ОСОБЕННОСТИ Т-КЛЕТОЧНОГО ЗВЕНА ИММУНИТЕТА И УРОВЕНЬ НАТУРАЛЬНЫХ КИЛЛЕРОВ У БОЛЬНЫХ, ПЕРЕНЕСШИХ COVID-19 С НАРУШЕНИЯМИ УГЛЕВОДНОГО ОБМЕНА

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Резюме. Пандемия новой коронавирусной инфекции COVID-19 создала чрезвычайную ситуацию в области общественного здравоохранения в РФ в 2020-2022 годах. Последствия COVID-19 многообразны и часто проявляются дисфункцией органов эндокринной системы. SARS-CoV-2 оказывает прямое цитотоксическое и опосредованное повреждающее действие на островки поджелудочной железы, что приводит к развитию гипергликемии. Установлено, что гипергликемия ассоциируется с провоспалительным уровнем иммунного статуса, увеличением количества циркулирующих маркеров воспаления, что приводит к изменениям врожденного и адаптивного иммунитета. Среди первых, кто реагирует на вирусные инфекции, клетки-натуральные киллеры (NK) обладают огромным терапевтическим потенциалом, образуя мост между врожденными и адаптивными реакциями. В целом Т-клеточный ответ в когорте long-COVID претерпевает как фенотипические, так и функциональные изменения. Актуальностью исследования являются недостаточные данные о Т- и NK-клеточном иммунитете у больных с гипергликемией после перенесенного COVID-19. Целью данного исследования явилось выявление особенностей Т-клеточного иммунитета у лиц с постковидным синдромом и нарушениями углеводного обмена, в зависимости от числа NK-клеток.

Пациенты с нарушениями углеводного обмена (НУО) в постковидном периоде (всего 64 человека) в зависимости от числа NK-клеток разделены на три группы: со сниженными показателями NK-клеток, нормальными и повышенными. НУО включали нарушенную толерантность к глюкозе (n = 36) и сахарный диабет 2 типа (n = 28). Группу сравнения составили лица в постковидном периоде без НУО в анамнезе (всего 60 человек). Оценка лимфоцитарного звена иммунитета включала определение: $CD45^+CD3^+$ (Т-лимфоциты), $CD45^+CD3^+CD4^+$ (хелперы индукторы), $CD45^+CD3^+CD8^+$ (цитотоксические Т-лимфоциты), $CD45^+CD3^+CD16^+CD56^+$ (Т-NK-клетки), $CD45^+CD3^-CD16^+CD56^+$ (натуральные киллеры), $CD45^+CD3^+CD4^+CD25^+$ (Т-лимфоциты – ранняя активация), $CD45^+CD3^+HLA\text{-}DR^+$ (Т-лимфоциты – поздняя активация).

Снижение количества натуральных киллеров сопровождалось более высоким уровнем Т-хелперов в группе с нарушениями углеводного обмена, последнее, вероятно, связано с компен-

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саторным повышением Т-лимфоцитов и нарушением регуляции Т-клеточного звена иммунной системы. Также можно сделать вывод, что у пациентов с long-COVID и НУО при нормальных показателях NK-клеток сохраняется измененный субпопуляционный состав, а именно, значимое повышение общих Т-лимфоцитов. Полученные данные о значимом снижении Т-NK-лимфоцитов многие авторы связывают со снижением противовирусной активности иммунной системы, что может приводить к некачественному ответу на новые вирусные агенты или способствовать активации хронических вирусных инфекций. Повышение значений Т-лимфоцитов ранней активации, вероятно, связано с каскадом реакций, направленных на активацию транскрипционных факторов NFAT, NF-κB и AP-1, которые отвечают за регуляцию продукции множества генов (в том числе гена IL-2), контролирующих пролиферацию и дифференцировку активированных лимфоцитов. Нарушения регуляции Т-клеточного звена у лиц с long-COVID и нарушениями углеводного обмена требует более детального изучения, в том числе с оценкой цитокинового профиля у данной категории пациентов.

