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Functional state of mononuclear phagocytes in patients with multiple sclerosis carrying disease-associated HLA-DR polymorphism

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One of the key factors in the pathogenesis of multiple sclerosis, which can affect the risk of its development, clinical manifestations and the nature of the course, is considered to be the polymorphism of disease-associated genes. The identification of genetic biomarkers in multiple sclerosis is the subject of current research in Europe, Asia, as well as in the United States and Canada, which are united in the International Multiple Sclerosis Genetics Consortium (IMSGC). The study included 137 patients with relapsing-remitting disease with haplotype AG, n = 137, and haplotype AA, n = 47; a group of patients with progressive multiple sclerosis with haplotype AG, n = 141, and haplotype AA, n = 17. Determination of the relative content of CD80, CD86 and PD-L1 positive peripheral blood monocytes was performed by immunofluorescence using PE-labeled monoclonal antibodies against CD14, FITC-labeled monoclonal antibodies against CD80, PE-Cy7-labeled monoclonal antibodies against CD86 and APC-labeled monoclonal antibodies against PD-L1 produced by EXBIO Praha, a. s. (Czech Republic). The content of cytokines (IFN-γ, IL-1β, IL-12, IL-10) in the supernatant of mononuclear macrophages was evaluated by enzyme-linked immunosorbent assay (ELISA). According to the results of the study, a method was proposed for identifying a genetic risk group for the development of multiple sclerosis by determining the SNP rs9271366 (AG) of the HLA-DRB1*1501-DQB1*0602 haplotype in individuals from the northeastern region of Ukraine. The minor allele was most often detected among patients with progressive PMS (89.2% vs. 10.8%), while among patients with relapsing-remitting disease the G allele was detected in 74.5% vs. 25.5%. The article deals with the antigen-presenting and cytokinesis properties of mononuclear phagocytes of patients with different types of multiple sclerosis depending on the presence of the disease-associated HLA-DR polymorphism. The level of CD86 expression was increased in all patients carrying the disease-associated allele, and the level of CD80 expression was increased only in heterozygous patients with progressive multiple sclerosis. The expression of PD-L1 molecules in patients with the disease-associated polymorphism was lower compared with both patients homozygous for the A-allele and the control group. The level of synthesis of cytokines IFN-γ, IL-1β, IL-12 in the supernatant of mononuclear macrophages of patients with progressive multiple sclerosis with the AG haplotype was more pronounced than in patients with relapsing-remitting multiple sclerosis with a disease-associated polymorphism with the AG haplotype, indicating the influence of genetic inheritance on the level of cytokine expression. It was found that the level of IL-10 in the supernatant of mononuclear macrophages of patients with relapsing-remitting multiple sclerosis with the AG haplotype indicates a less pronounced effect of this interleukin on the compensation of proinflammatory imbalance.

Keywords: multiple sclerosis; disease-associated polymorphism of HLA-DR; demyelinating disease; expression of co-signaling molecules; cytokines.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory immune-mediated multifactorial disease characterized by demyelination and axonal degeneration. The study of the pathogenesis of MS and the development of methods for its diagnosis are one of the most relevant areas of modern medicine and biotechnology. The extension of MS and the formation of different types of its course are the result of a complex interaction between environmental factors, genetic factors that determine individual susceptibility, and immunological and physiological characteristics of the patient (Dobson & Giovannoni, 2019; Kimoff et al., 2022).

As one of the key factors in the pathogenesis of MS, in terms of its ability to influence the risk of development, clinical manifestations and nature of the course of the disease, polymorphism of disease-associated genes is considered. The relationship of multiple sclerosis with genetic polymorphism is also the subject of modern research. To date, dozens of genes have been identified, the polymorphism of which is associated with a high risk of developing multiple sclerosis, an increased level of autoimmune disorders, and permeability of the blood-brain barrier. Identification of genetic biomarkers in PC is a very complex task, at which many modern studies in Europe, Asia, as well as in the USA and Canada are aimed. Researchers of polymorphic genetic variants that affect the risk of developing multiple sclerosis are united in the international consortium International Multiple Sclerosis Genetics Consortium (IMSGC). To date, 110 genetic risk variants for MS have been identified by IMSGC participants (Katsavos & Anagnostouli, 2013; Axisa & Hafler, 2016).

Due to the complexity of MS development, not only genetic markers of the disease, but a panel of biomarkers of different types may be needed to reflect pathological changes and study the mechanisms of this disease. In general, the definition of effective and adequate biomarkers of MS is a complex and difficult task, some aspects of which are addressed in this study. Recent studies using the method of genome-wide association studies (GWAS) have shown that polymorphic variants of genes of co-signaling molecules, such as CD40, CD86 and CD80, are also associated with multiple sclerosis (De Silvestri et al., 2019; Shepard et al., 2019).

