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Tc1/Tc2 balance in healthy individuals — the three-color flow cytometry analysis

Równowaga Tc1/Tc2 w grupie osób zdrowych — analiza z użyciem techniki trójkolorowej cytometrii przepływowej

INTRODUCTION

The existence of distinct cytokine-producing subsets among CD4 T cells has been demonstrated in the last years. Firstly Mosman et al. [1] identified mouse T helper clones, later human clones were detected [2]. Mouse Th1 cells secrete IL-2, IFNγ and TNFβ and are involved in cell-mediated immune response, while Th2 cells produce IL-4, IL-5, IL-6, IL-10 and play a role in humoral and allergic response. Human Th1 and Th2 cells produce similar cytokine patterns, however the synthesis of IL-2, IL-6, IL-10 and IL-13 are not firmly restricted to a single subset [3]. Thus human Th1 and Th2 subsets are usually defined according to IFNγ/IL-4 production [4]. In recent years it has become clear that cytokine secretion is not confined to CD4+ cells and the existence of distinct subset of CD8+ cells that are similar to their CD4 counterparts has been detected in mice, rats and humans [5]. They are termed Tc1 and Tc2, respectively. Tc1 cells secrete IL-2 and IFNγ, while Tc2 synthesize IL-4, IL-5, IL-10 [3]. Their role, although it still needs to be clarified, is probably similar to Th1-Th2 cells — in some cases, they provide help for antibody production by B-cells or be involved in the inflammatory responses, respectively [6].

This study presents an analysis of intracellular IFNγ and IL-4 expression by CD3+/CD8+ cells in healthy blood donors. The aim of it was to analyze the Tc1/Tc2 balance in healthy individuals according to the pattern of secreting cytokines.

MATERIALS AND METHODS

Cell separation and stimulation

Twelve normal healthy subjects (7 males and 5 females) were studied. Venous blood samples were collected after informed consent into heparinised tubes and tested immediately.

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation (Lymphoprep, Nycomed Pharma, Norway) then washed twice with PBS and finally resuspended at a concentration of 2×10^6 cells /ml in culture medium consisted of RPMI 1640 (Gibco Laboratories, USA) with 1% 2mM L-glutamine, 1% antibiotic, 10% heat-inactivated fetal calf serum (FCS). This culture medium was supplemented with stimulators 25 ng/ml Phorbol 12-myristate 13 acetate (PMA) together with 1.5 μg/ml ionomycin and 10 μg/ml Brefeldin A — the intracellular protein transport inhibitor. The cells were incubated at 37°C in 4% CO_2 atmosphere for 4 hours.

Cell staining and flow cytometry analysis

Following stimulation period the cells were collected, washed twice with PBS and divided at a concentration of about 5×10^5 cells into tubes containing 20 μl of ant-CD3 PerCP monoclonal antibody (MoAb) along with 20 μl anti-CD8 FITC MoAb (Becton Dickinson, USA). The cells were placed in the dark for 15 min. at room temperature (RT). Then the cells were submitted to fixation and permeabilisation procedure with IntraPrep kit (Immunotech, France) followed up the manufacture's instruction. Fixed and permeabilised cells were then incubated in the dark at RT for 15 min. with 20 ml of anti-IL4, anti-IFNγ MoAb or isotypic IgG1 control, all labeled with PE. The cells were then washed resuspended into 1% paraformaldehyd and analysed immediately by FACSCalibur (Becton Dickinson, USA) flow cytometer and Cell Quest Software. At least 5000 of gated CD3 positive cells of lymphocytic gate were accumulated and analyzed per test as percentages among the Tc cell subsets (CD3+/CD8+).

Evaluation of Tc1/Tc2 balance

The percentages of CD3+/CD8+/IFNγ+ and CD3+/CD8+/IL4+ cells were assumed Tc1 and Tc2 lymphocytes, respectively. The Tc1/Tc2 balance was evaluated by the ratio of % IFNγ to % IL4 expressing cells.

Table I. Intracellular IFNγ and IL-4 expression by CD3+/CD8+ cells in healthy individuals. The data are shown as MEAN±SD

Tc1 *CD3+/CD8+/IFNγ+*	*Tc2* *CD3+/CD8+/IL-4+*	*Tc1/Tc2*
14.98±6.39%	1.17±1.36%	1.17±1.36

