REVIEW

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IMMUNOLOGICAL MECHANISMS OF INCREASED SUSCEPTIBILITY TO COVID-19 DISEASE AND ITS SEVERE COURSE IN PATIENTS WITH DIABETES MELLITUS TYPE 2 AND OBESITY

K. P. ZAK[1], M. D. TRONKO[1], S. V. KOMISARENKO[2]✉

[1]V. P. Komisarenko Institute of Endocrinology and Metabolism, National Academy of Medical Sciences of Ukraine, Kyiv;
[2]Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv;
✉e-mail: svk@biochem.kiev.ua

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In this review, we analyze and summarize literature data and the results of our own research related to the immunity status of patients with type 2 diabetes mellitus (T2D) and those T2D patients who were infected with the SARS-CoV-2 virus. It was shown that in the blood plasma of T2D patients, especially those with elevated BMI, the level and ultrastructure of the main cellular components of natural immunity - neutrophils and monocytes - were affected accompanied by high levels of proinflammatory cytokines (IL-1β, IL-6, IL-17 and TNF-α). It was suggested that the increased susceptibility of T2D patients to SARS-CoV-2 infection is primarily due to a weakening of the innate immune defense against pathogens, whereas in T2D patients who have COVID-19, adaptive T-cell immunity disorders accompanied by a cytokine storm prevail. It was concluded that hyperinflammation in T2D+COVID19 patients is the result of enhancement of already existing before SARS-CoV-2 infection T2D-caused disorders of innate and adaptive immunity, in the mechanism of which cytokines and chemokines play a significant role.

K e y w o r d s: type 2 diabetes mellitus, COVID-19, cytokines, T-lymphocytes, neutrophils, innate and adaptive immunity.

Until very recently, the world experienced the greatest tragedy of our time – the coronavirus COVID-19 pandemic, which took the lives of millions on our planet. Thus, according to the WHO data, the number of COVID-19 patients global by May 2023 reached about 766 million people including 6.9 million deaths [1]. Yet it has been firmly established that old age, obesity and diabetes mellitus (DM) are the main risk factors for SARS-CoV-2 infection, the more severe its clinical course and increased mortality, especially upon acute respiratory distress syndrome (ARDS), as well as increased level of hyperglycemia and insulin resistance (IR) [2-4].

Analysis of the data obtained from the case histories of thousands of COVID-19 patients admitted to the Central Hospital of New York (USA) showed that 34% of these patients were simultaneously diagnosed with type 2 diabetes (T2D) [5]. A high rate (39.6%) of T2D among patients suffering from COVID-19 was also observed at Massachusetts General Hospital (USA) [6]. A significant percentage (23.3%) of T2D in patients with morbid COVID-19 was also reported in Lombardy (Italy) [7]. Moreover, a significantly higher mortality rate was observed in patients with COVID-19+T2D than patients with COVID-19 without T2D. According to some data, this ratio was 42.8% versus 21.7% ($P < 0.001$) [7], and to others - 20.3% versus 10.9% ($P < 0.01$), respectively [8].

A more severe clinical course of the disease and increased mortality rate were also observed in

obese (BMI > 30 kg/m^2) COVID-19 patients with T2D compared to normal patients (BMI ≤ 25 kg/m^2) [9, 10].

To answer the questions – why patients with T2D are more prone to SARS-CoV-2 infection and why in T2D patients who have already caught COVID-19, especially those with obesity, the disease is more severe with a higher mortality rate – more detailed information about the immune defense system in the T2D patients before their infection with COVID-19 is required.

For many years, we have studied natural and T-cell immunity in newly diagnosed untreated T2D patients with a range of body weights [11-16]. The present work summarizes and analyzes these numerous studies, as well as current literature data on the immunity of individuals with prediabetes (HbA1c ≥ 5.7-6.4%) and T2D (HbA1c > 6.5%) in comparison with the results of published studies in patients with COVID-19 of varying severity [17-28], which could be important for understanding the causes and immune mechanisms that make COVID-19 more severe in T2D patients as well as for developing more targeted treatments.

Natural and T-cell immunity in T2D patients with a range of body weight

According to modern concepts, T2D is a chronic low-gradient systemic immune-based inflammation [29-32]. Thus, most of the classic inflammatory biomarkers were found in patients with T2D, namely: leukocytosis, neutrophilia, monocytosis, increased level of C-reactive protein, NLR index, ferritin, D-dimer [33-35], as well as high levels of pro-inflammatory cytokines (IL-1β, IL-6, IL-17, TNFα, etc.) [14, 34, 36-43].

A statistically significant increase in similar inflammatory biomarkers, but to a lesser extent, was also observed in normoglycemic individuals with morbid obesity [44-49].

It should be noted that the vast majority of patients with T2D (almost 80% of cases) tend to suffer from comorbid obesity [50, 51], which is now recognized to be a low-gradient inflammation of adipose tissue (AT), similar to T2D [52]. Furthermore, the number of overweight and obese people is steadily increasing. About 2 billion people worldwide are obese, that is, every eleventh person on our planet has obesity [53]. It was also discovered that in many countries [54], including Ukraine [55], the curve of the annual global increase in the number of obese people and the curve of the annual increase in the number of people with T2D run in parallel. According to the IDF data, the number of T2D patients worldwide has quadrupled over the past 25 years and was 537 million people for 2021 and is predicted to rise to 643 million by 2030 [56]. It was also found that each increase in body mass index (BMI) by 5 kg/m^2 increases mortality among T2D patients by 30% [9, 57].

Although obesity is considered one of the main causes of insulin resistance (IR) resulting in the development of T2D in most individuals via the route AT → IR → PD → T2D, it is still unclear whether obesity is the primary cause of T2D or IR.

The age of the patient is also essential in the onset of type 2 diabetes. Thus, one in five people over 65 was diagnosed as having T2D [56].

Our study of the PB leukocyte composition showed [15], that untreated newly diagnosed T2D patients aged 40-65 years had statistically significant leukocytosis on account of an increase in the absolute number of neutrophils and monocytes. The most pronounced segmented neutrophilia (without "left shift") (Fig. 1) was observed in overweight (especially obese) T2D patients, which correlated with BMI values (35-37 kg/m^2). Similar changes were observed in the PB monocyte count, predominantly in obese women (Fig. 1) [58].

