Резюме

Беспалова О.Я. *Особливості клітинних факторів імунітету у хворих на дилатаційну кардіоміопатію.*

Проведені дослідження клітинних факторів імунітету показали, що у хворих дилатаційною кардіоміопатією спостерігався дисбаланс клітинної ланки імунної системи, що проявлявся достовірним зниженням відносної кількості CD3+ лімфоцитів, дисбалансом імунорегуляторних клітин підвищенням хелперної та супресорної популяції клітин, що призводило до підвищення імунорегуляторного індексу. Одночасно мало місце достовірне збільшення кількості CD16+ та CD95+ лімфоцитів. Зниження функціональної активності лімфоцитів, проявлялося достовірним зменшенням кількості CD 25+ клітин. Розвиток деструктивно-запального процесу в міокарді серця хворих дилатаційною кардіоміопатією призводить до порушень клітинних факторів імунітету.

Ключові слова: дилатаційна кардіоміопатія, лімфоцити, клітинний імунітет.

Резюме

Беспалова Е.Я. *Особенности клеточных факторов иммунитета у больных дилатационной кардиомиопатией.*

Исследования клеточных факторов иммунитета показали, что у больных дилатационной кардиомиопатией наблюдался дисбаланс клеточного звена иммунной системы, который проявлялся достоверным снижением относительного количества CD3+ лимфоцитов, дисбалансом иммунорегуляторных клеток повышением хелперной и супрессорной популяции клеток. Одновременно имело место достоверное увеличение количества CD16+ и CD95+ лимфоцитов. Снижение функциональной активности лимфоцитов, проявлялось достоверным уменьшением количества CD 25 + клеток. Развитие деструктивно-воспалительного процесса в миокарде больных дилатационной кардиомиопатией приводит к нарушениям клеточных факторов иммунитета.

Ключевые слова: дилатационная кардиомиопатия, лимфоциты, клеточный иммунитет.

Summary

Bespalova E. Ya. *Cellular factors of immunity peculiarities in patients with dilated cardiomyopathy.*

Studies of cellular immunity factors showed that in patients with dilated cardiomyopathy imbalance of cellular link of the immune system is observed, which revealed significant reduction in the relative number of CD3+ lymphocytes, imbalance of immunoregulatory cells increase in helper and suppressor cell population, which led to an increase in immunoregulation index. At the same time there has been a significant increase in CD16+ and CD95+ lymphocytes. Reduced of lymphocytes functional activity, manifested reliable decrease in CD 25+ cells. Development of destructive, inflammatory process in the myocardium of patients with dilated cardiomyopathy leads to disturbances of factors cellular immunity.

Key words: dilated cardiomyopathy, lymphocytes, cellular immunity.

Рецензент: д.мед.н., проф.М.О.Пересадін

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ALTERNATIVELY SPLICED INTERLEUKIN-4 PROTEIN, INTERLEUKIN-4 DELTA2, IS NATURALLY SECRETED BY T CELLS

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Introduction

Interleukin-4 (IL-4) is a pleiotropic cytokine produced predominantly by Th2 lymphocytes that is involved in the regulation of various functions of B cells [9], T cells [15], macrophages [3], and other hematopoietic [12] and nonhematopoietic cells [6]. IL-4 gene is expressed in two mRNA forms: 1) full-length form containing all four exons and 2) alternatively spliced mRNA form, known as interleukin-4 delta 2 (IL-4δ2), in which exon 2 (corresponds to amino acids 22 to 37) is omitted [10,18]. Expression of IL-4δ2 mRNA was described previously in peripheral blood mononuclear cells (PBMC), thymocytes and bronchoalveolar lavage cells. It was observed that IL-4δ2 mRNA is expressed in the PBMC in lower amounts than IL-4 mRNA. In contrast, IL-4δ2 mRNA is expressed in much higher levels than IL-4 mRNA in thymocytes and bronchoalveolar lavage cells, suggesting tissue specificity of expression [2,13]. It was also reported that the total levels of combined IL-4 + IL-4δ2 mRNAs and IL-4/IL-4δ2 mRNA ratios change in patients with asthma [7, 16], systemic sclerosis [17], associated with infectious diseases such as pulmonary tuberculosis [4], Helicobacter pylori infection [11], HIV-tuberculosis co-infection [5], and in patients with severe sepsis [8]. However, it still remains unknown whether IL-4δ2 is expressed as a protein in vivo. Comparative analysis between predicted molecular model of IL-4δ2 proteins and crystal structure of human recombinant interleukin-4 showed that they have similar conformational structure and nearly identical charge [20].