The expression of HLA-DR, as well as the cosignaling molecules CD40, CD80 and CD86, which are involved in the implementation of

intercellular synapses, is an important characteristic of the state of mononuclear phagocytes, particularly their ability to activate lymphocytes and present antigens. Connected with it, higher or lower levels of expression of different HLA-DR molecules determine the spectrum of their possible combinations with specific receptors and the levels of co-signaling molecules, which determines the strength and quality of the T-cell response, and the type of polarization of peripheral T-cells.

First of all, the action of autoreactive T-lymphocytes and autoantibodies to myelin proteins is associated with demyelinating processes in MS. At the same time, in terms of immunological mechanisms of MS development, a key pathogenetic role is assigned to innate immunity, in particular mononuclear phagocytes of the brain (microglia), as well as peripheral organs and blood (monocytes, macrophages, dendritic cells). It is known that mononuclear phagocytes take part in various pathological processes at almost all stages of the development of the disease. Thus, along with the activation of microglia cells, the most important mechanism for initiating and maintaining inflammation in the CNS is the infiltration of peripheral monocytes into brain tissue with their subsequent transformation into activated macrophages and dendritic cells (Katsavos & Anagnostouli, 2013; Hollenbach & Oksenberg, 2015).

T-cell activation requires two distinct but synergistic signals: the first signal is provided by the interaction of the T-cell receptor with the MHC/antigen peptide complex expressed on antigen-presenting cells (APCs), while the second signal is achieved by the binding of cosignaling receptors on the surface of T-cells to ligands presented on APCs. The interaction of CD80, CD86 with CD28 acts as a co-signaling action for T-cell activation.

It is known that in MS patients, increased expression of CD80 has been found in active lesions in multiple sclerosis, as well as on monocytes and B cells in the cerebrospinal fluid and peripheral blood (Wagner et al., 2015). It has been found that the expression levels of CD86 and CD40L are significantly increased on monocyte cells in patients with secondary progressive MS compared with these indicators in a group of patients with the remitting type of the disease (Wagner et al., 2015). The expression of co-signaling molecules, such as CD80 and CD86, is noted on microglia, astrocytes, vascular epithelial cells in areas of MS lesions (Wagner et al., 2015; Zadeh et al., 2017). An important function in the regulation of immune homeostasis and in maintaining peripheral tolerance through secondary co-signaling action of activated lymphocytes is played by PD-1 and its ligands (PD-L1 and PD-L2), transmembrane proteins that have different expression patterns and differ in their affinity (Javan et al., 2016; Li et al., 2021).

Managing a complex disease such as MS requires not only knowledge of the underlying physiological processes necessary for clinical decision-making or for the identification and evaluation of novel therapeutic targets, but also an understanding of the mechanisms underlying the disease. Imaging of disease activity by magnetic resonance imaging only partially correlates with clinical measures of disease progression, such as relapse rate or expanded disability score (Dobson & Giovannoni, 2019; Kimoff et al., 2022). There are still no clear objective clinical criteria for diagnosing or predicting the type of clinical course, signs of disease progression (e.g., transition from clinically isolated syndrome to clinically significant multiple sclerosis, or prognosis of malignancy in MS.

Connected with this, the aim of our work was to study the features of the functional state of peripheral blood mononuclear phagocytes in patients with multiple sclerosis with the presence of the disease-associated HLA-DR polymorphism among the population of the North-Eastern region of Ukraine by determining the single nucleotide polymorphism SNP rs9271366 (AG) haplotype, the number of cells expressing receptors for CD80, CD86, PD-L1 monocytes, and the level of secretion of IFN-γ, IL-1β, IL-12, IL-10 to monitor the risk group for the development of the disease.

Materials and methods

The study was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all participants, the protocol was approved by the Bioethics Commission of the State Institution "Mechnikov Institute of Microbiology and Immunology of the National Academy of Medical Sciences of Ukraine", Kharkiv, Ukraine, which confirms that the materials and methods of the conducted scientific research comply with the order of the Ministry of Health of Ukraine dated 01.11.2000 No. 281 (protocol No. 5 dated 05.05.2025). The data are anonymized and stored in accordance with GDPR requirements.

