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КОЭКСПРЕССИЯ МЕМБРАНОСВЯЗАННЫХ РЕЦЕПТОРОВ К ФАКТОРУ НЕКРОЗА ОПУХОЛИ АЛЬФА НА ОСНОВНЫХ ПОПУЛЯЦИЯХ ИММУНОКОМПЕТЕНТНЫХ КЛЕТОК ЗДОРОВЫХ И БОЛЬНЫХ РЕВМАТОИДНЫМ АРТРИТОМ И БРОНХИАЛЬНОЙ АСТМОЙ

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Резюме. TNFα – плейотропный цитокин, относящийся к важным воспалительным медиаторам ряда заболеваний и реализующий свои биологические функции через два его различных рецептора, TNFR1 и TNFR2. Важное значение в реализации ответа клетки на действие TNFα имеет изменение соотношения данных типов рецепторов, способствующее смещению баланса между проапоптотическими и пролиферативными сигнальными путями. Протекающие в организме патологические процессы могут изменять уровень экспрессии TNFR1 и TNFR2 на клетках, вовлеченных в развитие заболевания.

Цель исследования – изучение уровня коэкспрессии рецепторов 1-го и 2-го типа к TNFα на основных субпопуляциях клеток периферической крови у больных ревматоидным артритом и бронхиальной астмой.

Для оценки уровня коэкспрессии рецепторов 1-го и 2-го типа к TNFα использовалась цельная кровь условно-здоровых доноров больных ревматоидным артритом и бронхиальной астмой. Оценка фенотипических характеристик проводилась методом проточной цитометрии. Для одновременного определения количества рецепторов TNFα типов 1 и 2 на различных субпопуляциях проводили двойное мечение парных образцов. После цитометрического анализа подстчитывли количество рецепторов 1-го и 2-го типа, а также процент каждой фракции.

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Наиболее выраженными изменениями в соотношении процента клеток, экспрессирующих TNFR1 и TNFR2, отличалась популяция В-лимфоцитов. Для популяции Т-лимфоцитов характерны были различия по проценту клеток, экспрессирующих только TNFR1, у больных РА и БА по сравнению со здоровыми донорами и между собой. Для моноцитов характерен больший процент дубль отрицательных клеток при БА и РА по сравнению со здоровыми донорами. Полученные данные свидетельствуют о различии в профиле коэкспрессии рецепторов 1-го и 2-го типа к TNFα при РА и БА как по сравнению со здоровыми донорами, так и между данными заболеваниями.

Основные популяции иммунных клеток активно вовлечены в патогенез как ревматоидного артрита, так и бронхиальной астмы и полученные результаты могут свидетельствовать о возможностях этих клеток по-разному отвечать на действие TNFα при изменении соотношения процента и количества рецепторов на их поверхности.

Ключевые слова: клеточная иммунология, иммунная регуляция, коэкспрессия, рецепторы фактора некроза опухоли, ревматоидный артрит, бронхиальная астма

CO-EXPRESSION OF MEMBRANE-BOUND TUMOR NECROSIS FACTOR-α RECEPTORS IN MAJOR SUBPOPULATIONS OF IMMUNOCOMPETENT CELLS IN HEALTHY INDIVIDUALS AND PATIENTS WITH RHEUMATOID ARTHRITIS AS WELL AS BRONCHIAL ASTHMA

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Abstract. A pleiotropic cytokine TNFα is an important inflammatory mediator of a number of diseases; its biological functions are fulfilled through two different receptors, TNFR1 and TNFR2. Changes in the ratio between these types of receptors shifting the balance between the pro-apoptotic and proliferation signaling pathways play a crucial role in eliciting the cell response to TNFα. The pathological processes in the body can alter the levels of TNFR1 and TNFR2 expression on the cells involved in disease development. Therefore, this study was aimed at investigating the level of co-expression of type 1 and 2 TNFα receptors in the major subpopulations of peripheral blood cells in patients with rheumatoid arthritis (RA) and bronchial asthma (BA). The greatest changes in the percentage of cells expressing TNFR1 and TNFR2 were revealed for the B-lymphocyte subpopulation. For the T-lymphocyte subpopulation, there were some differences in the percentage of cells expressing exclusively TNFR1 in RA and BA patients compared with those in healthy subjects, as well as between the RA and BA groups. A higher percentage of double-negative monocytes was observed in patients with BA and RA compared to healthy subjects. These findings indicate that the co-expression profile of TNFR1 and TNFR2 receptors in patients with RA and BA differ within these groups as well as compared to that in healthy subjects. These immune cell populations are actively involved in the pathogenesis of both rheumatoid arthritis and bronchial asthma, so the results may indicate that these cells might show different responses to TNFα as the percentage and the number of receptors on their surface vary.

