Analysis of murine lamina propria TH17 cells

Koji Atarashi
Laboratory of Immune Regulation, Graduate School of Medicine, Osaka University, Japan
Kenya Honda
Laboratory of Immune Regulation, Graduate School of Medicine, Osaka University, Japan

Method Article

Keywords: TH17, TH1, lamina propria, intestine

Posted Date: September 16th, 2008

DOI: https://doi.org/10.1038/nprot.2008.205

Abstract

Introduction

IL-17-expressing CD4+ cells \(TH17 cells) constitute a considerable proportion of lymphocytes in the intestinal lamina propria \(LP), even in healthy mice kept under specific pathogen free conditions. TH17 cells are rarely observed in the spleen, mesenteric lymph node, or Peyer's patches. Germ-free mice or antibiotics \(vancomycin and metronidazole)-treated mice show marked reductions in numbers of LP TH17 cells; thus, intestinal commensal bacteria provide a particular environment for the development of LP TH17 cells. This protocol describes the procedure to isolate LP lymphocytes and to assay for IL-17 expression by intracellular cytokine staining.

Reagents

- Hank's Balanced Salt Solution \(HBSS) - RPMI1640 medium - PBS - EDTA - fetal bovine serum \(FBS) - Bovine serum albumin \(BSA) - collagenase type II \(Invitrogen) - dispase \(Invitrogen) - DNase I \(Roche Diagnostics) - Fluorescently labelled anti-CD4, anti-IL-17, anti-anti-IFN-γ, anti-TCR-β \(BD Biosciences) - Percoll \(GE Healthcare) - Phorbol 12-myristate 13-acetate \(PMA) \(Sigma) - Calcium Ionophore A23187 \(Sigma) - BD GolgiStop - BD Cytofix/Cytoperm

Equipment

- shaking water bath - Flow cytometer

Procedure

Isolation of LP lymphocytes \(see also ref. 1) 1. Open intestines longitudinally, and wash in PBS to remove faecal content. 2. Shake in HBSS containing 5 mM EDTA for 20 min at 37°C. 3. After removal of epithelial cells and fat tissue, cut the intestines into small pieces and incubate with RPMI1640 containing 4% FBS, 1 mg/ml collagenase type II, 1 mg/ml dispase and 40 µg/ml DNase I for 1 h at 37°C in a shaking water bath. 4. Wash the digested tissues with HBSS containing 5 mM EDTA, resuspend in 5 ml of 40% Percoll \(GE Healthcare) and overly on 2.5 ml of 80% Percoll in a 15-ml Falcon tube. 5. Centrifuge the samples at 2,000 rpm for 20 min at room temperature. 6. Collect LP lymphocyte from the interface of the Percoll gradient and wash with PBS containing 0.5% BSA and 2 mM EDTA) or RPMI1640. The cells can be used immediately for further experiments. **Intracellular cytokine staining**. 1. Incubate LP lymphocytes with 50 ng/ml PMA, 5 µM Calcium Ionophore A23187 and Golgistop in complete media at 37°C for 4 h. 2. Incubate the cells with a corresponding cocktail of fluorescently labeled antibodies, such as anti-CD4 and anti-TCR-β, for 20 min at 4°C. 3. Permeabilize the cells with Cytofix/Cytoperm solution for 20 min at 4°C. 4. Incubate cells with fluorescently labelled cytokine antibodies, e.g. anti-IL-17 and anti-IFN-γ, for 20 min at 4°C. 5. Wash cells with PBS containing 0.5% BSA and 2 mM EDTA buffer. 6. Analyze with Flow cytometer.

Timing

12 hrs

Anticipated Results

See attached Figure 1.

References

1. Ivanov, II _et al_. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. _Cell_ **126**, 1121-33 \(2006).

Figures

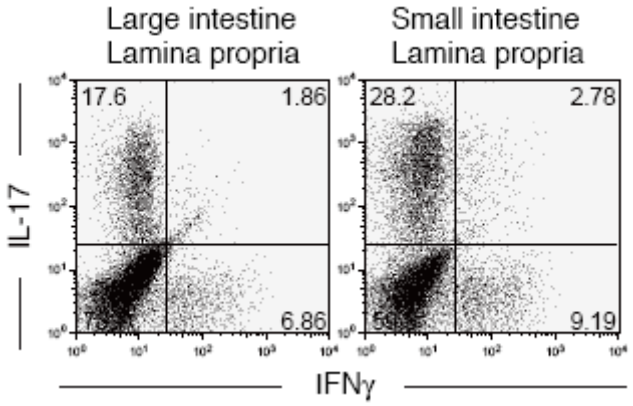

Figure 1

Lymphocytes were isolated from the lamina propria of the large and small intestine of SPF mice, and stimulated with PMA and calcium ionophore for 4 h. After the stimulation, cells were permeabilized, stained for CD4, IFN-γ and IL-17, and analysed by FACS. Representative dot plots gated on CD4+ cells are shown. Numbers indicate the percentages of cells in the quadrants.