Chapter 3

Isolation of T Cells from the Gut

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Abstract

The lymphocytes of epithelial and lamina proprial compartments of the intestine are phenotypically and functionally distinct and serve a wide range of functions in the intestinal mucosa like regulating intestinal homeostasis, maintaining epithelial barrier function as well as regulating adaptive and innate immune responses. To analyze the role of these cells in different disease states, it is necessary to isolate pure cell populations of the intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) of the gut. In this protocol we describe a method to isolate T cells from IEL and LPL, which can be used for further investigations like comparative studies of mRNA expression, cell proliferation assay, or protein analysis.

Key words Mucosal immunology, Lamina propria, Gut, T cells, Lamina propria lymphocytes, Intraepithelial lymphocytes

1 Introduction

The intestinal mucosal immune system actively contributes to the maintenance of mucosal homeostasis and defends against pathogenic microbes. It consists of three major lymphoid areas: (1) the lamina propria (LP) which lies just beneath the basement membrane in the intestinal villi and contains the lamina propria lymphocytes (LPL); (2) the intraepithelial compartment which is located just above the basement membrane between the columnar epithelial cells and comprises the intraepithelial lymphocytes (IEL); (3) the Peyer's patches (PP) which are organized in lymphoid nodules (akin to lymph nodes) embedded in the gut wall, separated from the LP and IEL. The LP, PP, and IEL lymphoid populations form a complex, interconnected network that responds to immunological insults in the intestine. Therefore, these lymphocyte populations should be analyzed when studying the immunological status of the intestine.

Various techniques have been described so far for the isolation of intestinal cells [1, 2]. IEL fractions have been isolated by gentle mechanical manipulation [3], by EDTA treatment [4–6], and by enzymatic treatment [7].

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In the present protocol, we describe a simple modification of a standard lamina propria lymphocyte and intraepithelial lymphocyte isolation technique [8] that involves the combination of mechanical dissociation with enzymatic degradation of the extracellular adhesion proteins. The protocol can be used to characterize T cell subpopulations isolated from LPL and/or IEL of the colon or of the small intestines or subparts of it like the duodenum, jejunum, or ileum. The isolated cells should be used directly for downstream applications such as magnetic cell separation for T cells, cellular or molecular analysis.

The stated volumes in this protocol are calculated for one intestine, either small intestine or colon. Do not pool the small intestine and colon for the purification procedure.

2 Materials

2.1 Equipment

1. Scissors.
2. Forceps.
3. Petri dishes.
4. 100 μm cell strainer.
5. 5 and 50 mL Falcon conical centrifugation tubes.
6. gentleMACS™ tubes (alternatively use 50 mL Falcon conical centrifugation tubes).
7. gentleMACS dissociator (alternatively use vortexer).
8. Vortexer.
9. Centrifuge used in cell culture.
10. Thermal incubator with rotation unit.
11. PIPETBOY.

2.2 Reagent Setup

1. Predigestion solution: 1× HBSS (without Ca^{2+} and Mg^{2+}, no Phenol Red) containing 10 mM HEPES, 5 mM EDTA, 1 mM DTT, and 5 % fetal calf serum (FCS).
2. Digestion solution: 1× HBSS (with Ca^{2+} and Mg^{2+}, no Phenol Red) containing 10 mM HEPES, 0.5 mg/mL Collagenase D, 0.5 mg/mL DNase I grade II, 3 mg/mL Dispase II, and 5 % FCS. Prepare the digestion solution just before use.
3. 1× HBSS (without Ca^{2+} and Mg^{2+}, no Phenol Red) with 5 % FCS.
4. Fluorescence-activated cell sorting (FACS) buffer: 2 % FCS in 1× PBS.
5. T cell medium: RPMI 1640 supplemented with 10 % FCS (decomplemented), 1 mM sodium pyruvate, 2 mM L-glutamine, 1× nonessential amino acids, 0.1 mM 2-β-mercaptoethanol, and 10 mM HEPES.

6. Percoll gradient 40 %: Use 42.01 mL of Percoll separation solution with 1.124 g/L density and dilute with 57.99 mL of 1× PBS.
7. Percoll gradient 80 %: Use 79.83 mL of Percoll separation solution with 1.124 g/L density and dilute with 20.17 mL of 1× PBS.
8. Sterile 1× PBS (without Ca^{2+} and Mg^{2+}).

