Video Article

Isolation of Lymphocytes from Mouse Genital Tract Mucosa

Janina Jiang[1], Kathleen A. Kelly[1,2]

[1]Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles

[2]California NanoSystems

Correspondence to: Kathleen A. Kelly at kkelly@mednet.ucla.edu

URL: https://www.jove.com/video/4391
DOI: doi:10.3791/4391

Keywords: Immunology, Issue 67, Mucosal immunity, sexually transmitted diseases, genital tract lymphocytes, lymphocyte isolation, flow cytometry, FACS

Date Published: 9/3/2012

Citation: Jiang, J., Kelly, K.A. Isolation of Lymphocytes from Mouse Genital Tract Mucosa. *J. Vis. Exp.* (67), e4391, doi:10.3791/4391 (2012).

Abstract

Mucosal surfaces, including in the gastrointestinal, urogenital, and respiratory tracts, provide portals of entry for pathogens, such as viruses and bacteria [1]. Mucosae are also inductive sites in the host to generate immunity against pathogens, such as the Peyers patches in the intestinal tract and the nasal-associated lymphoreticular tissue in the respiratory tract. This unique feature brings mucosal immunity as a crucial player of the host defense system. Many studies have been focused on gastrointestinal and respiratory mucosal sites. However, there has been little investigation of reproductive mucosal sites. The genital tract mucosa is the primary infection site for sexually transmitted diseases (STD), including bacterial and viral infections. STDs are one of the most critical health challenges facing the world today. Centers for Disease Control and Prevention estimates that there are 19 million new infectious every year in the United States. STDs cost the U.S. health care system $17 billion every year [2], and cost individuals even more in immediate and life-long health consequences. In order to confront this challenge, a greater understanding of reproductive mucosal immunity is needed and isolating lymphocytes is an essential component of these studies. Here, we present a method to reproducibly isolate lymphocytes from murine female genital tracts for immunological studies that can be modified for adaption to other species. The method described below is based on one mouse.

Video Link

The video component of this article can be found at https://www.jove.com/video/4391/

Protocol

1. Removal of the Genital Tract

1. Euthanize the mouse using approved guidelines. Make low midline incision and retract skin.
2. Isolate and remove whole genital tract, from oviduct to vaginal opening.
3. Open the entire tract longitudinally with scissors.
4. Cut the genital tract with fine surgical scissors into fine pieces (about <1 mm) in a Petri dish with Complete RPMI-10/EDTA (see the formula below) and stir the tissue in 125 ml flask at room temperature on a magnetic stirrer set for 15 min. This procedure is repeated twice and then the supernatant, which contains epithelium and other debris, is discarded.
5. Pour contents of flask through a cell strainer (40 µm). Wash off EDTA residue with complete medium.
   Complete RPMI/10: RPMI 500 ml (Gibco), 10% Fetal bovine serum (FBS), heat-inactivated, 5 ml penicillin/streptomycin, 5 ml 1M HEPES.
   Complete RPMI-10/EDTA: 10%RPMI (as above) with 5 µM EDTA

2. Digesting Genital Tract Tissue

1. Transfer tissue pieces to a new flask with 20 ml fresh RPMI-10/collagenase (see the recipe below) and digest the tissue at 37 °C for 1 hr on a magnetic stirrer set to vigorously stir the tissue.
   Complete RPMI-10/collagenase: Right before use, reconstitute collagenase type VIII (Sigma; store at -20 °C, or according to manufacturer's instructions.), add into complete RPMI/10 to a final concentration of 450-500U/ml.
2. After the first digestion, collect supernatants through 100 µm cell strainer, keep the cells on ice.
3. Transfer the undigested tissue to a new flask and repeat step 2.1 two more times using fresh complete RPMI-10/collagenase for a total of 3 separate digestions. At this point, no visible tissue pieces should be in solution.
4. Pool the all supernatants together from a sample and collect through 100 µm cell strainer. Spin the cells down. Cell pellets will be separated using Percoll gradients.

3. Separating Genital Tract Cells Using Percoll Gradients

1. Prepare each concentration of Percoll before use:
   - 100% isotonic Percoll: mix 9:1 stock Percoll (Pharmacia Biotech) vs. 10x PBS. pH it to 7.4 using HCl.
   - 40% Percoll solution: mix 40 ml of 100% isotonic Percoll and 60 ml of complete RPMI.
   - 70% Percoll solution: mix 70 ml of 100% isotonic Percoll with 30 ml of complete RPMI.

