

Isolation of $\gamma\delta$ T cells from mouse small intestine

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ABSTRACT

Gamma/delta T cells ($\gamma\delta$ T cells) are among the first lymphoid cells appearing in the ontogenesis. Many of $\gamma\delta$ T cells are located in the small intestine/lamina propria of mice and human, where they cooperate with different cells (B-1 cells, in particular) and influence their activity. However, such interactions are studied rather scanty, and the functional role of $\gamma\delta$ T cells in the intestine is not yet fully elucidated. To study the interactions of mouse $\gamma\delta$ T cells with other cells it is necessary to have purified cell populations. Unfortunately, most approaches used for isolation of human $\gamma\delta$ T cells are not suitable for isolation of mouse $\gamma\delta$ T cells. The aim of the present study is the modification of the method of mouse intestinal $\gamma\delta$ T cell isolation in the quantities sufficient for the *in vivo* and *in vitro* experiments.

Keywords: Gamma/Delta T Cells; Small Intestine; Intraepithelial Lymphocytes; Magnetic Separation

1. INTRODUCTION

Gamma/delta T cells ($\gamma\delta$ T cells) are among the first lymphoid cells appearing in ontogenesis. Many of them reside at the basolateral surfaces of intestinal epithelial cells [1]. From alpha/beta T lymphocytes, appearing a little later, $\gamma\delta$ T cells differ by their capacity to recognize unprocessed antigens. It was shown that $\gamma\delta$ T cells participated in the processes of oral tolerance [2] and possess immunoregulatory functions [3,4]. However, the interactions of $\gamma\delta$ T lymphocytes with other cells are studied rather scanty, and their functional role in the intestine is not yet fully elucidated.

It was suggested that the switch from IgM to IgA in T-independent intestinal B-1 cells might be provided with $\gamma\delta$ T cells factors. This suggestion was not experi-

mentally checked, and the question remains open till now. The answer may be obtained by the study of the direct B-1 and $\gamma\delta$ T cell interactions *in vitro*. To perform such experiments purified B-1 and $\gamma\delta$ T cells should be used.

It is known that peritoneal B-1 cells may migrate to the gut where many of them become IgA-producers [5]. Thus, peritoneal cavity may be used as a source of B-1 cells. Method of B-1 cell isolation is well developed routine procedure. On the contrary, the isolation of $\gamma\delta$ T cells from mouse intestine meets with significant difficulties. We tried to modify and simplify the method of mouse intestinal $\gamma\delta$ T cell isolation.

2. MATERIALS AND METHODS

2.1. Mice

Female CBA mice, 16 - 18 g were used. Mice were maintained in the animal house of Mechnikov Institute of Vaccines and Sera of Russian Academy of Medical Science.

2.2. Isolation of $\gamma\delta$ T Cells

Intraepithelial lymphocytes (IELs) were isolated from the small intestine; $\gamma\delta$ T cells were isolated from IELs. To isolate IELs in some experiments collagenase of *Clostridium histolyticum* (Gibco) was used.

2.3. Flow Cytometry

The different cell populations were analyzed by cytometry. IELs and $\gamma\delta$ T cells were prestained with anti-CD16-32 mAb (2.4G2) to block Fc γ RII/RIII receptors and stained at 4°C for 30 min with the following fluorochrome conjugated antibodies: FITC-anti-CD3 (17A2), PE-anti-CD19 (1D3), PE-anti- γ/δ T (GL3) (all reagents, BD-Pharmingen).

After washing away the excess of reagents stained cells were resuspended in staining medium containing 5 μ g/ml propidium iodide (PI) (Sigma). Cells stained with PI (dead cells) were excluded from analysis. Cells were

analyzed on Beckman Coulter EPICS XL. Results were treated with the Programmed SYSTEM II (Beckman Coulter).

3. RESULTS AND DISCUSSION

Over the years, a number of strategies have been developed to obtain enriched preparations of intraepithelial lymphocytes (IELs) and $\gamma\delta$ T cells. The method described are used mainly for isolation of human intestinal cells [6], although the methods of the isolation of IELs and T lymphocytes from monkey [7], rat [8], pig [9] and mice intestine are also described [10].

The most commonly used approaches involve the removal and washing of intestine, isolation of IELs and purification of T lymphocytes through Percoll density gradients. There are different modifications of these approaches concerning mainly the mode of tissue degradation, the time and the temperature of incubation of intestine segments with EDTA and dithiotreitol (DTT) and the concentrations of collagenase.

We have found that low viability of mouse IELs depended mainly on the high sensibility of intestinal mouse cells to the temperature and, especially, to the collagenase treatment. In addition, relatively many IELs (and $\gamma\delta$ T cells, respectively) were lost during centrifugation in Percoll gradients. Therefore we tried to develop the method of IEL isolation without collagenase treatment and without cell purification through Percoll gradient. Below the protocol used in our experiments is written in details.