3 Methods

1. Sacrifice mice, remove the intestine, and place it in ice-cold 1× PBS in a petri dish (*see* **Note 1**).
2. Clear the intestine of feces by holding it with forceps and flushing with a 1 mL syringe filled with ice-cold 1× PBS.
3. Remove residual mesenteric fat tissue, excise Peyer's patches carefully (*see* **Note 2**), and open the intestine longitudinally.
4. Cut the intestine into 0.5–1 cm pieces, transfer them into a 50 mL tube, and wash them several times by vortexing in ice-cold 1× PBS until the buffer appears clean.
5. Transfer the tissue pieces into a new 50 mL tube containing 20 mL of pre-digestion solution (*see* **Notes 3** and **4**) and incubate sample for 20 min at 37 °C with slow rotation (40 × *g*) on a horizontal tube rotator.
6. After the incubation remove the epithelial cell layer, containing the IEL, by intensive vortexing for 10 s and passing through a 100 μm cell strainer placed on a 50 mL Falcon tube. The flow-through contains the IEL and can be stored on ice for the isolation of IEL.
7. Transfer the tissue pieces into a new 50 mL Falcon tube containing new 20 mL of the pre-digestion solution and incubate a second time for 20 min at 37 °C with slow rotation (40 × *g*) on a horizontal tube rotator.
8. After the incubation mix the samples by intensive vortexing for 10 s and pass the sample through a 100 μm cell strainer placed on a 50 mL Falcon tube.
9. Transfer the tissue pieces into a new 50 mL Falcon tube containing 10 mL HBSS with 5 % FCS for 10 min at 37 °C with rotation on a horizontal tube rotator.
10. After the incubation mix the samples by intensive vortexing for 10 s and pass the sample through a 100 μm cell strainer placed on a 50 mL Falcon tube.

    If IEL isolation is desired, pool the supernatants of all pre-digestion treatments and the washing step. Wash once with FACS buffer and store them on ice (*see* **Notes 5** and **6**).

11. Transfer the intestine tissue pieces into a petri dish and cut them in 1 mm^2 pieces using scissors or razor blades. Place the remaining tissue in gentleMACS™ tubes and add 2.5 mL digestion solution. Incubate the samples at 37 °C for 30 min with rotation on a horizontal tube rotator (*see* **Note 7**).
12. Dissociate the LPL with the gentleMACS dissociator by running the program lamina propria. After termination of the program, add 10 mL of FACS buffer to each sample and isolate the cells by passing through a 100 μm cell strainer.
13. Wash the cell strainer with 10 mL of FACS buffer. Centrifuge the cell suspension for 10 min at 300 × *g*, discard the supernatant, and resuspend cells in FACS buffer and store them on ice.
14. Use cells immediately for further experiments, for example for T cell magnetic cell separation or flow cytometry.
15. For purification of lymphocytes resuspend LPL or IEL in 4 mL of the 40 % fraction of a 40:80 Percoll gradient, and overlay them carefully on 8 mL of the 80 % fraction in a 15 mL Falcon tube.
16. Perform Percoll gradient separation by centrifugation for 20 min at 1,000 × *g* at room temperature without brakes.
17. After the centrifugation, LPL or IEL should be visible in a white ring at the interphase of the two different Percoll solutions. Collect the cells carefully and transfer them into a new 15 mL Falcon tube. Add FACS buffer and centrifuge the samples for 10 min at 300 × *g* at 20 °C.
18. Resuspend the cells directly in FACS buffer or T cell medium and store them on ice.

    Use the cells immediately for experiments, for example for flow cytometry or T cell magnetic cell separation. The isolated T cells can be used for comparative stimulation assays, proliferation assays, RNA extraction, or protein isolation.

4 Notes

1. Small intestines are considered from the beginning of the duodenum/pylorus to the caecum. Colon is considered from the caecum to the anus. To extract the whole colon break the pelvis laterally.
2. Peyer's patches are found only in the small intestines. Remove Peyer's patches before cleaning the intestine, because otherwise it might be hard to identify the Peyer's patches.
3. Preheat the pre-digestion solution to 37 °C before adding to the tissue.
4. Per digestion a volume of 40 mL of the pre-digestion is required.

5. In the meantime: Preheat the digestion solution to 37 °C before adding to the tissue.
6. Per digestion a volume of 2.5 mL of the digestion solution is required.
7. Alternatively: If you do not use the gentleMACS dissociator, place in **step 11** the tissue in a 50 mL Falcon tube for the digestion step. After the incubation time (**step 12**), vortex the Falcon tube intensively for 20 s and pass the cell solution through a 100 μm cell strainer set over a 50 mL Falcon tube. Ideally, all tissue pieces should be digested to invisible pieces.

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