Recombinant human IL-4 delta 2 protein (rhIL-4δ2p) was expressed in yeast [2] and in Escherichia coli [14] and effects of rhIL-4 δ2p in vitro were studied. As described previously, rhIL-

4d2p is an antagonist of the hIL-4-induced synthesis of IgE and expression of CD23 in B cells [1]; blocks inhibitory action of hIL-4 on LPS-induced cyclooxygenase-2 expression and subsequent prostaglandin E2 secretion in monocytes [1]; has no independent effect on proliferation of T cells, B cells, or Mφ and competes with IL-4 effects on T cell proliferation [2, 20]. These data suggest that IL-4δ2p acts as a regulator of the cytokine net, being the natural antagonist of IL-4. The purpose of this study was to investigate whether natural IL-4δ2p produced and secreted by T cells. In this paper we demonstrate that alternatively spliced IL-4δ2p naturally produced and secreted by human T lymphocytes.

Patients and Methods

Patients and controls. Seven adult patients with allergic asthma and five healthy volunteers were included in the study. The diagnosis of asthma was made following The National Asthma Education and Prevention Program (NAEPP) guidelines. Healthy controls were defined as current non-smokers who did not smoke in the past 3 years, with no known allergies and no asthma, and were older than 21 years. Peripheral blood from patients and controls was obtained by venipuncture.

Cell preparation and culture condition. Human embryonic kidney (HEK-293) was obtained from the American Type Culture Collection (ATCC, Manassas, VA). Cells were grown in RPMI 1640 (Gibco, Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, 2 mM glutamine, 2 mM sodium pyruvate, and antibiotic-antimicotic. HEK 293 cells were maintained at 37°C in T75 culture flasks in humidified atmosphere with 5% CO_2. T cells were isolated from whole blood by negative selection using RosetteSep® Human T Cell Enrichment Cocktail according to the protocol of the manufacturer (StemCell Technologies Inc., Vancouver, British Columbia, Canada). RPMI1640 culture medium supplemented with 10% dialyzed fetal bovine serum, 2 mM glutamine, 2 mM sodium pyruvate and antibiotic-antimicotic was used to culture T cells at 5x106 cells/ml.

Transfection and Plasmids. Primary T-cells from healthy donors were transfected with IL-4 or IL-4δ2 or Null-encoding constructs using electroporation technique (Amaxa, Gaithersburg, MD). Transfection of T cells with resulted in IL-4 or IL-4δ2

protein secretions confirmed by ELISA, and Western blotting using antibodies purchased from R&D Systems (Minneapolis, MN, USA) and BD Pharningen (San Jose, CA, USA).

Immunodepletion of IL-4. For IL-4 immunodepletion experiment T cells from 4 healthy donors and 9 asthma patients were activated for 24 hrs with 50 ng/ml Phorbol 12- myristate 13-acetate (PMA) and 1μM Ionomycin (Sigma, St. Louis, MO). After 24 hrs of culture the supernatants were harvested for determination of IL-4 and IL-4δ2. Monoclonal anti-human IL-4 antibody was purchased from R&D Systems (Minneapolis, MN) and BD Pharningen (San Jose, CA, USA). Protein G agarose was purchased from Thermo Scientific (Rockford, IL). Immunoprecipitation of rhIL-4 or native human IL-4 was performed according to the protocol of the manufacturer (Thermo Scientific, Rockford, IL) with slight modifications. Briefly, protein G agarose slurry and anti-human IL-4 antibody were incubated with gentle mixing for 2 hours at RT under rotary agitation. Protein G agarose was washed three times with IP Buffer (25 mM Tris, 150 mM NaCl; pH 7.2). Recombinant IL-4 and IL-4δ2 or supernatants of PMA/Ionomycin stimulated T lymphocytes were incubated with Protein G-antibody complex for 2 hrs at RT. Supernatants were collected and sandwich ELISA with indiscriminate anti-human IL-4 antibody (BD Pharmingen, San Diego, CA) was performed.