At the same time, the absolute number of lymphocytes was slightly but reliably decreased [15], and in some severe patients, especially women, the most pronounced lymphocytopenia was observed. Moreover, the NLR index, an important inflammatory biomarker [59], was significantly increased. The results of our study are fully consistent with the data obtained in the research conducted by immunological centers in various European countries during which a massive number of T2D patients were examined [31, 35].

Our own research [15] also showed that the degree of leukocytosis, neutrophilia, monocytosis and NLR index in T2D patients greatly depended on body weight. Thus, the comparison of leukocyte count in PB of normoglycemic normal weight individuals (BMI ≤ 25.5 kg/m^2) with normal weight T2D patients (BMI ≤ 25.5 kg/m^2) revealed a significant increase in total leukocyte count by 11.5%, in neutrophil count by 20.7%, and in monocyte count by 11.1%, NLR index by 19.4%, whereas in obese T2D patients (BMI > 35.5 kg/m^2) lymphocyte count was higher by 67.3%, neutrophil count - higher by 93%,

monocyte count – higher by 97%, and NLR index – higher by 54.5% compared to normoglycemic individuals (Fig. 1).

In the normoglycemic control group, similar differences in PB counts between normal weight and obese individuals were detected, but the differences were less pronounced than in T2D patients.

It was revealed, using electron microscopy, that the increased neutrophil count in PB of T2D patients, especially with concomitant obesity, was accompanied by significant changes in neutrophil ultrastructure, indicating a disorder in the functional activity of this type of leukocytes [13].

The PB neutrophils of T2D patients, in comparison with those of normoglycemic individuals, contain a greater number of destructive primary (azurophilic) granules with matrix residues (phagolysis) and many vacuoles (lysosomes) in the cytoplasm (Fig. 2). Neutrophils with an altered structure of mitochondria and nuclei with an increased number of appendages are more common. The observed changes in neutrophil ultrastructure upon T2D were also confirmed by other methods for studying such cells upon diabetes: phagocytosis, ketosis (extracellular traps), cytotoxicity, which also demonstrated a decrease in the protective anti-inflammatory activity of neutrophils in diabetes [60, 61]. Increased vacuolization of neutrophil cytoplasm, which is a sign of increased secretory activity of cells, is consistent with published data that this type of leukocytes is responsible for increasing production of some proinflammatory cytokines (IL-1β, IL-6) and chemokines (IL-8) upon T2D [28, 62, 63].

Electron microscopy analysis of leukocytes in T2D patients also revealed pronounced submicroscopic changes in another integral cellular component of natural immunity – monocytes/macrophages. PB monocytes in T2D patients, compared to those

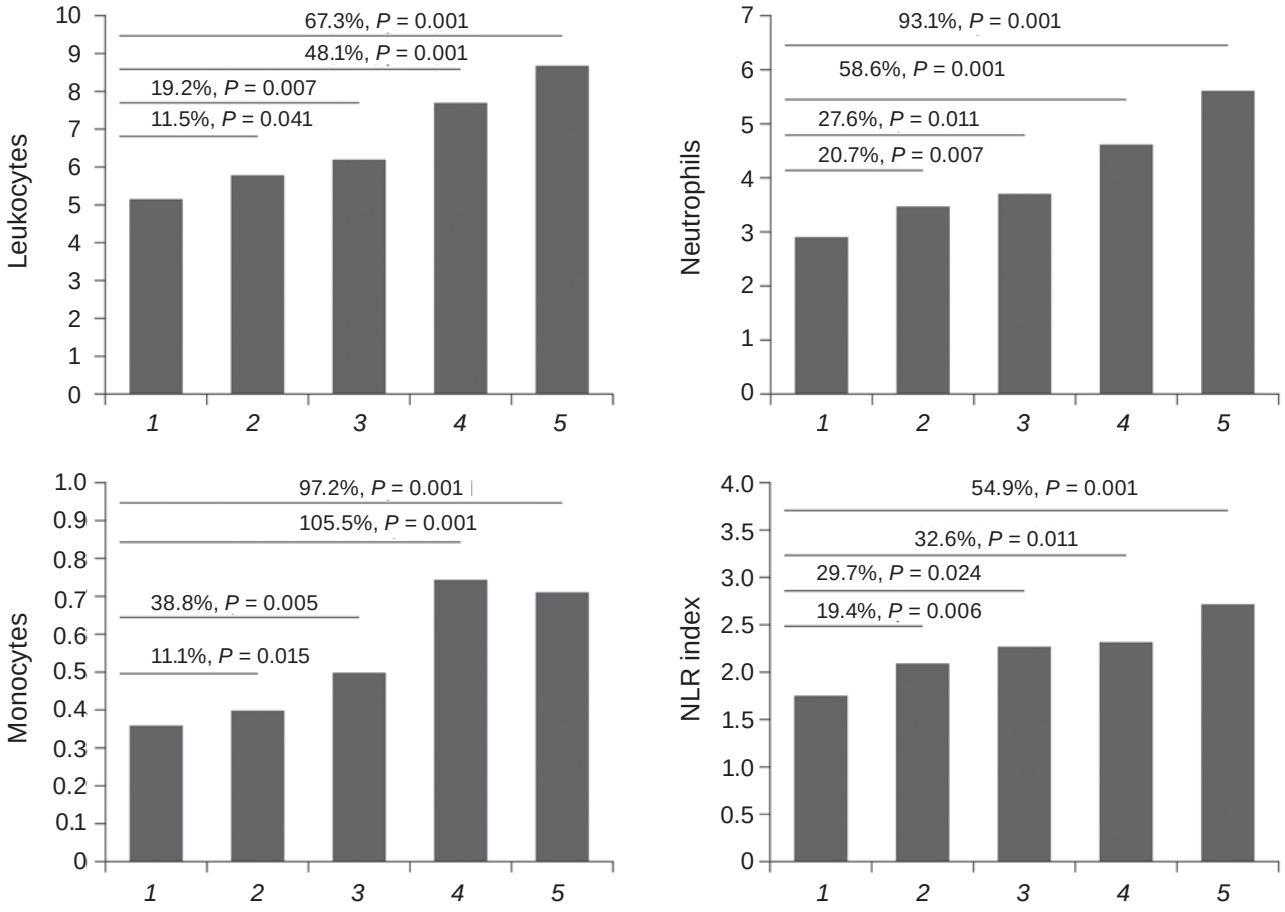

Fig. 1. Total leukocyte count (10^9/l), absolute neutrophil count, monocyte count and NLR index in newly diagnosed T2D patients in relation to BMI values: 1 – healthy individuals (BMI < 25.5 kg/m2); 2 – T2D patients (BMI < 25.5 kg/m^2); 3 – T2D patients (BMI 25.5 to 29.9 kg/m^2); 4 – T2D patients (BMI 30 to 34.9 kg/m^2); 5 – T2D patients (BMI > 35 kg/m^2) [15]

in normoglycemic individuals, contain a significantly smaller number of specific granules and an increased number of pinocytotic vacuoles and vesicles, involved in the transport and secretory activity of macrophage inflammatory cytokines [13]. Particularly significant changes in the monocyte submicroscopic structure were observed in T2D patients with concomitant obesity.