Ключевые слова: Т-лимфоциты, натуральные киллеры, постковидный синдром, нарушенная толерантность к глюкозе, сахарный диабет 2 типа, иммунная дизрегуляция

CHARACTERISTICS OF T CELL IMMUNITY AND LEVEL OF NATURAL KILLER CELL CONTENT IN COVID-19 CONVALESCENTS WITH CARBOHYDRATE METABOLISM DISORDERS

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Abstract. The pandemic of the new COVID-19 coronavirus infection has created a public health emergency in the Russian Federation in 2020-2022. COVID-19 causes various consequences, often manifested by the endocrine system dysfunction. The rationale for our study is insufficient data on T and NK cell immunity in patients with hyperglycemia after COVID-19. The study was aimed at the features of T cell immunity in individuals with post-COVID syndrome and disorders of carbohydrate metabolism, depending on the NK cells count. Materials and methods: Sixty-four post-COVID patients with carbohydrate metabolism disorders were divided into three groups: with reduced, normal, or elevated NK cell counts. Carbohydrate metabolism disorders included impaired glucose tolerance (n = 36) and type 2 diabetes mellitus (n = 28). The comparison group comprised 60 post-COVID persons with no history of carbohydrate metabolism disorders. The assessment of the lymphocytic link of immunity included the definition of: $CD45^+CD3^+$ (T lymphocytes), $CD45^+CD3^+CD4^+$ (T helpers), $CD45^+CD3^+CD8^+$ (T cytotoxic), $CD45^+CD3^+CD16^+CD56^+$ (T-NK cells), $CD45^+CD3^-CD16^+CD56^+$ (natural killers), $CD45^+CD3^+CD4^+CD25^+$ (T lymphocytes – early activation), $CD45^+CD3^+$HLA-DR^+ (T lymphocytes – late activation). Results and Discussion. As the study showed, a decrease in the number of natural killers was accompanied by a higher level of T helpers in the group with carbohydrate metabolism disorders, the latter is probably associated with a compensatory increase in T lymphocytes and dysregulation of the T cell link of the immune system. It can also be concluded that in patients with long-COVID and CMD, with normal NK cell counts, an altered subpopulation composition remains, namely, a significant increase in total T lymphocytes. Many authors associate the obtained data on a significant decrease in T-NK lymphocytes with a decrease in the antiviral activity of the immune system, which can lead to a poor response to new viral agents or contribute to the activation of chronic viral infections. Dysregulation of the T cell link in individuals with long-COVID and disorders of carbohydrate metabolism requires a more detailed study, including an assessment of the cytokine profile in this category of patients.

Keywords: T lymphocytes, natural killers, long-COVID, impaired glucose tolerance, diabetes mellitus type 2, immune dysregulation

Introduction

The pandemic of the new COVID-19 coronavirus infection has created a public health emergency in the Russian Federation in 2020-2022. The wave-like spread of COVID-19 has led to high morbidity in all regions of the Russian Federation. COVID-19 causes various consequences, often manifested by the endocrine system dysfunction. SARS-CoV-2 has a direct cytotoxic and indirect damaging effect on the pancreatic islets, which leads to hyperglycemia [3]. Prediabetes and diabetes mellitus (DM) are often diagnosed in patients infected with SARS-CoV-2, who had no history of carbohydrate metabolism disorders (CMD) or of glucocorticoid treatment. Hyperglycemia has been found to be associated with an increase in proinflammatory immune status and in the circulating markers of inflammation, leading to changes in innate and adaptive immunity.

Among the first to respond to viral infections, natural killer cells (NK) have enormous therapeutic potential, bridging the innate and adaptive immune responses [5]. M. Galan et al. showed a 1.7-fold ($p = 0.032$) increase in the NK cells population expressing the CD16 marker on the surface ($CD3^-CD56^+CD16^+$) in the long-COVID group [2]. M. Dobrynina et al. showed a sharp three-fold decrease in the NK cells count in over a third of the patients with post-covid syndrome of immunopathology. This decrease was accompanied by a higher relative level of T lymphocytes and T helper cells [1]. The T cell response in the long-COVID cohort undergoes both phenotypic and functional changes. The rationale for our study is insufficient data on T and NK cell immunity in patients with hyperglycemia after COVID-19. The study was **aimed** at the features of T cell immunity in individuals with post-COVID syndrome and disorders of carbohydrate metabolism, depending on the NK cells count.

Materials and methods

Sixty-four post-COVID patients with carbohydrate metabolism disorders were divided into three groups: with reduced, normal, or elevated NK cell counts. Carbohydrate metabolism disorders included impaired glucose tolerance ($n = 36$) and type 2 diabetes mellitus ($n = 28$). The comparison group comprised 60 post-COVID persons with no history of carbohydrate metabolism disorders. Diagnosis of diabetes mellitus met the criteria of Algorithms of specialized medical care for patients with diabetes mellitus (2021).