2. Resuspend each cell pellet from step 6 in 40% Percoll solutions. An equal volume of 70% Percoll will be used to make the gradients. There are two ways to set up gradient tubes:
   1. Under-layer: add cell suspension with 40% Percoll into a gradient tube first, then carefully under-layer 70% Percoll.
   2. Over-layer: add 70% Percoll into tube first, then carefully and slowly load 40% Percoll containing the cells on the top.

3. Gradient samples will be centrifuged at 900 x g, room temperature, for 20 min with the brake off.
4. The lymphocytes will be at the interface layer between 40% and 70% Percoll layers. Carefully harvest the interface layer, wash with RPMI 1:3 and spin down at 740 x g. The lymphocytes will be in the cell pellet and are ready for further use.

4. Representative Results

An example of isolation of lymphocytes from mouse genital tract and flow cytometry (FACS) analysis is shown in **Figure 1**. Genital tract was removed from *Chlamydia muridarum* intravaginally infected mouse 7 days after infection. Two genital tracts were pooled together in order to have enough lymphocytes for functional and phenotype examination by FACS. Dissected tissues were processed by the digestion and isolation steps as outlined above. The single cell suspension was stained for various fluorochrome-conjugated monoclonal antibodies against mouse CD3, CD4 and CD8. **Figure 1** shows a dot-plot presentation of CD4 positive T cells versus CD8 positive T cells gated on CD3 positive T lymphocytes. Our procedure can examine lymphocytes isolated from genital tracts of different disease models to assess various immune cell functional and phenotypic characteristics in different diseases in mouse model. These include diverse immune cells, such as dendritic cells, neutrophil, macrophages NK, NKT, T cells (Ag-specific T cells), B cells etc.[3-10].

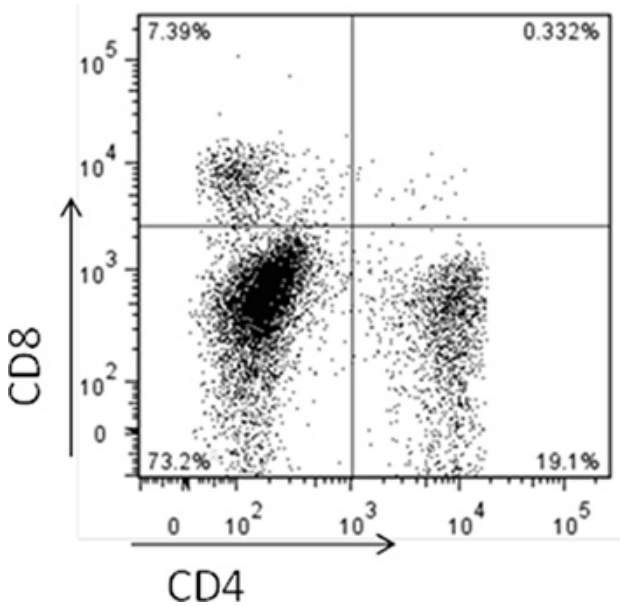

Figure 1. Lymphocytes were isolated from genital tracts of mice infected with *Chlamydia muridarum*, using the method presented here. After the complete separation procedure, lymphocytes were stained with fluorochrome-conjugated CD3, CD4 and CD8. The quadrant data were gated on CD3+ lymphocytes, showing CD4+ versus CD8+ with and percentage of gated cells.

Discussion

In this protocol, it is shown how to prepare lymphocytes from mouse genital tracts and is easily mastered. The most critical point of this process is to maintain cell viability by keeping the cells on ice. The cell yield from this method depends on technique, handling skill, the infectious status of the mouse (naïve or day after infection), the pathogen (bacterial, viral, fungal) and the section of the murine reproductive tract examined (oviduct, uterus, vaginal segment or entire genital tract). Our group and others have published various studies on different lymphocyte populations from mouse genital tract [5-7]. On our hands, from one naïve BALB/c mouse, entire genital tract can generate 2×10^6 cells with 90% viability. In many occasions, the lymphocytes will be used for further functional and/or flow cytometric analysis [3-10]. In comparison with the commonly used isolation method [11], by our method, the cell yield is increased 10 times. This procedure is relatively long, but it can be simplified depending on the further use of the isolated lymphocytes. When the cells are used for non-functional assays such as flow cytometry analysis, the method can be shorted by skipping Percoll gradient step. This method provides a powerful and an efficient tool to study mucosal lymphocytes and provide insight into infectious disease research, facilitate subsequent vaccine development.

Disclosures

No conflicts of interest declared.

Acknowledgements

We like to thank NIH for funding grants R01-AI026328 (KAK) and R01-AI079004 (KAK).

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