Thus, our data on pronounced changes in the number and submicroscopic structure of neutrophils and monocytes, essential cellular elements of natural immunity, in T2D patients suggest a significant weakening of the innate (hereditary) antibacterial defense system in this disease.

In studying the number of T-lymphocytes of various immunophenotypes (CD3+T, CD4+T, CD8+T, CD20+ and CD56+ cells) in PB of newly diagnosed T2D patients by flow cytometry using a FACS-tar plus laser flow cytometer and a panel of labeled monoclonal antibodies for cluster differentiation (CD), a significant increase in the total number of T-lymphocytes (CD3+T-cells) and their major subclasses: CD8+T and especially CD4+T-cells were observed, especially in women (Fig. 3).

Some reports noted that a significant increase in the number of PB CD4+T-cells and their recruitment to the AT was observed mainly in obese women with T2D [64, 65] and less pronounced in normoglycemic ones [47, 66, 67]. Moreover, it was found that CD4+T-cells affecting the Th17 cell production are the main producers of pro-inflammatory cytokine IL-17 [47]. However, there are reported data on a decrease in the regulatory fraction of CD4+, CD25+Fox3 lymphocytes in PB of obese patients both with and without T2D [68].

Using electron microscopy analysis of an enriched concentrate of CD4+T-cells isolated by flow cytometry we revealed that most lymphocytes of this subpopulation contained a specific organelle in the cytoplasm, the Goll bodies, which is a reliable cytoplasmic biomarker of CD4+T-cells [69, 70]. The Goll body cluster (GB) of CD4+ T-cells consists of a large rounded granule surrounded by smaller electron-dense satellite granules (Fig. 4, *A* and *B*). The GB cluster is reported to play an important role in the CD4+T cells secretory function, including the production of IL-6 and IL-17 cytokines [47, 71]. In T2D patients, especially those with morbid obesity (BMI > 30 kg/m^2), significant changes in the submicroscopic structure of the GB cluster (arrows) in the form of an increased number of smaller electron-dense satellite granules and an increased number of transport vesicles and vacuoles were detected (Fig. 4, *A*, *B*). This confirmed the data on significant changes in CD4+T-cell function in T2D patients [13, 72].

It is now well established that cytokines, hormone-like low-molecular-weight proteins or polypeptides that regulate most vital physiological and pathophysiological processes in the body, play a key role in the pathogenesis of inflammatory diseases, including T2D. Cytokines exhibiting apocrine, paracrine and distant (i.e., endocrine) effects are involved in cell signaling in the immune response and protection against various pathogens and stress and are also crucial in the regulation of the cardiovascular system, renal function, hematopoiesis, obesity, carcinogenesis, atherosclerosis, aging, as well as T2D [13, 27, 73].

The central role in the development of IR, PD and clinically evident T2D is attributed to the main proinflammatory cytokines, such as IL-1β, IL-6, IL-17 and TNFα, considered classic biomarkers of inflammation [27, 28, 36, 74].

Patients with T2D were shown to have elevated levels of the proinflammatory macrophage IL-1β (Fig. 5, *A*) in both the preclinical and clinical stages of the disease [14, 75]. Significant increases in IL-1β M1 macrophage levels in the AT of T2D patients in the postprandial stage were also reported [75]. Especially high levels of IL-1β were observed in obese T2D patients (BMI = 35-40 kg/m^2) [76]. There was also evidence of a significant decrease in the level of IL-1β in PB of normoglycemic obese individuals following their long-term diet and weight loss [77]. It was reported that the blockage of IL-1 receptor antagonist (IL-1Ra), which can neutralize the effect of IL-1, led to a sharp decrease in IL-1 concentrations in PB and a reduction in the incidence of T2D in patients with high IL-1 levels [78-80].

Numerous prospective studies such as widely known international projects MONICA, EPIC [27. 36, 74, 81, 83] and our own research [13, 14] conducted on patients with newly diagnosed and chronic T2D showed that patients with T2D are characterized mostly by an increased level of the proinflammatory cytokine IL-6 in PB compared to healthy individuals. The meta-analysis of published data fully confirmed the above data [74]. Moreover, elevated levels of IL-6 can be detected long before the development of T2D and, therefore, can be used as a reliable biomarker for predicting the risk of the disease [81]. It was also demonstrated that the de-