The post-COVID syndrome was diagnosed based on polymerase chain reaction confirmed SARS-CoV-2 infection, detection of IgA, IgM, or IgG to the SARS-CoV-2 virus, and computed tomography-confirmed pneumonia. The study was conducted at least 6 months after the pneumonia caused by SARS-CoV-2 infection. Before the inclusion, a general practitioner and an endocrinologist examined each patient to identify concomitant diseases. Lymphocyte immunological indices were studied by flow cytometry on the Navios cytofluorimeter (Beckman Coulter, USA) using a standardized technology: $CD45^+CD3^+$ (T lymphocytes), $CD45^+CD3^+CD4^+$ (T helpers), $CD45^+CD3^+CD8^+$ (T cytotoxic), $CD45^+CD3^+CD16^+CD56^+$ (T-NK cells) $CD45^+CD3^-CD16^+CD56^+$ (natural killers), $CD45^+CD3^+CD4^+CD25^+$ (T lymphocytes – early activation), $CD45^+CD3^+$HLA-DR^+ (T lymphocytes – late activation).

The study was approved by the Independent Local Ethics Committee at the Autonomous Healthcare Institution Order of the Red Banner of Labor City Clinical Hospital No. 1, Chelyabinsk, Record No. 8 of April 11, 2022, the base of the studies. IBM SPSS Statistics, Version 19 software was used for statistical data processing. Correlation analysis within the groups was performed by calculating Spearman's rank correlation. The differences in the distribution of values between the groups were calculated using the Mann–Whitney U test. The differences between the groups were considered significant at $p < 0.05$.

Results and discussion

As the study showed, a decrease in the number of natural killer cells was accompanied by a higher level of T helper cells in the group with carbohydrate metabolism disorders; the latter is probably associated with a compensatory increase in T lymphocytes and dysregulation of the T cell link of the immune system (Table 1). It can also be concluded that in patients with long-COVID and CMD, with normal NK cell counts, an altered subpopulation composition remains, namely, a significant increase in total T lymphocytes.

Many authors associate the obtained data on a significant decrease in T-NK lymphocytes with a decrease in the antiviral activity of the immune system, which can lead to a poor response to new viral agents or contribute to the activation of chronic viral infections [6]. An increase in the values of early activation T lymphocytes is probably associated with a cascade of reactions aimed at activating the

TABLE 1. COMPARISON OF T CELL IMMUNITY INDICES IN PATIENTS WITH POST-COVID SYNDROME AND DISORDERS OF CARBOHYDRATE METABOLISM (CMD), DEPENDING ON NK CELL COUNT

Measures	Group of patients with post-COVID syndrome and CMD (n = 64)			p
NK cells	1 reduced (n = 16)	2 normal (n = 30)	3 elevated (n = 18)	
NK cells ($CD45^+CD3^-CD16^+CD56^+$), 10^6 cells/L	86.130±7.586	246.110±14.466	546.440±25.089	
T lymphocytes ($CD45^+CD3^+CD19^-$), %	79.688±2.735	74.716±1.427	62.000±1.909	$p_{2,5} = 0.029$
T lymphocytes ($CD45^+CD3^+CD19^-$), 10^6 cells/L	1668.500±198.340	1781.630±145.475	1625.890±150.595	$p_{2,5} = 0.041$
T helpers ($CD45^+CD3^+CD4^+$), %	53.330±2.751	49.730±2.353	41.590±2.424	$p_{1,4} = 0.019$
T helpers ($CD45^+CD3^+CD4^+$), 10^6 cells/L	1121.380±149.424	1089.680±93.715	1108.110±141.759	$p_{1,4} = 0.006$
T cytotoxic ($CD45^+CD3^+CD8^+$), %	24.263±2.295	25.484±2.658	20.000±1.432	
T cytotoxic ($CD45^+CD3^+CD8^+$), 10^6 cells/L	496.380±52.527	621.160±98.659	522.330±57.936	
Immunoregulatory index (Tx/Tc)	2.400±0.339	2.416±0.336	2.200±0.249	
T-NK lymphocytes ($CD45^+CD3^+CD16^+CD56^+$), %	2.233±0.643	5.626±0.649	5.911±1.281	$p_{1,4} = 0.010$
T-NK lymphocytes ($CD45^+CD3^+CD16^+CD56^+$), 10^6 cells/L	54.140±14.647	126.110±15.616	149.670±30.769	$p_{1,4} = 0.048$
T lymphocytes $CD45^+CD3^+CD4^+CD25^+$ (early activation), %	7.367±0.916	5.642±0.682	7.000±0.991	$p_{1,4} = 0.026$
T lymphocytes $CD45^+CD3^+CD4^+CD25^+$ (early activation), 10^6 cells/L	79.290±14.615	58.210±7.556	79.110±15.478 norm	$p_{1,4} = 0.010$
T lymphocytes $CD45^+CD3^+CD4^+HLA\text{-}DR^+$ (late activation), %	3.350±0.563	4.974±0.839	7.567±2.213	
T lymphocytes $CD45^+CD3^+CD4^+HLA\text{-}DR^+$ (late activation), 10^6 cells/L	67.250±31.099	51.680±9.564	80.560±20.843	