Keywords: cellular ummunology, immune regulation, co-expression, tumor necrosis factor receptors, rheumatoid arthritis, bronchial asthma

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Introduction

TNFα is a pleiotropic cytokine playing a crucial role in maintaining immune homeostasis and contributing to disease development [1]. This cytokine is one of the important inflammatory mediators in a number of diseases such as autoimmune, cardiovascular, and allergic diseases, cancer, etc. [6]

It is believed that the biological functions of TNFα are fulfilled through its two different receptors, TNFR1 and TNFR2 [5, 6]. Along with the cytokine TNFα per se, these receptors significantly contribute to the development of immune-mediated diseases [7, 10]. Changed ratio between the different receptor types shifting the balance between the pro-apoptotic and proliferation signaling pathways play a crucial role in eliciting the cell cytokine response. It is noteworthy that the pathological processes occurring in the body can alter the expression levels of different receptor types, which may be one of the mechanisms explaining why immunocompetent cells exhibit different responses. This was supported by a number of publications showing that the major immune cell subsets differ in their expression and co-expression levels in healthy subjects and in patients with immune-mediated diseases being also associated with inflammation severity [2, 8, 9].

Co-expression of different types of TNFα receptors has been studied only for few diseases, so it seems rather promising to broaden the range of investigated pathologies and compare data both with those for healthy subjects and between the groups of patients with diverse diseases. The findings obtained in this study will contribute both to understanding the foundations of the cytokine network function and elaborating novel approaches to the diagnostics and prediction of the course of immunopathological conditions due to identified changes compared to healthy subjects. Therefore, the aim of this study was to investigate the co-expression level of type 1 and type 2 TNFα receptors on the major peripheral blood cell subsets in patients with rheumatoid arthritis (RA) and bronchial asthma (BA).

Materials and methods

The co-expression levels of type 1 and 2 TNFα receptors were assessed using whole blood samples from otherwise healthy subjects with rheumatoid arthritis and bronchial asthma receiving treatment at the Clinic of Immunopathology of the Research Institute of Fundamental and Clinical Immunology.

The study involved 46 apparently healthy subjects aged 18-77 years (median (IQR): 35.6 (30-54) years), including 16 (34.8%) males and 30 (65.2%) females; 64 patients with rheumatoid arthritis aged 22-83 years (median (IQR): 55 (45-65) years), predominantly including females (54 (85.4%)) and 10 males; as well as 22 patients with bronchial asthma aged 22-70 years (median (IQR): 46 (32-49) years), including 16 (72.7%) females and 6 males.

Fasting venous blood samples were collected from the median cubital vein under sterile conditions into 6 ml vacuum tubes containing K3-EDTA anticoagulant (EDTA tripotassium salt, Vacuette K3-EDTA, Greiner Bio-One GmbH, Austria)

Sample preparation was performed by using BD FACS Lysing Solution (Catalog # 349202; BD, USA) according to the manufacturer's protocol.

Flow cytometry

The phenotypic traits were assessed by flow cytometry on a FACSVerse cytofluorometer (BD, USA) using anti-human CD3 APC/Cy7, anti-human CD19 PE/Cy7, anti-human CD14 PerCP, anti-human TNFRI-PE, anti-human TNFRII-PE, anti-humanTNFRI-APC, and anti-humanTNFRII-APC monoclonal antibodies (R&D Systems, Minneapolis, MN). The data on fluorescence intensity were processed and calculated by using the FacsDiva software (BD, USA).

In order to simultaneously quantify type 1 and 2 TNFα receptors on different cell subpopulations, sample pairs were double-labeled ($TNFR1^+TNFR2^-$, $TNFR1^+TNFR2^+$, $TNFR1^-TNFR2^+$, and $TNFR1^-TNFR2^-$). Each sample containing a certain quantity of rhTNF (or a control sample without TNF) was split and placed into two tubes to be stained with TNFR1-PE and TNFR2-APC or TNFR2-PE and TNFR1-APC.