Statistical analysis of data. The results were statistically analyzed with a statistical package IBM SPSS version 19 (Chicago, IL). For all statistical analysis, the level of significance was set at $P<0.05$.

Results

Patient and control characteristics. Patients and controls did not differ in their gender or age distributions. Approximately one third of patients were smokers, whereas all healthy volunteers were non-smokers. More than half of the patients had mild disease. Blood samples from subsets of this cohort were used in the experiments presented below, with characteristics of the subsets presented in each case.

Primary human T lymphocytes secrete IL-4δ2 protein. Previous studies focused on expression levels of IL-4δ2 mRNA (15-21), whereas the expression of the corresponding protein has not been addressed. It was previously reported that a cell line HEK293 infected with IL-4-encoding or IL-4δ2-encoding adenoviral constructs or

transfected with corresponding plasmid constructs produces and secretes IL-4 or IL-4δ2 in cell culture. In that study, antibodies were used that react selectively with IL-4 but not IL-4δ2 (selective Ab) or with both IL-4 and IL-4δ2 (indiscriminate Ab). Although those results suggested that IL-4δ2 protein is produced in the cells expressing IL-4δ2 mRNA, limitations were that HEK293 is a transformed cell line and that expression of IL-4δ2 mRNA was forced by the gene delivery procedures. We hypothesized that primary T cells also can produce and secreted IL-4δ2 protein. To confirm this hypothesis primary T cells from two separate donors were purified and electrophoretically transfected with IL-4δ2-encoding or Null constructs. 48 hrs after transfection supernates were collected and ELISA assays with selective anti-IL-4 Ab and indiscriminate anti-IL-4/IL-4δ2 Ab were performed (Figure 1). Thus, primary T cells can produce and secreted IL-4δ2p but there is still limitation here - the production of IL-4δ2 was induced artificially. So, it was unclear whether primary T cell produced and secreted IL-4δ2p without artificial manipulations.

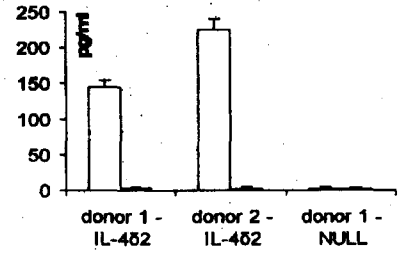

Figure 1. ELISA of cell culture supernates of purified primary T cells from two separate donors transfected with IL-4δ2-encoding or NULL constructs.

Detection of naturally produced IL-4δ2 by T cells. Scheme of the immunodepletion experiments is shown in Figure 2.

Initially, two rounds of immunodepletion of recombinant human IL-4 and IL-4δ2 proteins with known concentration 50 or 100 pg/ml were performed, and the relative amounts of cytokines in the solutions before and after immunodepletion were calculated by ELISA with indiscriminate antibody (Figure 3).

Concentrations of rhIL-4 before immunodepletion were 50 or 100 pg/ml and after immunodepletion IL-4 protein was not detected. In contrast, essentially the same concentrations of rhIL-4δ2 in the solutions were detected before and after immunodepletion. Thus, IL-4p but not IL-4δ2p may be completely removed from solution by

immunodepletion. In next experiment primary T cell from 4 healthy donors and 8 asthma patients were purified and activated with PMA/Ionomycin for 24 hrs. T cells supernatants were collected and immunodepletion of IL-4 was performed. Concentration of IL-4 and IL-4δ2 in the supernatants before and after immunodepletion were calculated by ELISA with indiscriminate antibody (Figure 3).

