C, 5 % CO₂.
17. Change medium every 3 days by removing 2.5 ml of medium and replacing with 2.5 ml complete RPMI medium with 1.5 ng/ml GM-CSF. Be careful not to disturb the cells at the bottom of the well.
18. On day 6–9, add 5 µl of 1 µg/ml lipopolysaccharide (LPS) per well and incubate for 16–18 h for maturation.
19. Harvest cells using cell scraper.
20. Wash cells 3× with complete RPMI medium to remove all LPS and resuspend in 20 ml complete RPMI medium.
21. Irradiate cells (3000 rad) and keep at 37 °C, 5 % CO₂ until ready to use.

3.2 Activation of CD8 Cells

Day 0:

1. Collect spleens from either G9 or 8.3 cells in complete RPMI medium.
2. Homogenize spleens using glass homogenizer.
3. Remove macroscopic tissue debris.
4. Spin cells at $400 \times g$, 5 min, 4 °C.
5. Lyse RBC by adding 900 µl dH₂O immediately followed by 100 µl 10× PBS and 4 ml MACS Buffer.
6. Spin cells at $400 \times g$, 5 min, 4 °C.
7. Resuspend in MACS buffer (10 ml or more depending on number of spleens being processed).
8. Count cells.
9. Spin cells at $400 \times g$, 5 min, 4 °C.
10. Use CD8-microbeads (Miltenyi) to sort CD8⁺ spleen cells following the manufacturer's guidelines.
11. After separation, spin cells and resuspend cells in 10 ml complete RPMI medium.
12. Count cells.

13. Calculate volume for final concentration of CD8⁺ cells at 10⁶/ml
14. Calculate number of DCs needed (1:20 DC:CD8 ratio or final concentration of DCs is 0.5 × 10⁵/ml)
15. Add CD8⁺ cells and DCs together, top up to total volume to give 10⁶/ml CD8 cells
16. Add insulin B_{15–23} peptide at 1 µg/ml or IGRP_{206–214} peptide at 10 ng/ml, depending on CD8 cell type to be used
17. Add 5 ml cell suspension per well of 6-well plate
18. Incubate for ~24–36 h (after this the cells start to die)

Day 1–2:

1. Cells should have an activated appearance (clusters of blasting cells surrounded by larger single cells)
2. Harvest cells with 1 ml micropipette
3. Rinse each well with 1 ml sterile PBS
4. Spin at 400 × *g*, 5 min, RT
5. Resuspend cells in 10 ml saline
6. Spin at 400 × *g*, 5 min, RT
7. Repeat this 2×; count cells prior to final spin
8. Resuspend cells at 5–10 × 10⁶/µl PBS, dependent on the number of cells required. For G9 cells, 7 × 10⁶ cells will be sufficient to induce diabetes in 7–10 days. For 8.3 cells, fewer cells may be sufficient.
9. Inject 200 µl cell suspension IV per NOD SCID mouse

Day 5/6:

1. Check mice on a daily basis from day 5/6 onwards for glucose in urine
2. If high for 2 consecutive days, test blood glucose and cull mouse if blood glucose is >13.9 mmol/l (>250 mg/dl).

4 Notes

1. Reconstitution of peptides: do not use PBS instead of saline as this will result in the peptide coming out of solution

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