Таблица 1 (окончание)
Table 1 (continued)

Measures	Group of patients with post-COVID syndrome without CMD (n = 60)			p
NK cells	4 reduced (n = 14)	5 normal (n = 38)	6 elevated (n = 8)	
NK cells ($CD45^+CD3^-CD16^+CD56^+$), 10^6 cells/L	62.290±10.167	229.110±14.561	657.75±189.051	
T lymphocytes ($CD45^+CD3^+CD19^-$), %	79.414±2.608	70.961±1.125	57.975±6.143	$p_{2,5} = 0.029$
T lymphocytes ($CD45^+CD3^+CD19^-$), 10^6 cells/L	1427.140±294.744	1342.440±77.867	1636.000±247.748	$p_{2,5} = 0.041$
T helpers ($CD45^+CD3^+CD4^+$), %	40.800±3.327	48.560±1.501	34.050±2.282	$p_{1,4} = 0.019$
T helpers ($CD45^+CD3^+CD4^+$), 10^6 cells/L	548.600±58.391	1018.840±83.168	966.500±146.301	$p_{1,4} = 0.006$
T cytotoxic ($CD45^+CD3^+CD8^+$), %	28.871±5.271	22.826±1.638	22.9500±5.8487	
T cytotoxic ($CD45^+CD3^+CD8^+$), 10^6 cells/L	467.290±72.987	480.740±55.944	634.250±149.956	
Immunoregulatory index (Tx/Tc)	2.300±0.731	2.384±0.229	1.700±0.274	
T-NK lymphocytes ($CD45^+CD3^+CD16^+CD56^+$), %	8.350±1.601	6.653±0.942	5.450±1.648	$p_{1,4} = 0.010$
T-NK lymphocytes ($CD45^+CD3^+CD16^+CD56^+$), 10^6 cells/L	116.400±22.997	145.580±35.064	142.000±32.432	$p_{1,4} = 0.048$
T lymphocytes $CD45^+CD3^+CD4^+CD25^+$ (early activation), %	4.033±0.457	6.053±0.571	8.775±2.051	$p_{1,4} = 0.026$
T lymphocytes $CD45^+CD3^+CD4^+CD25^+$ (early activation), 10^6 cells/L	28.200±2.518	61.420±8.103	88.250±32.082	$p_{1,4} = 0.010$
T lymphocytes $CD45^+CD3^+CD4^+HLA\text{-}DR^+$ (late activation), %	4.700±1.549	3.705±0.474	6.850±1.687	
T lymphocytes $CD45^+CD3^+CD4^+HLA\text{-}DR^+$ (late activation), 10^6 cells/L	33.860±8.860	39.370±6.393	68.250±25.477	

transcription factors NFAT, NF-κB and AP-1, which are responsible for regulating the production of many genes (including the IL-2 gene) that control proliferation and differentiation of activated lymphocytes [4]. Dysregulation of the T cell link in individuals with long-COVID and disorders of carbohydrate metabolism requires a more detailed study, including an assessment of the cytokine profile in this category of patients.

Conclusions

1. Decreased and normal values of NK cells are accompanied by a change in the subpopulation composition of T lymphocytes in the group of people with long-COVID and concomitant pathologies, both in impaired glucose tolerance and in type 2 diabetes mellitus.

2. In individuals with impaired carbohydrate metabolism in the post-COVID period, a decrease in the level of NK cells is accompanied by a significantly higher level of T helper cells.

3. With normal NK cells, the level of total T lymphocytes is significantly higher with concomitant disorders of carbohydrate metabolism in the long-COVID group.

4. In the group of people with post-COVID syndrome and disorders of carbohydrate metabolism, a significant increase in T lymphocytes of early activation was revealed.

The obtained data showed that in the post-COVID period, in the group of patients with disorders of carbohydrate metabolism, dysregulation of the immune system is observed, which in the future can lead to an inadequate immune response, which requires a more detailed study, including an assessment of the cytokine profile.

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