After the cytometric analysis, the quantity of TNFR1 (for the $TNFR1^+TNFR2^-$ and $TNFR1^+$ TNFR2 fractions) was counted in the tubes containing TNFR1-PE and TNFR2-APC. The quantity of TNFR2 (for the $TNFR1^-TNFR2^+$ and $TNFR1^+TNFR2^+$ fractions) was counted in the tubes containing TNFR2-PE and TNFR1-APC. The percentage of each fraction was determined as the mean value for two samples.

Statistics

Statistical analysis of the data was performed using the STATISTICA 7.0 software (StatSoft, USA). The data are presented as the median values normalized to the mean values. Independent samples were compared and statistical significance of the differences was determined using the Kruskal–Wallis non-parametric ANOVA test by ranks with multiple comparisons of

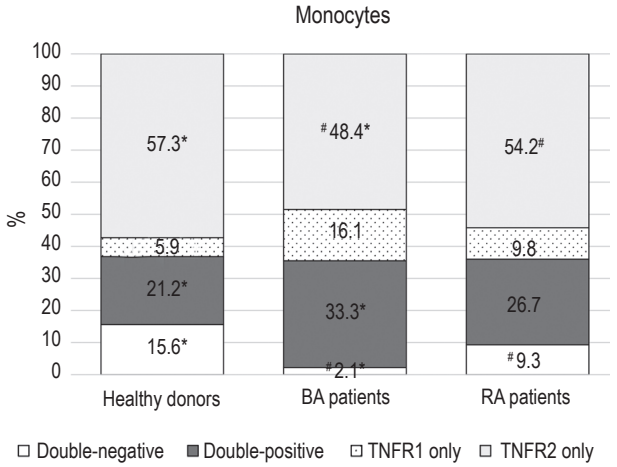

Figure 1. Percentage of cells carrying the receptors among monocytes in healthy donors and patients with BA and RA

Note. The data are presented as the medians normalized to the mean values.*, healthy subjects *vs* BA patients ($p < 0.05$); #, patients with RA and BA ($p < 0.05$).

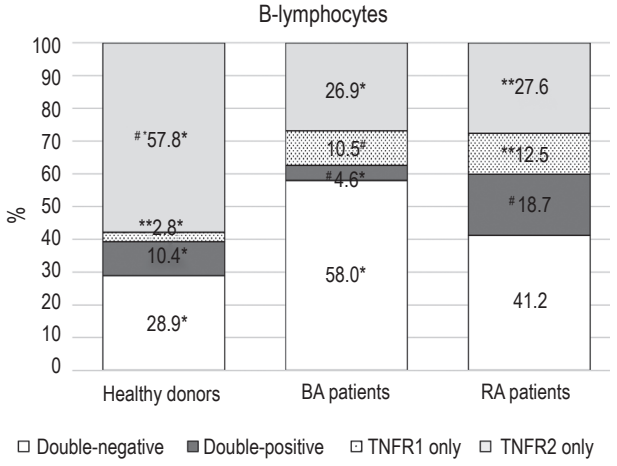

Figure 2. Percentage of cells carrying the receptors among B-lymphocytes in healthy donors and patients with BA and RA

Note. The data are presented as the medians normalized to the mean values. *, healthy subjects *vs* BA patients ($p < 0.05$); **, healthy subjects *vs* RA patients ($p < 0.05$); #, patients with RA and BA ($p < 0.05$).

the medians (when comparing identical parameters for different subpopulations.)

Results and discussion

Earlier studies focused on expression of TNFR1 and TNFR2 on the major mononuclear cell subsets (such as the total monocyte pool, total B-cell and T-cell pools), revealed that expression levels of receptors for immunomodulatory cytokines differ in healthy subjects and in patients with rheumatoid arthritis, atopic dermatitis, and pulmonary tuberculosis [3, 4], thus indicating that not only the parameters of produced mediators and soluble receptors are changed during inflammatory diseases, but expression of membrane-bound receptors on the surface of immunocompetent cells is affected as well. In order to deeper understand a role played by the ratio between TNFR1 and TNFR2, we compared the percentage of cells carrying receptors on the major subsets of immunocompetent cells in healthy subjects and in patients with RA and BA.