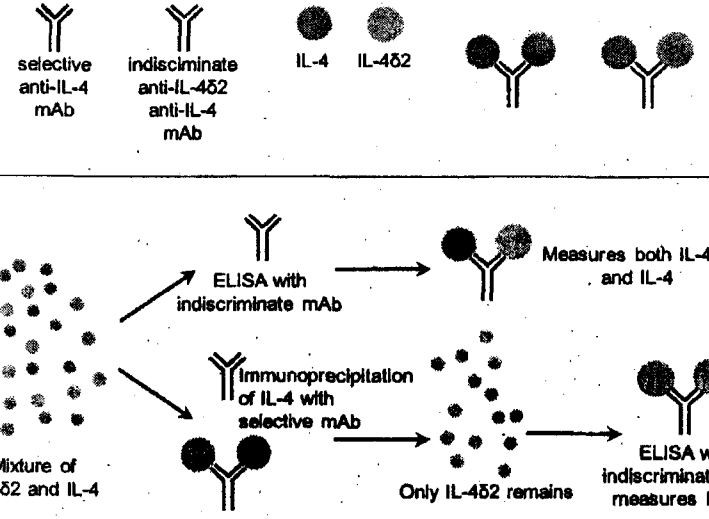

Figure 2. Scheme of the immunodepletion experiment.

Healthy donors before immunodepletion had low levels of IL-4 and IL-4δ2 (20-30 pg/ml) and after immunodepletion we could not detect any product. In contrast, the levels of IL-4 and IL-4δ2 in T cells supernatants from asthma patients before immunodepletion were higher (40-120 pg/ml) and even three rounds of immunodepletion could not remove reactivity of indiscriminate Ab. Thus, we suggest that this reactivity of indiscriminate Ab caused by IL-4δ2 that was naturally secreted by activated T cells.

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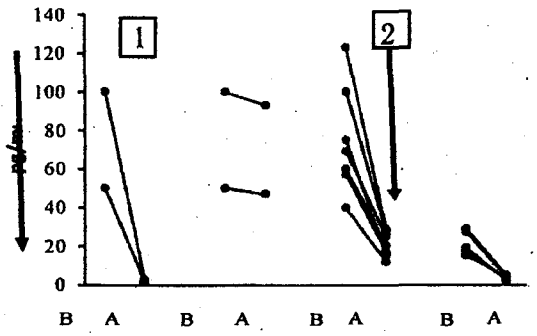

Figure 3. Indiscriminate ELISA (detects both IL-4δ2 and IL-4) of indicated samples before (B) and after (A) immunodepletion of IL-4 with selective anti-IL-4 antibody attached to solid support. Notice that rhIL-4 but not rhIL-4δ2 added to fresh cell culture medium was completely depleted (arrow 1) by immunoprecipitation (details described in the text). In the samples from atopic asthmatics, such selective immunodepletion of IL-4 did not completely eliminate the signal (arrow 2). The remaining signal is likely due to naturally produced IL-4δ2. Additional two cycles of immunodepletion of IL-4 did not lower the remaining reactivity of these samples. The entire experiment was repeated with newly derived T cells from new blood samples of the same control and atopic asthmatic volunteers on three independent occasions with one week intervals between the repetitions, with similar results (second repetition is shown).

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Summary

Bocharov A. *Alternatively spliced interleukin-4 protein, interleukin-4 delta2, is naturally secreted T cells.*

An alternatively spliced isoform of IL-4 mRNA that omits exon 2 (IL-4δ2) was previously described. It was reported that the total levels of combined IL-4 + IL-4δ2 mRNAs and IL-4/IL-4δ2 mRNA ratios change in patients with scleroderma, asthma, and various infections. Natural IL-4δ2 protein was not previously studied because most commercial anti-IL-4 antibodies (Ab) do not react with IL-4δ2. We established mammalian expression systems (HEK293 stably transfected or infected with recombinant adenoviral constructs) producing naturally processed (folded and glycosylated) human IL-4 or IL-4δ2. By screening a library of commercial anti-IL-4 Abs, we identified a selective Ab reacting exclusively with IL-4, and an indiscriminate Ab reacting equally with IL-4 and IL-4δ2. PBMC and T lymphocyte (> 95% purity) cultures from 3 donors with allergic asthma and 3 controls were stimulated with PMA/ionomycin for 24 hrs. The supernates were immunodepleted of wild-type IL-4 using four cycles of depletion with the selective antibody. These immunodepleted supernates were negative in ELISAs with the selective anti-IL-4 Ab. However, the IL-4-depleted supernates from asthmatics, but not controls, were positive in ELISAs with the indiscriminate antibody that now detected only IL-4δ2. The levels of IL-4δ2 were 5 to 10 fold lower than the levels of IL-4 in the initial undepleted supernates. Thus, IL-4δ2 is naturally produced not only in the mRNA form, but also as a secreted protein.