Biological material was blood and buccal epithelium samples from MS patients and clinically healthy people. In addition to assessing the clinical status of patients, the study design also included an assessment of systemic immunity indicators: the level of expression of co-signaling molecules CD80, CD86 and PD-L1 on peripheral blood monocytes, determination of cytokine content (IFN-γ, IL-1β, IL-12, IL-10) in the supernatant of cultured mononuclear cells and analysis of HLA polymorphism, in particular, the detection of the HLA-DR15 haplotype. for its specific SNP marker rs9271366.

The immunological study was conducted in the Laboratory of Clinical Immunology and Allergology of the Mechnikov Institute of Microbiology and Immunology of the National Academy of Medical Sciences of Ukraine. The diagnosis was verified in the Department of Neuroinfection and Multiple Sclerosis of the Institute of Neurology, Psychiatry and Narcology of the National Academy of Medical Sciences of Ukraine. The survey involved 342 patients with a verified diagnosis of "multiple sclerosis", residents of Kharkiv and Kharkiv region, of whom: 97 men aged 33.7 ± 7.7 years and 245 women aged 42.1 ± 11.9 years, respectively. All patients were divided into the following groups depending on the clinical course of the disease: 1) RRMS – a group of patients with relapsing-remitting type of MS, with haplotype AG, n = 137; 2) RRMS – a group of patients with relapsing-remitting MS, with haplotype AA, n = 47; 3) PMS – a group of patients with progressive MS with haplotype AG, n = 141; 4) PMS – a group of patients with progressive MS, with haplotype AA, n = 17. The criterion for including patients in the study was the absence of therapy with disease-modifying drugs or other immunosuppressive therapy for at least six months prior to the study.

The control group consisted of 364 clinically healthy individuals, men and women with a mean age of 30.1 ± 8.2 years. The inclusion criteria for the control group were the absence of acute infections for at least 1 month before taking the biological material, the absence of chronic inflammatory, allergic and autoimmune diseases.

Molecular genetic methods. Isolation of high-molecular-weight DNA was performed on a magnetically sensitive sorbent using the NeoPrep100 DNA Magnet kit (NeoGene, Ukraine). SNP polymorphism rs9271366 A/G was typed by allele-specific amplification with subsequent electrophoretic detection of the results. Primers were selected according to the method (Liu et al., 2012), adding a mismatch at the third position at the 3' ends. Primers MS92AF 5`-CACGTA ATATAAATGGTTGCAAAGGA-3`, MS92GF 5`-CACGTAATA TAAATGGTTGCA-AAGGG-3` and MS92R5` AACCTGATGTA ACAGA(C/T)CTCTA-3` (Eurofins Genomics), as well as Taq-mut polymerase (Liteh), were used in the study. Amplification was performed on a Tercyc multichannel amplifier. Amplification mode: denaturation at 96 °C for 3 minutes and 35 cycles, which included denaturation at 95 °C for 30 seconds, annealing at 58 °C for 30 seconds and synthesis at 72 °C for 30 seconds. The length of the amplicon was 233 bp. Electrophoresis was performed in 2% agarose gel in TAE buffer. Electrophoresis analysis was performed on a UVT 1 "Biocom" transilluminator.

Identification of single nucleotide polymorphism (SNP) rs9271366 using allele-specific PCR. Molecular genetic research within the framework of determining the presence of disease-associated polymorphic variants of HLA-DR as markers of risk of MS development included: collection of biological material, preparation of DNA samples, development and validation of a method for typing a single polymorphism rs9271366 A/G using allele-specific PCR, determination of the haplotype HLA-DRB1*1501-DQB1*0602 (HLA-DR15) in 342 patients with MS and 364 healthy individuals.

The presence of the "risk" haplotype HLA-DRB1*1501-DQB1*0602 in patients with MS was established using one of the

specific markers of this gene variant – the G allele of SNP rs9271366, which belongs to non-synonymous single nucleotide polymorphisms and is located on chromosome 6p21.3 in the HLA locus with localization 32 619 080. SNP rs9271366 is characterized by the presence of two alleles – A and G (A>G).

Methods for detecting HLA-DR15 based on typing of tagSNPs specific to this haplotype allow achievement of sufficiently high levels of sensitivity and specificity (Javan et al., 2016). For typing of SNP rs9271366 (AG), a method based on allele-specific PCR was developed using a newly created, exclusive primer system that allowed for clear differentiation of alleles of this locus.