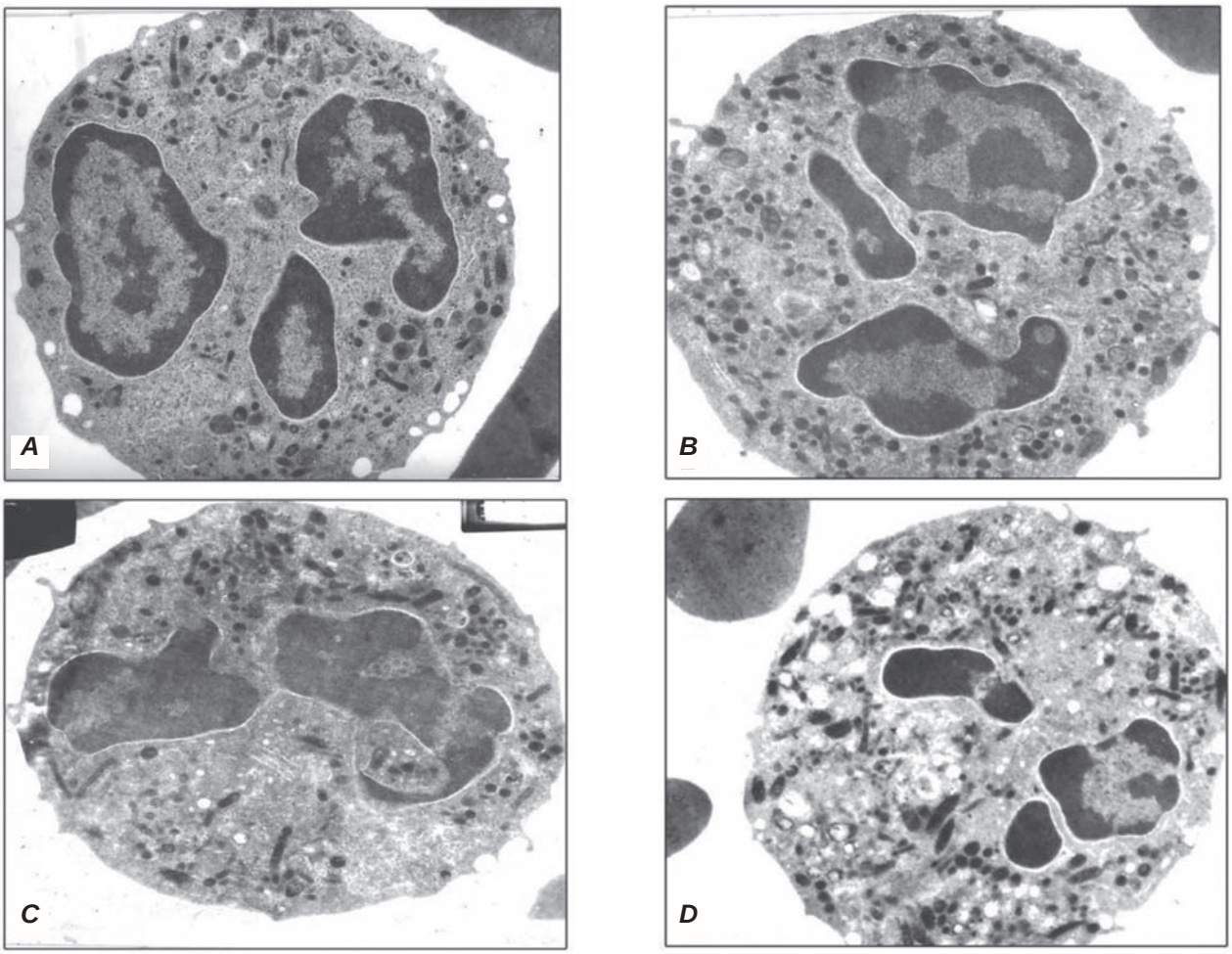

Fig. 2. Segmentonuclear neutrophils in PB: ***A*** *– healthy individual (control) with normal BMI (23.5 kg/m²);* ***B*** *– a newly diagnosed T2D patient with normal BMI (22.8 kg/m²);* ***C*** *– individual with metabolic syndrome/obesity (BMI 35.0 kg/m²);* ***D*** *– T2D patient with metabolic syndrome/obesity (BMI 37.0 kg/m²), ×9000 [42]*

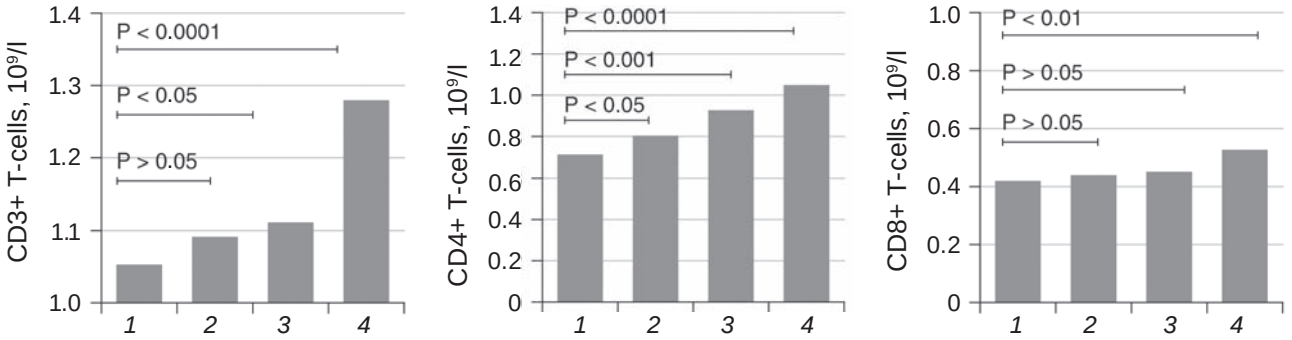

Fig. 3. Absolute counts (10⁹/l) of CD3+ T-, CD4+ T- and CD8+ T-cells in 1 – normoglycemic individuals with BMI < 25 kg/m² and T2D patients with a range of body weights: 2 – BMI < 25 kg/m²; 3 – BMI 25-30 kg/m²; 4 – BMI > 30 kg/m² [16]

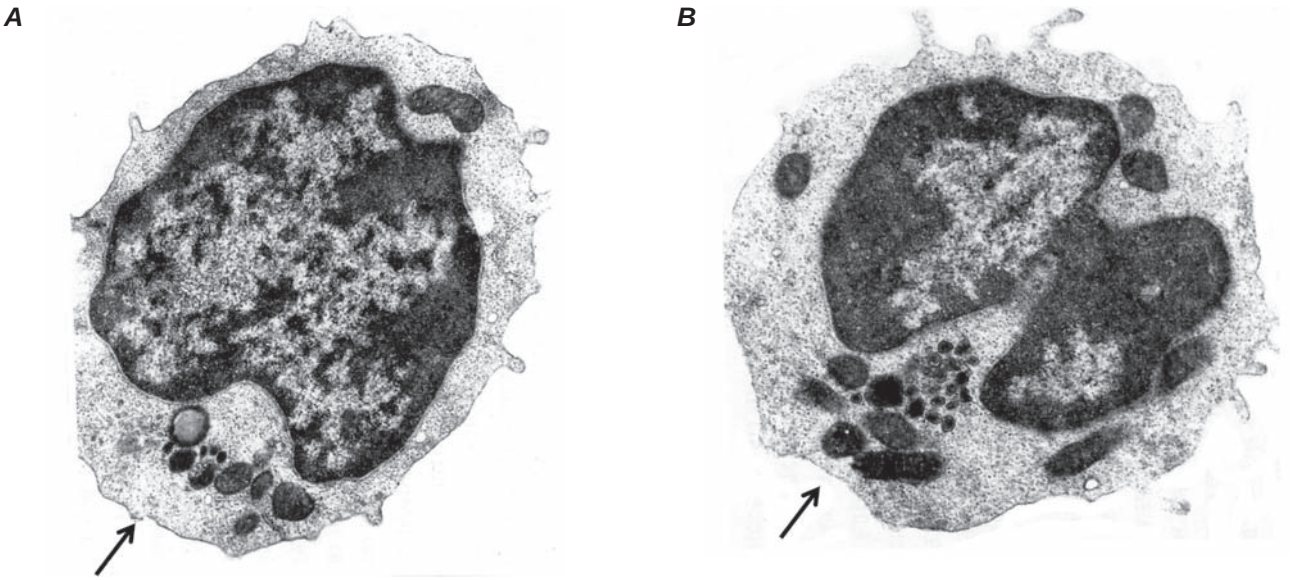

Fig. 4. Ultrastructure of CD4+ T-cells containing Goll bodies (arrows). ***A*** *– healthy individuals (BMI < 25 kg/m²);* ***B*** *– T2D patients with obesity [13]*