Key words: interleukin-4δ2, immunoprecipitation, T-lymphocytes, allergic asthma.

Резюме

Бочаров А. А. *Продукция и секреция альтернативного сплайс-варианта интерлейкина-4, интерлейкин-4 дельта 2, Т-лимфоцитами.*

Альтернативный сплайс-вариант интелейкина-4, интерлейкин-4 дельта 2, ранее был описан только в форме мРНК. Продукция и секреция альтернативной формы белка ИЛ-4δ2 не была описана ранее в связи с тем, что большинство коммерческих анти-ИЛ-4 антител не реагируют с ИЛ-4δ2. Скрининг коммерчески доступных анти-ИЛ-4 антител позволил выявить селективные антитела, которые способны взаимодействовать только с ИЛ-4 и антитела реагирующие, как с ИЛ-4 так и с ИЛ-4δ2. Культуры Т-лимфоцитов периферической крови, выделенные от доноров с аллергической астмой и здоровых людей стимулировали ФМА/иономицином в течение 24 часов. Супернатанты использовали для иммунопреципитации интелейкина-4 с селективными анти-ИЛ-4 антителами. Концентрации ИЛ-4 в супернатантах Т-лимфоцитов определяли до и после иммунопреципитации методом ИФА. В супернатантах Т-лимфоцитов контрольной группы после иммунопреципитации ИЛ-4 не обнаруживался, в то время как исследование супернатантов Т-лимфоцитов от пациентов с астмой показало положительную реакцию в ИФА при использовании неселективных антител даже после 3 циклов иммунопреципитации ИЛ-4. Этот факт позволил сделать обоснованное предположение о наличии в супернатантах ИЛ-4δ2. Уровни ИЛ-4δ2 были в 5-10 раз ниже, чем уровни ИЛ-4 в непреципитированных супернатантах. Таким образом, установлено, что альтернативный сплайс-вариант интерлейкина-4, интерлейкин-4 дельта 2, экспрессируется в Т-лимфоцитах не только в форме мРНК, но и на уровне белка.

Ключевые слова: интерлейкин-4 делта 2, иммунопреципитация, Т-лимфоциты, аллергическая астма.

Резюме

Бочаров О. А. *Продукція і секреція альтернативного сплайс-варіанту інтерлейкіна-4, інтерлейкін-4 дельта 2, Т-лімфоцитами.*

Альтернативний сплайс-варіант інтерлейкіна-4, інтерлейкін-4 дельта 2, раніше був описаний лише у формі мРНК. Продукція і секреція альтернативної форми білка ІЛ-4δ2 не була описана раніше у зв'язку з тим, що більшість комерційних анти-ІЛ-4 антитіл не реагують з ІЛ-4δ2. Скринінг комерційно доступних анти-ІЛ-4 антитіл дозволив виявити селективні антитіла, які здатні взаємодіяти тільки з ІЛ-4 і антитіла, що реагують як з IL-4 і з IL-4δ2. Культури Т-лімфоцитів периферичної крові, виділені від донорів з алергічною астмою і здорових людей стимулювали ФМА/іономіцином протягом 24 годин. Супернатанти використовували для імунопреципітації інтелейкіна-4 з селективними анти-ІЛ-4 антитілами. Концентрації ІЛ-4 в супернатантах Т-лімфоцитів визначали до і після імунопреципітації методом ІФА. В супернатантах Т-лімфоцитів контрольної групи після імунопреципітації ІЛ-4 не виявлявся, в той час як дослідження супернатантів Т-лімфоцитів від пацієнтів з астмою показало позитивну реакцію при використанні неселективних антитіл навіть після 3 циклів імунопреципітації ІЛ-4. Цей факт дозволив зробити обґрунтоване припущення про наявність в супернатантах ІЛ-4δ2. Рівні ІЛ-4δ2 були в 5-10 разів нижче, ніж рівні ІЛ-4 в непреципітованих супернатантах. Таким чином, встановлено, що альтернативний сплайс-варіант інтелейкіна-4, інтерлейкін-4 дельта 2, експресується в Т-лімфоцитах не тільки у формі мРНК, а й на рівні білка.

Ключові слова: інтерлейкін-4 дельта 2, імунопреципітація, Т-лімфоцити, алергічна астма.

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