Mouse small intestine was washed by RPMI 1640 medium with 8% FCS on ice, everted and cut into several 2 - 3 cm segments to avoid winding. (Note! The intestine should not be minced or cut into very small pieces, otherwise the yield of cells would be greater but their viability would be less). The segments were placed into a 50-ml conical tube containing RPMI 1640 medium with 8% FCS, 1 mM EDTA, 1 mM DTT (Sigma), and 250 μ l of penicillin + streptomycin (1/100) (Pen Strep, Gibco). The tube was shaken horizontally on an orbital shaker for 30 - 60 min at 37°C. The tissue suspension was passed through 4 layers of gauze to remove mucus and centrifuged for 6 min. at 400 g. The yield of IELs CD3⁺ T cells and $\gamma\delta$ T cells after different time of isolation is present in the **Figure 1**. It may be seen that the increase of the time of tissue incubation from 30 to 60 min. permits to isolate from the intestine of 3 mice about 38 - 40 $\times 10^6$ cells instead of 1 - 2 $\times 10^6$ after 30 min. incubation.

To purify $\gamma\delta$ T cells commercial TCR γ/δ T cell Isolation Kit (Miltenyi Biotec) was used. The yield of CD3⁺ T cells in 2 experiments was equal to 27 and 20 mln, and the purity of CD3⁺ T cells was about 63% - 67%. The yield of $\gamma\delta$ T cells was 1.0 - 1.2 $\times 10^6$ (from

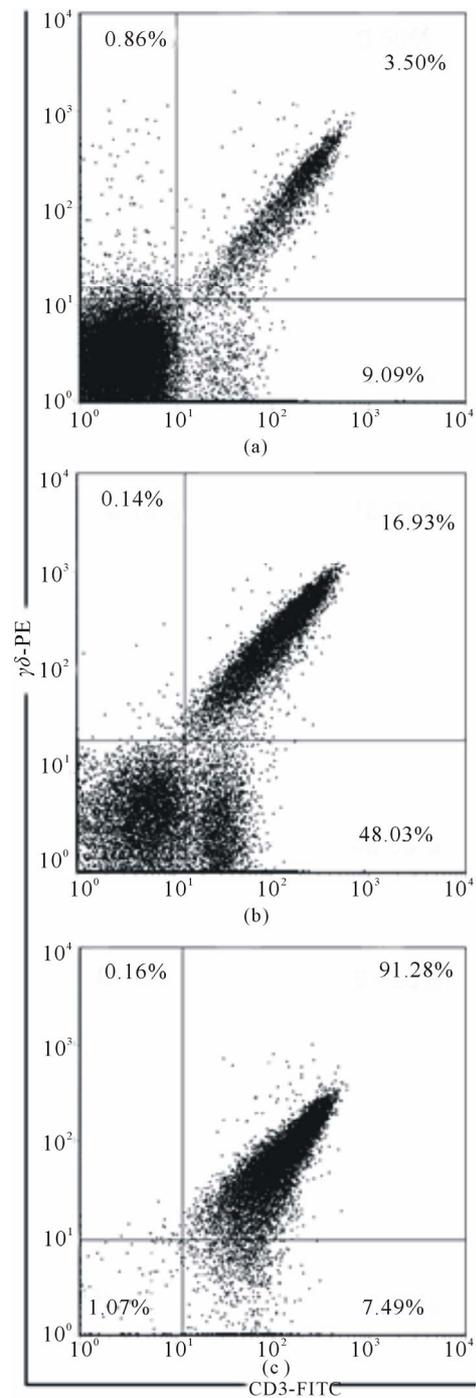


Figure 1. The percentage of $\gamma\delta$ T cells at different stages: (a) after 30 min of incubation; (b) after 60 min of incubation; (c) after magnetic cell sorting.

intestines of 3 mice). After one-step purification the purity of $\gamma\delta$ T cells was about 50%, but after two step purification it increased to 90% - 92%. The viability of cells was not less of 85%. These data do not differ from those recently published [11,12], where IELs were purified by centrifugation through Percoll gradient, and

$\gamma\delta$ T cells were isolated by immunomagnetic method. We believe that modification described here may be used for the isolation of $\gamma\delta$ T cells for *in vivo* and *in vitro* studies.

4. CONCLUSION

The method of $\gamma\delta$ T cell isolation from mouse small intestine without collagenase treatment and without centrifugation through Percoll gradients is described. The procedure permits obtaining viable $\gamma\delta$ T cells of 90% purity.

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