gree of the IL-6 increase in the PB of diabetic patients depended largely on the value of body mass index (BMI), i.e., concomitant obesity (Fig. 5, *B*) [13, 83, 84].

In T2D patients, a significant increase in the content of recently discovered proinflammatory cytokine IL-17A, a highly active immunomodulator of natural and T-cell immunity, was found [47, 85, 86]. Moreover, it was shown that in patients with IR and obesity, the level of IL-17 generation in AT increases almost 8-10-fold [47]. We also revealed a similar increase in IL-17 level (Fig. 5, *B*) in patients with newly diagnosed T2D compared to normoglycemic individuals.

Another characteristic feature of T2D patients was found to be a significant increase in the proinflammatory cytokine TNFα, secreted mainly by the AT cells [13, 14, 84, 87, 88]. It was shown that elevated plasma levels of TNFα in normoglycemic individuals may precede clinically diagnosed T2D and can serve as a marker for identifying a risk group among them [36]. A particularly pronounced increase in TNFα level in PB was observed in T2D patients with concomitant obesity [45, 84, 89-91].

Our studies showed that the degree of increase in all proinflammatory cytokines in T2D patients is largely correlated with the value of body weight/obesity (Fig. 5), i.e. the higher the patient's BMI, the higher PB cytokine levels [12, 13]. These findings suggest that since most T2D patients usually have an elevated BMI, hypercytokinemia is not only the result of the pathogenesis of T2D itself but is also largely because of the concomitant excess body weight. Further research is needed to support this conclusion.

The role of anti-inflammatory cytokines (IL-4, IL-10, IL-13, IL-38, TGFβ) in T2D pathogenesis has been discussed only in a few ambiguous publications [92]. Among them, the most well-known are studies of the cytokine contents of IL-4 and IL-10 in the PB and AT of T2D patients. These types of cytokines are characterized by high pluripotency and gene polymorphism [93-95].

The pleiotropic anti-inflammatory cytokine IL-4, produced mainly by Th-2 and mastocytes, exhibits the ability to bind to specific cell receptors and is involved in the signaling cascade resulting in a direct protective effect of pancreatic islets against pathogen damage. Incubation of IL-4 with adult pancreatic islets prevents apoptosis caused by the "cocktail" of proinflammatory cytokines IL-1β + TNFα + IFNγ [96].

Some research investigating the IL-4 level in PB of adult T2D patients with normal BMI reported conflicting data. Some studies [97, 98] found decreased levels of IL-4 in PB, while others observed elevated or near-normal levels [99, 100]. A particularly significant increase in IL-4 level was observed in African-American women with high HbA1c levels [91]. The authors attributed the differences in IL-4 levels in PB of T2D patients across continents to genotype polymorphism [101].

