

Т-ЛИМФОЦИТЫ 2-ГО ТИПА ИММУННОГО ОТВЕТА И ИХ РОЛЬ В УСИЛЕНИИ ВОСПАЛЕНИЯ ПРИ ВЫПОЛНЕНИИ ПРОФЕССИОНАЛЬНОЙ ДЕЯТЕЛЬНОСТИ ПОЖАРНЫХ

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Резюме. Т-лимфоциты 2-го типа иммунного ответа способствуют развитию и обострению воспаления, главным образом аллергического. Усиление воспаления при длительном воздействии неблагоприятных факторов при работе пожарных может привести к развитию различных заболеваний. Оценка иммунитета пожарных важна для назначения адекватного лечения и профилактики инфекционных и аллергических заболеваний. Целью работы стал анализ показателей иммунитета у сотрудников государственной противопожарной службы МЧС России в зависимости от возраста и интенсивности профессиональной нагрузки. Обследованы мужчины (n = 79), средний возраст 31 год, стаж работы от 1 года до 22 лет с различной интенсивностью нагрузки. В периферической крови методом проточной цитометрии (Navios, FC 500, Beckman Coulter) оценивали субпопуляции моноцитов, относительное количество Т-лимфоцитов 2-го типа иммунного ответа CD3⁺CD294⁺. Определяли концентрацию общего иммуноглобулина Е (Immulite). В секрете носовых ходов оценивали содержание секреторного иммуноглобулина А (Вектор Бест) (n = 30). Статистическую обработку результатов проводили с помощью пакета Statistica 12.0 (StatSoft). Увеличение количества CD3⁺CD294⁺ клеток наблюдали в 16,5%. Выявили прямую корреляционную зависимость количества Т-лимфоцитов 2 и возраста обследованных лиц (p < 0,05). В группе пожарных с более интенсивной нагрузкой в 5 раз выше встречали повышение количества CD3⁺CD294⁺ клеток (p < 0,05). Среди пациентов, имевших какое-либо заболевание респираторного тракта, увеличение этой популяции наблюдали статистически значимо чаще – в 26% случаев против 11,5%. Выявили сильную прямую корреляционную зависимость количества Т-лимфоцитов 2 и длительности стажа курения (p < 0,05). Установили прямую корреляционную зависимость количества Т-лимфоцитов 2 и концентрации общего IgE (p < 0,05). Снижение секреторного IgA в секрете из носовых ходов наблюдали у 23% пожарных, у 13% обследо-

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важных показателей выходил за верхнюю границу референтного интервала. Значимо чаще выявляли отклонение этого показателя от референтных значений у пожарных с высокой нагрузкой. Установили повышение субпопуляции классических моноцитов в группе обследованных с высоким количеством CD3⁺CD294⁺ клеток ($p < 0,05$). Таким образом, с увеличением профессиональной нагрузки пожарных в неблагоприятных условиях несения службы отмечается угнетение противоинойфекционной защиты и усугубление повреждения респираторного тракта при усилении 2-го типа иммунного ответа. Оценка количества Т-лимфоцитов 2-го типа в периферической крови позволит выявить предрасположенность к Т2 профилю иммунного воспаления, что будет способствовать персонализированному подходу к ведению пациентов.

Ключевые слова: Т-лимфоциты 2, CD3⁺CD294⁺ клетки, пожарные, воспаление, секреторный иммуноглобулин А, общий иммуноглобулин Е

T LYMPHOCYTES OF THE 2nd TYPE OF THE IMMUNE RESPONSE AND THEIR ROLE IN ENHANCING INFLAMMATION DURING THE PROFESSIONAL ACTIVITIES OF FIREFIGHTERS

