Induction of IBD by transferring CD4+ CD45RB high cells in to SCID or Rag-/- mice

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Method Article

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Abstract

Introduction

The mouse model of inflammatory bowel disease \(IBD) is a well-established system for studying Treg cell-mediated suppression _in vivo_ \(Asseman et al., 1999).

Procedure

Cell transfers _in vivo_ 1| Harvest splenocytes from 5 to 8 week old mice. 2| Osmotically-lyse the erythrocytes and incubate the single cell suspensions with FITC-conjugated anti-CD4 and biotin-conjugated anti-CD45RB followed by incubation with anti-FITC microbeads. 3| Purify CD4+ T cells by magnetic isolation using the Auto MACS sorter \(Miltenyi Biotec). For isolation of CD4+CD45RB high naive cells, after releasing the beads, incubate the purified CD4+ T cell suspension with α-biotin microbeads followed by separation using the Auto MACS. In all of our experiments, 99% of these cells were positive for CD4 and high for CD45RB. 4| For the first intra peritoneal \(i.p.) injection, inject Thy1.2+ C.B-17 scid mice with 4×10^5 fresh Thy1.1+CD25– CD45RBhighCD4+ congenic cells. 5| After 6 or 7 days, administer an i.p. injection of PBS or 0.8-1 $\times 10^6$ freshly isolated Thy1.2+ Treg cells. Control mice receive PBS in both the injections. C57BL/6 Rag1–/– or Rag2–/– mice \(CD45.1) can be used as recipients C57BL/6 donor cells were transferred. After 6 or 7 days, they received an i.p. injection of PBS or 1 × 10 6 freshly isolated CD45.1+ Treg cells. Cells were transferred in 100ul of PBS/ mouse. On day 6-10 after Treg cells were transferred, mesenteric lymph node cells or lamina propria cells were isolated \(using the protocol from Current protocols in Immunology) and Annexin V and Propidium iodide staining was performed to measure apoptosis using flow cytometry. TUNEL staining was also done on cryo-sections of the gut to measure apoptosis of the effector cells in the lamina propria of the gut followed by confocal immunofluorescence analyses.

Timing

Cell purification 4-5 hours, Cell injection 3 min/mouse

Critical Steps

All the steps of cell purification and injection should be carried out in sterile conditions. Disease induction may be difficult if the purity of CD45RB high cells is not good. The recipient mice should also be 8 weeks old. Female mice were used both as donors and recipients of cells.

Anticipated Results

12-15 million cells may be obtained from 5 spleens.

References

Asseman, C., Mauze, S., Leach, M. W., Coffman, R. L. & Powrie, F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J Exp Med. 190, 995-1004 \(1999).