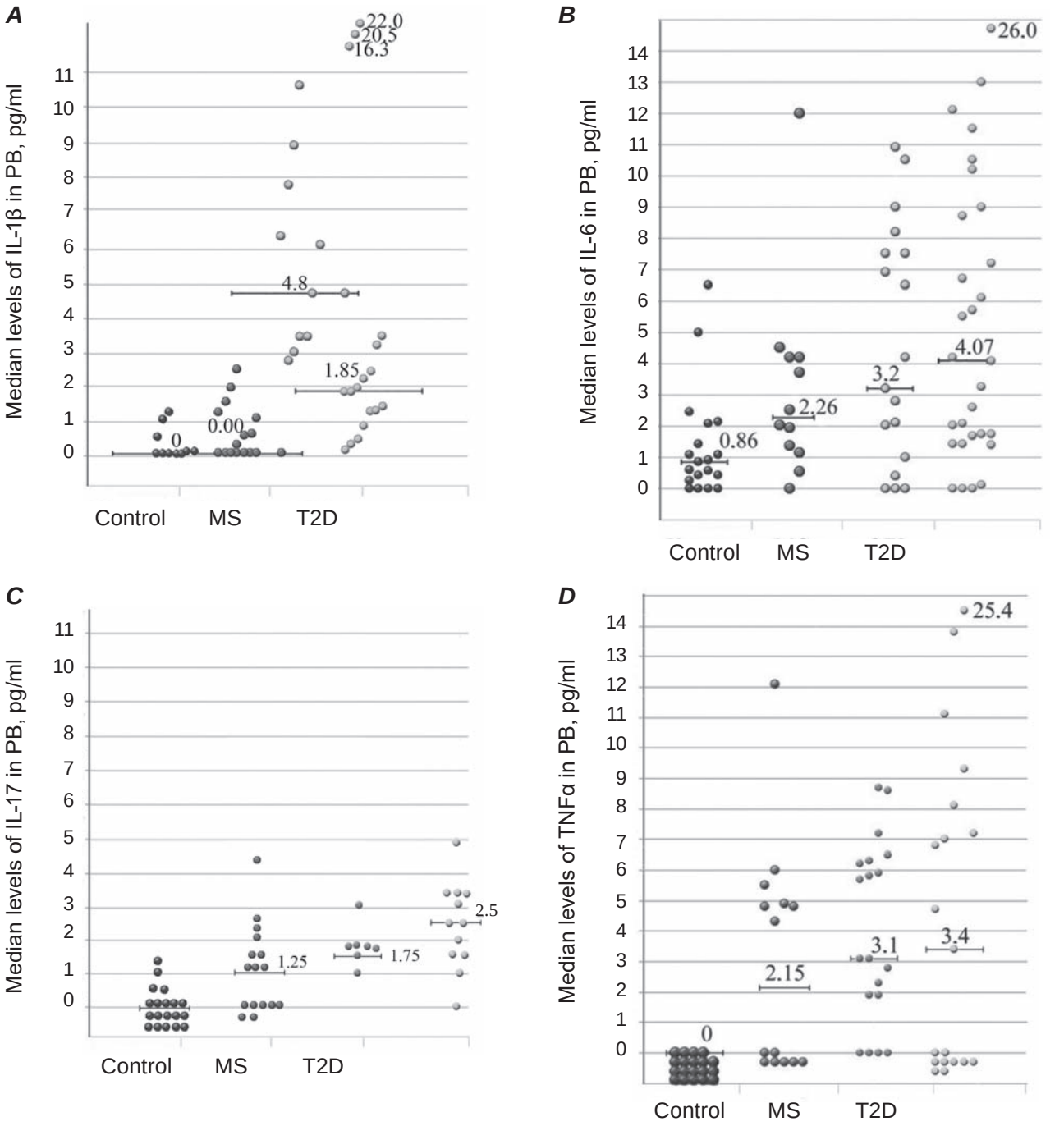

*Fig. 5. The median levels of IL-1β (**A**), IL-6 (**B**), IL-17 (**C**), and TNFα (**D**) in the PB of normoglycemic individuals (control, BMI < 25 kg/m^2); T2D patients with BMI < 25.0 kg/m^2; normoglycemic individuals with metabolic syndrome/obesity (BMI > 30 kg/m^2); and T2D patients with concomitant metabolic syndrome/obesity (BMI > 30 kg/m^2)*

The pleiotropic anti-inflammatory cytokine IL-10, produced mainly by monocytes and to a lesser extent by Th2 cells, B-lymphocytes and mast cells, has the ability to inhibit inflammation and autoimmune diseases [102]. Moreover, IL-10 can suppress the production of IL-6 and TNFα. In individuals with IR as well as T2D patients, a decrease in the IL-10 production and PB plasma level, especially pronounced in metabolic syndrome and obesity [103, 104], were observed [33, 103]. However, there

are data that in T2D patients, especially Brazilians and Italians, a slight increase in PB IL-10 levels were registered [83, 105].

The paradoxical action of the anti-inflammatory cytokines IL-4 and IL-10 in T2D is believed to be a protective compensatory response of the body to reduce the high level of pro-inflammatory cytokines so as to maintain a normal balance of pro- and anti-inflammatory cytokines [83, 92].

An important role in T2D pathogenesis is also performed by numerous chemokines. The level of CCL-2 in PB of T2D patients was found to be significantly increased [106, 107]. There was demonstrated a direct correlation between the CCL-4 level in PB and IR: CCL-4 deficiency significantly improves IR [108]. A marked increase in the level of chemokine CCL-5 in T2D patients was shown [109]. The RANTES/CCL-5 chemokine was reported to contribute to the IR development and was detected in MS and T2D [107].

A marked increase in the chemoattractant CXCL-8 (IL-8) upon T2D was also shown. It was established that this chemokine precedes the development of T2D [110]. In an *in vitro* study of isolated Langerhans islets in T2D patients, it was revealed that they secreted 33.5-fold more IP-10/CXCL-10 chemokine. A significant increase in CX3CL-1 in PB of T2D patients was observed [111]. These findings are considered to support the hypothesis of a significant role of the CX3CL-1 CX3CP-1 system in relation to AT inflammation, IR, and T2D.

For the first time, we showed that the chemokine IL-16/CXCL-16 is also actively involved in T2D pathogenesis [11], and it was confirmed by foreign researchers [112].

In conclusion it is worth noting that the above described changes in immunity indices were also observed in individuals with IR and the metabolic syndrome (prediabetes).

Natural and T-cell immunity in SARS-CoV-2-infected T2D patients

COVID-19 is an acute severe immune-mediated inflammatory disease caused by the SARS-CoV-2 virus or its mutants.

The mechanism of SARS-CoV-2 virus penetration into uninfected human cells is rather complicated and has not yet been sufficiently studied. The hypothesis based on studies [113-116] suggests that the key participants of SARS-CoV-2 virus penetration into epithelial cells are Spike proteins, which cover the virus spike surface, S-protein with glucose-regulating protein and receptor to angiotensin-converting enzyme (ACE).

At the same time, it was discovered that the proinflammatory transcription factor NF-kB and the signal transducer JNK including the activator of transcription 3 (STAT3) also play a critical role in the mechanism of SARS-CoV-2 virus entry [10].

Among COVID-19 patients, as it has already been noted, considerable percentages were patients with morbid T2D. In these COVID-19 patients, as in COVID-19 patients without diabetes, high levels of inflammatory biomarkers such as neutrophilia [13, 15], C-reactive protein [18, 20, 28, 117], ferritin [28, 119], D-dimer [20, 118], NLR index [28, 63], and increased proinflammatory cytokines [17, 19, 20] were also commonly observed.

Changes in natural and adaptive immunity parameters in COVID-19 patients were largely similar but more pronounced than in T2D patients, especially with concomitant obesity [2, 3].

In the hematological study of COVID-19 patients, pronounced changes in the leukocytic composition of PC were shown to be similar to those in T2D, namely by an increase in the total number of leukocytes [21, 23, 28, 63] the absolute number of neutrophils and partly monocytes [21, 23, 63, 120] with a decrease in the absolute number of lymphocytes [17, 19, 20, 120].

Significant neutrophilia, greater than that in T2D, was found in COVID-19 patients with existing complications, especially upon acute respiratory distress syndrome (ARDS) [2, 9, 10, 21, 23, 25], as well as concomitant T2D and obesity (BMI > 30 kg/m^2) [5-7, 10, 121, 122]. It is also believed that hyper neutrophilia in COVID-19 may be the most reliable biomarker of ARDS risk [22, 23].

At the same time, it should be noted that neutrophils are important cellular components involved in innate immunity [69, 123] and serve as producers of a range of major proinflammatory cytokines and chemokines [28, 63].

COVID-19 patients, compared to patients with T2D, are also characterized by marked lymphocytopenia, especially high in acute respiratory distress syndrome (ARDS), which largely results from the interaction between AP-1 located on SARS-CoV-2 and renin-angiotensin system receptors located on pneumocytes. Thus, very high lymphocytopenia can be an indicator of ARDS and high mortality risk [17-20, 23, 28, 120].