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Abstract. T lymphocytes of the 2nd type of immune response contribute to the development and exacerbation of inflammation, mainly allergic. Increased inflammation with prolonged exposure to adverse factors during the work of firefighters can lead to the development of various diseases. Evaluation of the immunity of firefighters is important for the appointment of adequate treatment and prevention of infectious and allergic diseases. This paper aimed to analyze the indicators of immunity in employees of the state fire service of EMERCOM of Russia, depending on the age and intensity of the professional workload. The surveyed were men ($n = 79$), mean age 31 years, work experience from 1 to 22 years, with different workload intensity. In peripheral blood, flow cytometry (Navios, FC 500, Beckman Coulter) was used to evaluate subpopulations of monocytes, the relative number of T lymphocytes of the 2nd type of immune response CD3⁺CD294⁺. The concentration of total immunoglobulin E (Immulite) was determined. From nasal secretions, the content of secretory immunoglobulin A (Vector Best) was evaluated ($n = 30$). Statistical processing of the results was performed using the Statistica 12.0 package (StatSoft). An increase in the number of CD3⁺CD294⁺ cells was observed in 16.5%. A direct correlation was found between the number of T lymphocytes 2 and the age of the examined persons ($p < 0.05$). In the group of firefighters with a more intense workload, an increase in the number of CD3⁺CD294⁺ cells were 5 times higher ($p < 0.05$). Among patients who had any disease of the respiratory tract, an increase in this population was observed statistically significantly more often – in 26% of cases *versus* 11.5%. A strong direct correlation was found between the number of T lymphocytes 2 and the duration of smoking experience ($p < 0.05$). A direct correlation was established between the number of T lymphocytes 2 and the concentration of total IgE ($p < 0.05$). A decrease in secretory IgA in the secret from the nasal passages was observed in 23% of firefighters, in 13% of the examined, the indicator went beyond the upper limit of the reference interval. Significantly more often revealed the deviation of this indicator from the reference values in firefighters with a high workload. An increase in the subpopulation of classical monocytes was established in the group of those examined with a high number of CD3⁺CD294⁺ cells ($p < 0.05$). Thus, with an increase in the professional workload of firefighters in unfavorable conditions of service, inhibition of protection to infection and aggravation of damage to the respiratory tract with an increase in the 2nd type of immune response are noted. Evaluation of the number of type 2 T lymphocytes in peripheral blood will reveal a predisposition to the T2 profile of immune inflammation, which will contribute to a personalized approach to patient management.

Keywords: T lymphocytes 2, CD3⁺CD294⁺ cells, firefighters, inflammation, secretory immunoglobulin A, total immunoglobulin E

Introduction

In the pathogenesis of autoimmune, oncological, allergic, and infectious diseases, a significant role is given to disorders in the immune system [8] because it is one of the three regulatory systems of the human organism. Inflammation is a fundamental biological process underlying almost all immune responses [2]. T lymphocytes of the 2nd type of immune response contribute to the development, maintenance, and exacerbation of inflammation, mainly allergic, with the participation of several mechanisms. This cell population is involved in prolonging immediate and delayed types of inflammation, as well as eosinophilic and neutrophilic [1]. An increase in type 2 T lymphocytes in the peripheral blood has been described in patients with allergic [9] and autoimmune diseases [13]. Recently, a hypothesis has been proposed [5] that cells of the 2nd type of immune response, in addition to the development of allergic reactions, control chronic inflammatory diseases, and metabolic homeostasis, take part in the repair and fibrosis of tissues, etc.

Strengthening the processes of local and systemic inflammation, which develop as a result of prolonged exposure to adverse factors in the respiratory tract of firefighters, can lead to the development of diseases of the respiratory and cardiovascular systems and can result in the occurrence of oncological diseases [14]. The complex of unfavorable professional factors for firefighters includes exposure to burning products, high temperatures, physical overstrain, stress, disturbed sleep and wakefulness, nutrition, and others not directly related to the fire extinguishing process. The respiratory tract is primarily involved in counteracting adverse factors of various origins, including pathogens and toxic substances from the environment. In the mucous of the respiratory tract, powerful immune defense mechanisms are used, but unfavorable professional factors that contribute to chronic inflammation can lead to the suppression of immunity to infection and the development of allergic pathology in the performance of firefighters.

Evaluation of cellular and humoral immunity in persons exposed to occupational hazards is important not only for prescribing adequate treatment for advanced diseases but also for determining the type of immune response to prevent possible diseases, including infectious and allergic ones.