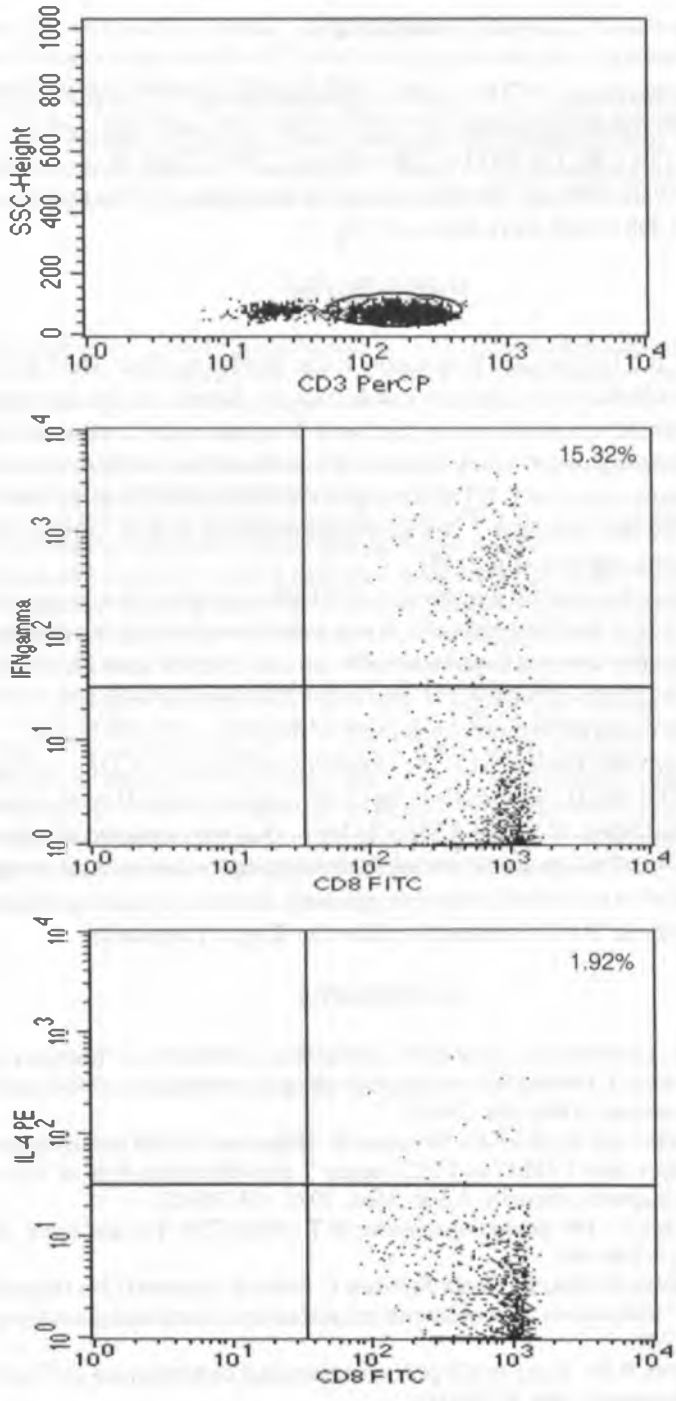

Fig. 1. The flow cytometry dot-plots of IFNγ and IL-4 expression by CD3+/CD8+ cells

RESULTS

The mean percentage of CD3+CD8+ cells expressing IFNγ in studied group was 14.98±6.39. The mean percentage of CD3+CD8+ cells with intracellular IL-4 expression was 1.17±1.36. The CD3+CD8+IFNγ+/CD3+CD8+IL-4+ ratio, however, was 26.72±19.02 (Tab. I). The flow cytometry dot-plots of IFNγ and IL-4 expression by CD3+/CD8+ cells were shown in Fig. 1.

DISCUSSION

The role of Tc1-Tc2 lymphocytes seems to be similar to their Th1-Th2 counterparts, they provide help for antibody production by B-cells or are involved in the inflammatory responses, respectively [6]. Both T helper and T cytotoxic cells play important immunoregulatory roles, producing cytokines that are critical to the effectiveness of immune response [7]. The dysregulated expansion of one of these subsets may contribute to the impaired T cell mediated response in such clinical states like infections, autoimmunity or cancer [8].

In this study we focused on assessment of T CD8+ lymphocytes in aspect of their polarization to T type 1 or T type 2 cells. It was possible with using the flow cytometry technique to identify the cytokine expression on the level of individual cell [9]. By simultaneously labeling with anti-CD3, and anti-CD8 together with anti-IL-4 or anti-IFNγ MoAbs we could determine the subsets of interest — Tc1–Tc2 cells.

Our results provide evidence for the capability of human T CD8+ cell subset to produce both IFNγ and IL-4 similarly to its CD4+ counterparts. However, we detected significant expansion of CD3+/CD8+/IFNγ+, that may indicate the dominance of Tc1 in the analyzed group. Further studies are required to determine the role of the T CD8+ cells subsets in immune response precisely, however it can be concluded that in healthy individuals Tc1/Tc2 balance is shifted to T type 1 response.

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STRESZCZENIE

Podział limfocytów T CD4+ na populacje Th1 i Th2, potwierdzony w ciągu ostatnich 10 lat licznymi badaniami, jest powszechnie znany i akceptowany. Stosunkowo niedawno pojawiły się doniesienia wskazujące na istnienie odrębnych populacji komórek T CD8+, odpowiednio Tc1 i Tc2, definiowanych na podstawie produkowanych przez nie cytokin (IL4/IFNγ). Komórki T typu 1 i T typu 2 odgrywają istotną rolę immunoregulacyjną, a produkowane przez nie cytokiny odpowiadają za efektywność odpowiedzi immunologicznej.

Celem pracy była ocena równowagi subpopulacji limfocytów Tc1/Tc2 definiowanych na podstawie produkowanych cytokin w grupie zdrowych dawców krwi.

Badaniem objęto grupę 12 osób. Limfocyty wyizolowane z krwi obwodowej poddano stymulacji PMA i jonomycyną, w obecności brefeldyny A. Następnie wykonano procedurę zewnątrz i wewnątrzkomórkowego znakowania przy użyciu przeciwciał monoklonalnych (anti-CD3 PerCP, anti-CD8 FITC, anti-IFNγ PE, anti-IL-4 PE). Barwione komórki oceniono przy użyciu techniki trójkolorowej cytometrii przepływowej. Średni odsetek limfocytów CD3+CD8+IFNγ+ oraz CD3+CD8+IL-4+ wynosił odpowiednio 14.98±6.39% i 1.17±1.36%. Stosunek CD3+CD8+IFNγ+/CD3+CD8+IL-4+ wynosił natomiast 26.72±19.02.

Wyniki przeprowadzonych oznaczeń wskazują na istotną dominację w badanej grupie subpopulacji komórek T CD8+ zdolnych do produkcji IFNγ. Jakkolwiek ocena roli komórek T CD8+ w regulacji odpowiedzi immunologicznej wymaga dalszych badań, przedstawione wyniki mogą sugerować przesunięcie w grupie osób zdrowych równowagi Tc1/Tc2 w kierunku populacji Tc1.