The human lymphocyte population comprises many subpopulations, often characterized by different functional activities, and so the question arises: which subpopulation is responsible for lymphocytopenia? There are ambiguous publications on the study of lymphocyte immunophenotype in patients infected with the SARS-CoV-2 virus that have not yet given a clear insight into the T-cell immunity status in this disease [114]. Thus, according to some data [20, 120], in patients with severe COVID-19, a decrease in the total number of T-lymphocytes (CD3+ T-cells) was due to a decrease in both of main lymphocyte subclasses, i.e. CD4+T- and CD8+ T-cells. According to other data [25], lymphocytopenia in patients with the severe form of COVID-19 was mainly due to a decrease in the CD4+ T-cell subpopulation, while the CD8+ T-cell subpopulation decreased to a much lesser degree. Moreover, the decrease in the number of CD4+ T-cells occurred mainly due to a decrease in the subpopulation of memory CD45 Ro+ cells and regulatory CD25+ CD127 low+ cells, while the number of naïve CD45 Ro- cells increased. According to these authors, the high number of naïve memory cells in patients with moderate illness indicates that the ratio of different immune cells may change during the COVID-19 disease.

Some studies revealed a significant decrease in all T-lymphocyte subpopulations (CD3+T-, CD4+T- and CD8+T-cells) in patients with COVID-19, which correlated with disease severity and mortality rate. Based on their study, the authors concluded that determining the counts of various T-lymphocyte subpopulations is a reliable biomarker for early diagnosis of COVID-19 and potential mortality risk [24].

In COVID-19 patients with moderate and especially severe disease, as in T2D patients, there were detected simultaneously both neutrophilia and lymphocytopenia that leads to a significant increase in the NLR index [28, 63, 124, 125], also called the "death index" [59, 128].

Also, it was shown [2, 26] that the increased levels of monocytes/macrophages in COVID-19 patients contribute greatly to ARDS pathogenesis through the hypersecretion of cytokines IL-1β and IL-6.

Summarizing our studies on significant disorders in the content and ultrastructure of PB neutrophils and monocytes, the main cellular components of natural immunity, in T2D patients, we can conclude that the increased susceptibility of T2D patients to SARS-CoV-2 infection is the result of the weakening of natural immune protection probably driven, in large part, by genetic predisposition.

Still, one of the most marked immune dysfunctions in T2D patients, especially those with developed COVID-19, is a sharp increase in PB content of various proinflammatory cytokines and chemokines, an event known as cytokine storm syndrome [17, 19, 20, 116].

About 20 different types of cytokines have already been studied in COVID-19 patients. The most studied are proinflammatory cytokines (IL-1β, IL-6, IL-7, IL-17, IL-18, TNFα), anti-inflammatory (IL-4, IL-10), immunomodulators (IL-2, IFNα and IFNγ), regulators of blood formation (CRP-H) and chemokines (CCL-2, CCL-4, CCL-5, CCL-8, CCL-10).

The degree of changes in the content of different types of cytokines in SARS-CoV-2-infected patients depends largely on the stage of the disease, its severity, the medical care quality and intensity, the complications developed, especially lung injury (ARDS), and comorbid morbidities, especially obesity and T2D [17, 22, 26, 116, 127].

The most pronounced changes in proinflammatory cytokines in COVID-19 patients, as well as in T2D patients, were found in cytokines IL-1β, IL-6, TNFα, and IL-17.

Thus, some publications reported that in COVID-19 patients, elevated levels of the proinflammatory macrophage cytokine IL-1β were detected [26, 128]. According to these data, in severe COVID-19 patients, the cytokine IL-1β activates and promotes the secretion of inflammasomes, a complex of intracellular multiproteins, leading to lytic cell death (pyroptosis) [128].

According to the majority of researchers [18, 17, 19, 20, 114], the highest content in PB in COVID-19, among different types of proinflammatory cytokines, was observed in IL-6, also called the "king of cytokines". At critical ICU and ARDS occurrence, the level of IL-6 in PB plasma in COVID-19 patients can reach 150 pg/ml or more (at norm: 1.4 ± 1.2 pg/ml), which is associated with the worst scenario, that is an increase in mortality rate [112]. In this regard, it was suggested that IL-6 should be considered the most accurate biomarker of COVID-19 disease severity and mortality risk [118, 120].

In COVID-19 patients, as well as in T2D patients, a significant increase in the content of TNFα, another major proinflammatory cytokine, secreted mainly by AT macrophages, was found [17, 19, 20, 118]. A particularly high level of this cytokine was

observed in obese women with COVID-19. Moreover, the high level of TNFα significantly decreased after therapy with metformin, which simultaneously lowered both hyperglycemia and BMI value [129].

There are also few reports on an increase in the proinflammatory cytokine IL-17 in COVID-19, similar to that in T2D [112].

Some unexpected data demonstrated a significant increase in the anti-inflammatory cytokines IL-4 and IL-10 in SARS-CoV-2-infected patients in contrast to T2D patients [17, 20, 25, 118].

Given this paradox, it was suggested that the increased level of anti-inflammatory cytokines IL-4 and, especially, IL-10 in COVID-19 patients resulted from the activation of protective compensatory mechanisms to restore the balance between pro- and anti-inflammatory cytokines [83, 91].

Important roles in protecting the body against SARS-CoV-2 were shown to play also by the immune-regulating cytokine IFNγ, which exerts a modulating effect and changes immunotolerance upon infection [117, 130], as well as the colony-stimulating cytokine CSF-H, which controls neutrophilopoiesis [17, 25, 118].

In COVID-19 patients, a significant increase in PB chemokines, such as CCL2, CCL5, CXCL8, CXCL10, CXCL16, was also detected that may explain the mechanism of mass migration of various types of immunological cells into inflammation focus [17, 19, 112, 118].

Until recently, the possibility of a direct inhibitory effect of the SARS-CoV-2 virus on insulin production by pancreatic beta cells remained subject to debate. Based on the analysis of case histories, a number of practitioners suggested that the SARS-CoV-2 virus could directly affect the mechanisms involved in the onset of diabetes in COVID-19 patients [122, 131, 132]. However, this assumption has been met with reasonable criticism [133].

Thus, using molecular and ultracytochemical methods, pronounced replication, differentiation, and degradation of beta cells in the pancreas, taken at biopsies from COVID-19 patients, were first described [134].

These data were confirmed by the study [135], showing that in COVID-19 patients, high susceptibility of beta cells to SARS-CoV-2 and secretion of cytokines and chemokines along with pronounced beta cell transdifferentiation, their degranulation and low insulin expression were observed [135].