This paper **aim** to analyze the indicators of cellular and humoral immunity in employees of the state fire service of EMERCOM of Russia, depending on the age and intensity of the professional workload.

Materials and methods

The study concluded 79 employees of the federal fire service of the state fire service of the EMERCOM of Russia, men, with an average age of 31 years (from

21 to 47 years), and work experience in the specialty from 1 to 22 years. The intensity of the load was calculated depending on the time spent by the fire and rescue team in personal respiratory protection equipment.

In the peripheral blood of all examined individuals, monocyte subpopulations were analyzed, the relative number of T lymphocytes of the 2nd type of immune response, the concentration of total immunoglobulin E (IgE), and the amount of secretory immunoglobulin A (sIgA) in the secretion of nasal passages were measured in 30 firefighters (average age and work experience corresponded to the main group).

The evaluation of the relative number of T lymphocytes of the 2nd type in peripheral blood, as well as subpopulations of monocytes, was carried out by flow cytometry. To determine the subpopulation composition of monocytes, whole blood aliquots were stained with a cocktail of anti-CD14, anti-CD16, and anti-CD45 monoclonal antibodies (Beckman Coulter, USA) according to the manufacturer's instructions. VersaLyse (Beckman Coulter, USA) was used for the lysis of erythrocytes. Samples were analyzed in a multicolor protocol on a Navios flow cytometer (Beckman Coulter, USA). The monocyte population was defined as CD45⁺SSCmodCD14⁺ cells. Depending on the CD16 expression density, three subpopulations were distinguished among CD14⁺ monocytes: CD14⁺CD16⁻ (classical), CD14⁺CD16⁺ (intermediate), and CD14^{dim}CD16⁺ (non-classical). To assess type 2 T lymphocytes, whole blood aliquots were stained with a cocktail of anti-CD3 and anti-CD294 monoclonal antibodies (Beckman Coulter, USA) according to the manufacturer's instructions. For the lysis of erythrocytes, OptiLyse C (Beckman Coulter, USA) was used. The population of T lymphocytes 2 was determined as CD3⁺CD294⁺ cells and their relative number from the total pool of lymphocytes was estimated. Samples were analyzed on a Cytomics FC 500 flow cytometer (Beckman Coulter, USA).

The chemiluminescent method (Immulite 2000, Siemens, Germany) was used to determine total immunoglobulin E in blood serum. Determination of the concentration of secretory IgA in nasal secretions was carried out by enzyme immunoassay (IgA secretory-ELISA-BEST, Vector Best, Russia). The material for the study was obtained by blotting nasal secretions with a standard circle of filter paper, after which 500 µL of the RPMI 1640 medium was added to the samples, and this material was subsequently used in enzyme immunoassay (INFINITE F50, TECAN, Austria).

Statistical processing of the results was carried out using the Statistica 12.0 (StatSoft) with the determination of descriptive statistics (mean values, standard error). The statistical significance of differences in the groups was assessed using a non-

parametric Mann–Whitney U test for independent variables. Spearman's non-parametric correlation analysis was used to identify and assess the relationship between quantitative traits. Frequency analysis was performed using four-field contingency tables based on Pearson's X² test. Differences in the compared parameters were considered statistically significant at $p < 0.05$.

Results and discussion

It is known that lymphocytes of the 2nd type of immune response with the CD3⁺CD294⁺ phenotype are effector memory T cells [1]. An increase in the relative number of this cell population above the upper limit of the reference interval (0.5–1.5%) was observed in 13 examined individuals (16.5%). In the group of firefighters with normal values of T lymphocytes of the 2nd type, this parameter was $0.7 \pm 0.3\%$, in the other group – $2.9 \pm 1.6\%$, the age of the examined in these groups differed statistically significantly – $30.3 \pm 6.6\%$ and $36.5 \pm 8.4\%$, respectively, $p < 0.05$. In firefighters younger than 35 years old, on average, $0.83 \pm 0.6\%$ of cells of this population among lymphocytes were detected in the group compared with persons older than 35 years old, where this figure was twice as high and amounted to $1.6 \pm 1.6\%$ ($p < 0.01$), which exceeds the upper limit of the reference interval. Correlation analysis revealed a direct correlation between the relative number of T lymphocytes of the 2nd type of immune response and the age of the examined individuals (Spearman's correlation coefficient 0.3, $p < 0.05$).