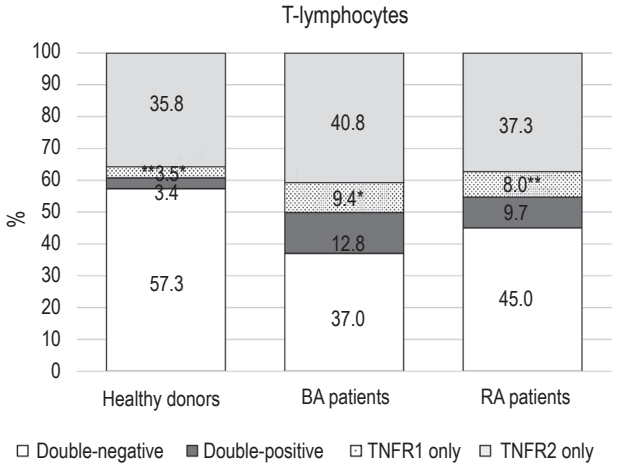

Figure 3. Percentage of cells carrying the receptors among T-lymphocytes in healthy donors and patients with BA and RA

Note. The data are presented as the medians normalized to the mean values.*, healthy subjects *vs* BA patients ($p < 0.05$); **, healthy subjects *vs* RA patients ($p < 0.05$).

It was found that the main subpopulations (monocytes, B-lymphocytes, the total T-lymphocyte pool, and the regulatory T-cells) in patients with BA and RA significantly differed both in terms of co-expressed TNFR1 and TNFR2 between the groups of patients with such diseases compared with healthy subjects.

In monocytes (Figure 1), significant difference in percentage of double-negative cells were revealed between patients with BA and healthy subjects (2.1 *vs* 15.6; $p < 0.0001$), as well as between patients with RA or BA (9.3 *vs* 2.1; $p = 0.0094$).

Healthy subjects and patients with BA significantly differed in percentage of double-positive cells (21.2 *vs* 33.3; $p = 0.0011$). The percentage of cells carrying solely TNFR1 differed in healthy subjects and in patients with BA (57.3 *vs* 48.4; $p < 0.0001$) as well as patients with BA and RA (48.4 *vs* 54.2; $p = 0.0006$).

Differences in the percentage of B-lymphocytes (Figure 2) are typically observed between healthy subjects and patients with BA and RA. The percentage of double-negative cells was higher in patients with BA (50.8 *vs* 28.8; $p < 0.0001$); in contrast, the percentage of double-positive cells was higher in healthy subjects (10.4 *vs* 4.6; $p = 0.0069$). The percentage of cells

expressing solely TNFR2 was significantly higher in healthy subjects compared to that in patients with BA (57.8 *vs* 26.9; $p < 0.0001$) and RA (57.8 *vs* 27.6; $p < 0.0018$). Groups of patients with BA and RA differed in percentage of double-positive cells (4.6 *vs* 18.7; $p = 0.0005$). The percentage of cells expressing solely TNFR1 differed between healthy subjects and patients with BA (2.8 *vs* 10.5; $p = 0.0006$) and RA (2.8 *vs* 12.5; $p < 0.0001$).

For the T-lymphocyte subpopulation (Figure 3), the significant changes involved only variation in the percentage of cells expressing solely TNFR1 between healthy subjects and patients with BA (3.5 *vs* 9.4; $p < 0.0001$) and RA (3.5 *vs* 8.0; $p = 0.0012$).

Conclusion

The study showed that the peak changes in the number of cells carrying TNFα receptors were observed in the B-lymphocyte subpopulation: the significant difference in distribution of receptor-carrying cells in the group of BA patients compared to healthy subjects was observed. This cell subpopulation is actively involved in the pathogenesis of both diseases. The findings indicate that these cells might differ in their response to TNFα as the percentage and number of receptors vary. Differences in the percentage of cells expressing solely TNFR1 in patients with RA and BA compared to healthy subjects and between the groups of RA and BA patients were typical to T-lymphocyte subpopulation. A higher percentage of double-negative cells in patients with BA and RA compared to that in healthy subjects was revealed for monocytes.

These findings demonstrate that TNFR1 and TNFR2 are redistributed within the major subpopulations of immunocompetent cells in patients with rheumatoid arthritis and bronchial asthma compared to those in healthy subjects, as well as within the groups of patients having BA and RA.

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