A retrospective analysis of data obtained in a study of about 40,000 COVID-19 patients showed that the occurrence of primary T2D a short time after infection with SARS-CoV-2 was found to be 12.3 people per 1,000 patients [136].

T2D combined with overweight/obesity is considered one of the main risk factors for COVID-19 development [2, 6, 10]. Moreover, symbiosis of all three types of inflammation (COVID-19+T2D+obesity) resulted in the most severe course of COVID-19 disease and dramatically increased mortality [7, 9, 10].

In recent years, important new data have also been published that support the concept that T2D and obesity are chronic low-grade inflammatory diseases that give clues to the mechanism of effectiveness of present-day COVID-19 therapy. First of all, it was found that a number of widely-used hypoglycemic antidiabetic drugs such as insulin, glycogen-like polypeptide agonists, thiazolidinediones exhibit a pronounced anti-inflammatory effect, leading to a decrease in the elevated levels of proinflammatory cytokines (IL-1β, IL-6, TNFα) [32]. A particularly significant anti-inflammatory and immunosuppressive effect, as well as a positive therapeutic effect, and a decrease in the mortality rate among T2D patients infected with SARS-CoV-2 were shown for the antidiabetic drug metformin [129, 137, 138]. This can be attributed to the ability of metformin, along with its hypoglycemic effect, to normalize the levels of proinflammatory cytokines and other inflammatory biomarkers, as well as to reduce BMI [140]. It was very surprising discovery that the synthetic anti-inflammatory corticosteroids dexamethasone, prednisolone, and hydrocortisone possessed a particularly high positive therapeutic effect in severe COVID-19 patients. It was reported that the treatment of severe COVID-19 patients with massive doses of dexamethasone resulted in a significant reduction in the mortality rate, from approximately 40% (placebo) to 20% [140, 141].

Conclusion. The currently available data on the status of natural and adaptive immunity in patients with COVID-19, as well as T2D, accompanied by severe inflammation show that their immune parameters, in comparative terms, are very similar in many ways. This view was confirmed by increased levels of almost all major inflammation biomarkers such as neutrophilia, monocytosis, NLR inflammatory index, C-reactive protein, ferritin, D-dimer and proinflammatory cytokines in both diseases.

For T2D and COVID-19 patients, a significant increase in the absolute number of neutrophils and monocytes, the main cellular components of natural

immunity, is very common. Our study of neutrophils and monocytes in T2D patients using electron microscopy revealed pronounced submicroscopic changes in their cytoplasm in the form of degranulation of azurophilic granules and cytoplasm vacuolization. This indicates a significant decline in their function, i.e., the earliest primary stage of the immune defense against pathogens. The findings give a reasonable explanation for the increased susceptibility of T2D patients to SARS-CoV-2 infection.

In T2D patients with developed COVID-19, there predominates a disorder of adaptive immunity in the form of a significant decrease in the absolute number of lymphocytes in PB determined by a decrease in the number of T-lymphocytes (CD3+T-cells) at the expense of their subclass CD4+T-cells. Moreover, in the cytoplasm of CD4+ T-cells in T2D patients were found significant changes in the ultrastructure of Goll bodies, which are their cytological markers. The highest lymphocytopenia was observed in COVID-19 patients with concomitant T2D and its frequent comorbidity – obesity (COVID-19 + T2DM + obesity).

The most dramatic changes in the immune state upon both types of the disease (COVID-19 and T2D) were found to be in the increased levels of cytokines in the PB, particularly pro-inflammatory cytokines (IL-1β, IL-6, IL-17 and TNFα) and chemokines (CCL-2, CCL-4, CCL-5, CXCL-8, CXCL-10, and CX3CL-16) resulting in the development of cytokine storm syndrome, the degree of which in PB is associated with the severity of the clinical course of the disease and a risk factor for increased mortality rate.

Thus, analysis of the literature data and the results of our own studies showed that the increased susceptibility of T2D patients to SARS-CoV-2 infection is mainly attributed to weakening natural immunity, whereas in T2D patients with developed COVID-19, adaptive immunity disorders predominate. Moreover, the extremely severe clinical course and increased mortality rate are considered to be a consequence of summation and potentiation of hyperinflammation and oxidative stress already occurring in T2D resulting from impaired both natural and T-cell immunity, in the mechanism of which cytokines and chemokines play a crucial role.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

ІМУНОЛОГІЧНІ МЕХАНІЗМИ ПІДВИЩЕНОЇ СХИЛЬНОСТІ ДО ЗАХВОРЮВАННЯ НА COVID-19 ТА ЙОГО ТЯЖЧОГО ПЕРЕБІГУ У ХВОРИХ НА ЦУКРОВИЙ ДІАБЕТ 2 ТИПУ ТА ОЖИРІННЯ

К. П. Зак[1], М. Д. Тронько[1], С. В. Комісаренко[2]✉

[1]Інститут ендокринології та обміну речовин ім. В. П. Комісаренка НАМН України, Київ;
[2]Інститут біохімії ім. О. В. Палладіна НАН України, Київ;
✉e-mail: svk@biochem.kiev.ua

Огляд присвячено аналізу літературних даних та результатів власних досліджень авторів щодо стану імунітету у хворих на цукровий діабет типу 2 (ЦД2) та у хворих на ЦД2, інфікованих вірусом SARS-CoV-2. Показано, що у хворих на ЦД2, особливо, з надлишковим індексом маси тіла, порушеними є вміст та ультраструктура основних клітинних елементів природного імунітету – нейтрофілів та моноцитів, що супроводжується підвищеним вмістом прозапальних цитокінів (IL-1β, IL-6, IL-17 і TNF-α) у плазмі крові. Припускається, що підвищена схильність хворих на ЦД2 до зараження вірусом SARS-CoV-2 обумовлена, перш за все, ослабленням вродженого імунного захисту організму, тоді як у хворих на ЦД2 з розвиненим COVID-19 превалюють порушення адаптивного Т-клітинного імунітету, що супроводжується синдромом “цитокінового шторму”. Зроблено висновок, що гіперзапалення у хворих на ЦД2+COVID-19 є результатом потенціювання вже існуючих порушень вродженого імунітету у хворих на ЦД2 ще до їх зараження SARS-CoV-2, та адаптивного імунітету, у механізмі якого суттєву роль відіграють цитокіни та хемокіни.

Ключові слова: цукровий діабет типу 2, COVID-19, цитокіни, Т-лімфоцити, нейтрофіли, вроджений та адаптивний імунітет.

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