In addition, it was shown that in the group of firefighters with a more intense load, the frequency of occurrence of an increased (more than 2%) relative number of T lymphocytes of the 2nd type of immune response was 5 times higher (17.1% versus 3.2%, $p < 0.05$, Pearson's contingency coefficient C 0.242, which indicated the average strength of the connection). An increase in this population of cells in several examined individuals, especially among firefighters with an intensive professional workload, indicated an increase in type 2 immune response and indicated the influence of unfavorable working conditions on the deviation of the immune response.

It should be mentioned that the analysis included mainly young and middle-aged people, but at the same time, a quarter of them had diseases of the upper (in 85% of the total number of diseases) and lower (in 15% of cases) respiratory tract diseases at the time of the study. Among the diseases of the upper respiratory tract dominated allergic and chronic rhinitis, tonsillitis, and rhinosinusitis. Diseases of the lower respiratory tract included mainly chronic bronchitis, bronchial asthma, and chronic obstructive pulmonary disease. When conducting a frequency analysis, it was shown that among patients who had any disease, an increase in the population of type 2

T lymphocytes was observed statistically significantly more often – in 26% of cases versus 11.5% in persons without diagnosed diseases ($p < 0.05$).

A strong direct correlation was found between the number of T lymphocytes of the 2nd type and the duration of the smoking experience (Spearman correlation coefficient 0.49, $p < 0.05$). Tobacco smoke activates the epithelial cells of the respiratory tract, as a result of which they produce pro-inflammatory cytokines – TNF, IL-1 β and IL-8, granulocyte-macrophage colony-stimulating factor, as well as TSLP (thymic stromal lymphopoietin), which directs the immune response in type 2 [16]. A long time of smoking provokes the appearance of sensitization to allergens [4] and increases the incidence of chronic non-allergic rhinosinusitis [3]. Exposure to various toxic substances contained in tobacco smoke enhances the adverse effect of occupational hazards in firefighters and contributes to the persistence of chronic inflammation, mainly type 2.

In 30% of the firefighters, an increase in the concentration of total immunoglobulin E in the blood serum was revealed. A trend towards an increase in this indicator was shown in the group of patients with an increased relative number of type 2 T lymphocytes in peripheral blood – 144 IU/mL versus 103 IU/mL (reference interval 0–85 IU/mL). A direct correlation was found between the number of T lymphocytes of the 2nd type and the concentration of total IgE (Spearman correlation coefficient 0.24, $p < 0.05$). The results confirm the participation of this population of T cells in the switch of B lymphocytes to the synthesis of IgE and indicate a more pronounced IgE-mediated allergic inflammation in the group of firefighters with an increase in the number of type 2 T lymphocytes.

It should be noted that the concentration of total immunoglobulin E in both groups, on average, went beyond the upper limit of the reference interval, which confirmed the general trend of increased allergic inflammation in the group of firefighters compared to healthy individuals in the general population. The development of a specific allergic immune response to inhaled combustion products in firefighters has been described, as evidenced by an increase in the number of eosinophils, eosinophilic cationic protein, and pro-inflammatory cytokines IL-4 and IL-13 in sputum and bronchoalveolar lavage [6].

In addition to assessing the indicators of systemic cellular and humoral immune response in 30 firefighters in the upper respiratory tract, the concentration of secretory IgA, the main immunoglobulin contained in mucous secretions (tears, saliva, sweat, colostrum, discharge from the genitourinary tract, etc.) was determined. It was shown that a decrease in its amount in the secret from the nasal passages was observed in 23% of firefighters, and in 13% of the examined, the indicator went beyond the upper limit of the reference interval (5–30 $\mu\text{g/mL}$). The main function of secretory IgA is the binding of antigens

(both infectious agents and allergens that can cause sensitization) [7]. At the same time, it is not excluded that the reason for the excessively high content of secretory immunoglobulin A may be an increase in its synthesis in damaged organs [11]. It has been shown in animal models that exposure to high temperatures leads to a decrease in this important humoral mucosal protection factor [10], and adaptation to moderately high temperatures increases the concentration of sIgA locally [12]. Therefore, secretory immunoglobulin A can be considered a marker of the body's adaptation to external influences.

There was a pronounced tendency to increase the amount of sIgA in the secret from the nasal passages in the group of firefighters with work experience of 6-14 years compared with the group, which included persons working less than 5 years – 52 ± 59 $\mu\text{g/mL}$ and 19 ± 59 $\mu\text{g/mL}$, respectively, $p = 0.07$. When conducting a frequency analysis, it was shown that in the group of firefighters with a high load, a deviation of this indicator from the reference values was significantly more often ($p < 0.05$) – in 8 out of 14 examined persons (in 5 people a decrease was detected, in 3 an increase in sIgA in the secret) against 3 out of 16 people with a lower professional load (2 people showed a decrease, 1 had an increase in sIgA in secret). The results obtained prove the influence of unfavorable working conditions on the lesion of the parameters of the immune system of the mucous of the upper respiratory tract. In the group of firefighters, multidirectional trends can be distinguished – both a decrease immunity to infection with a decrease in one of the main humoral factors of mucosal immunity, and excessive synthesis of sIgA that accompanies damage to the epithelial lining of the respiratory tract. In both cases, as a result, there is a high probability of developing inflammation, both infectious with a decrease in the protection of mucous and aseptic with the toxic effects of burning products.

There was a trend towards an increase in the frequency of occurrence of a reduced amount of sIgA in the secret from the nasal passages in the group of firefighters with an increased number of type 2 T lymphocytes – 3 out of 7 people (43%) versus 4 out of 23 (17%). Probably, in several examined individuals, an increase in type 2 immune response leads to inhibition of the synthesis of protective immunoglobulin A with an increase in the production of immunoglobulin E in the mucous, which will contribute to the development of allergic inflammation.

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An increase in inflammation in firefighters with a deviation of the immune response towards type 2 was also evidenced by a statistically significant increase in the relative number of classical monocytes ($92 \pm 3.7\%$ versus $88 \pm 5.2\%$, $p < 0.05$), which went beyond the upper limit reference interval (81-90%), in the group examined with a high number of CD3⁺CD294⁺ cells. The main function of classical monocytes is to promote homeostasis by eliminating apoptotic bodies, maintaining inflammation, and participating in tissue repair [15].

Conclusion

In our study, it was found that an increase in the peripheral blood of firefighters T lymphocytes with the CD3⁺CD294⁺ phenotype is observed not only with age, but also with an increase in professional workload, and is also associated with smoking experience and an increase in the incidence in the examined group. In firefighters with an increased number of T lymphocytes of the 2nd type, an increase in the concentration of immunoglobulin E and the relative number of classical monocytes with the phenotype CD14⁺CD16⁻ was found, as well as a tendency to decrease in the secretion from the nasal passages of secretory immunoglobulin A. Therefore, an increase in the relative amount of T lymphocytes type 2 in peripheral blood will contribute to a more likely development of allergic reactions, a decrease in protective forces, and possible fibrosis of lung tissue during prolonged contact with burning products and other inhaled allergens during the professional activities of firefighters, especially with an increase in the smoking experience.

The revealed tendencies of inhibition of immunity to infection and aggravation of damage in the respiratory tract with an increase in type 2 immune response with an increase in professional workload in adverse working conditions contribute to the deterioration of the health of firefighters. Regular in-depth dispensary monitoring of this contingent should be recommended, using laboratory and instrumental methods of examination to prevent the development of diseases and timely detection of severe complications. The use of the relative amount of type 2 T lymphocytes in peripheral blood as a laboratory biomarker to characterize the dominant type of immune inflammation will make it possible to identify in firefighters a predisposition to the T2 profile of the immune response, which will contribute to a personalized approach to patient management.

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