The sequence CACGTAATATAAATGGTTGCAAAAG (AG) was chosen as the starting oligonucleotide for the forward primer, the last nucleotide of which was either A or G, depending on the reaction setup. Due to the low content of CG (30.77%), the length of the primer was increased to 26 steps, which provided a sufficiently high melting temperature T_m = 57.1 °C. Although the oligonucleotide had a high self-complementarity index (6), the most unfavorable 3'-self-complementarity was 0. The reverse primer 5`-AACCCTGATGTAA CAGA(C/T)CTCTA-3` was selected using the PrimerBlast program (NCBI), its characteristics: length 23 nucleotides, T_m = 58.32 °C, GC content 43.48%, self-complementarity – 5 and 3'-self-complementarity – 2. Checking the primers against the NCBI databases indicated their high specificity for the selected region of the human genome.

Since a single nucleotide difference cannot completely exclude amplification of an alternative allele, additional changes were made to the sequence of the 3'-end of the forward primers to ensure the specificity of allele-specific PCR, which directly recognize allelic variants of the studied polymorphism. When introducing an additional mismatch, we were guided by the recommendations ofJavan et al. (2016), which, based on the study of more than 2000 primer pairs, showed that the highest specificity is provided by introducing a mismatch in the third position from the 3'-end of the primer (Javan et al., 2016). In our case, this was ensured by replacing A with G in the 3rd position from the 3' end of the forward primer. The final structure of the primers produced by Eurofins Genomics that were used in the study is given below:

MS92AF 5`-CACGTAATATAAATGGTTGCAAAGGA-3` 26
MS92GF 5`-CACGTAATATAAATGGTTGCAAAGGG-3` 26
MS92R5`-AACCCTGATGTAACAGA(C/T)CTCTA-3` 23

Results with a reproducibility level of more than 95% were obtained when using a mutant recombinant Taq polymerase of the company, specially adapted for allele-specific PCR with electrophoretic detection of reaction results.

The developed method for determining the presence of the HLA-DRB1*1501-DQB1*0602 haplotype by typing SNP rs9271366 A/G by allele-specific PCR is characterized by a significant reproduction speed (up to 4 hours), high indicators of specificity, sensitivity and reproducibility of reaction results (not less than 95%) and can be used to assess the increased risk of MS development among certain cohorts of the Ukrainian population for early diagnosis of MS, as well as diagnosis of the disease in subclinical types of its course, prediction of the effectiveness of MS therapy by genetic markers.

Isolation of the monocytic fraction of peripheral blood mononuclear cells was performed according to the method (Repnik et al., 2003), adapted to small blood volumes.

The relative content of CD80, CD86 and PD-L1 positive peripheral blood monocytes was determined by immunofluorescence using PE-labeled monoclonal antibodies against CD14, FITC-labeled monoclonal antibodies against CD80, PE-Cy7-labeled monoclonal antibodies against CD86 and APC-labeled monoclonal antibodies against PD-L1 manufactured by EXBIO Praha, a.s. (Czech Republic).

The assessment of cytokine content (IFN-γ, IL-1β, IL-12, IL-10) in the supernatant of mononuclear macrophages was carried out by solid-phase enzyme-linked immunosorbent assay (ELISA) using Ukrainian-certified test systems manufactured by Vector Best-Ukraine (Ukraine) using the Stat-Fax 303 enzyme-linked immunosorbent assay.

The normality of data distribution in groups was checked using the Shapiro-Wilk test. To determine the significance of differences between indicators in the studied samples, the Mann-Whitney U test was used, with a normal distribution, the Pearson test (χ^2 coefficient) (McHugh, 2013), Spearman correlation coefficient (r) (De Winter et al., 2016) diagnostic odds ratio (DOR) (Böhning et al., 2010; McHugh, 2013).

Results

Based on the obtained statistical data on the presence of the G allele of SNP rs9271366 in MS patients (PMS and RRMS), the work shows a positive correlation (r = 0.65; r = 0.75, respectively) between this disease-associated G-allele and MS. The values of the Pearson test in MS patients (PMS and RRMS) with the presence of the disease-associated HLA-DR polymorphism have a statistically significant difference (219.60; $P < 0.0001$; 301.73; $P < 0.0001$) compared to the control group of healthy individuals with the AG haplotype, respectively. Furthermore, in RRMS patients with the presence of the disease-associated HLA-DR polymorphism (haplotype AG), the probability of developing MS increases by 73 times (DOR = 72.87), and in PMS patients the probability of developing MS increases by 208 times (DOR = 207.77), respectively.

To identify the carriership of the HLA-DRB1*1501-DQB1*0602 haplotype and the frequency of occurrence among the population of the northeastern region of Ukraine in the presence of the single nucleotide polymorphism rs9271366 to determine the risk group for MS, 342 patients with MS were examined, including 158 patients with PMS, 184 with RRMS, respectively, in comparison with the control group of 364 healthy individuals.

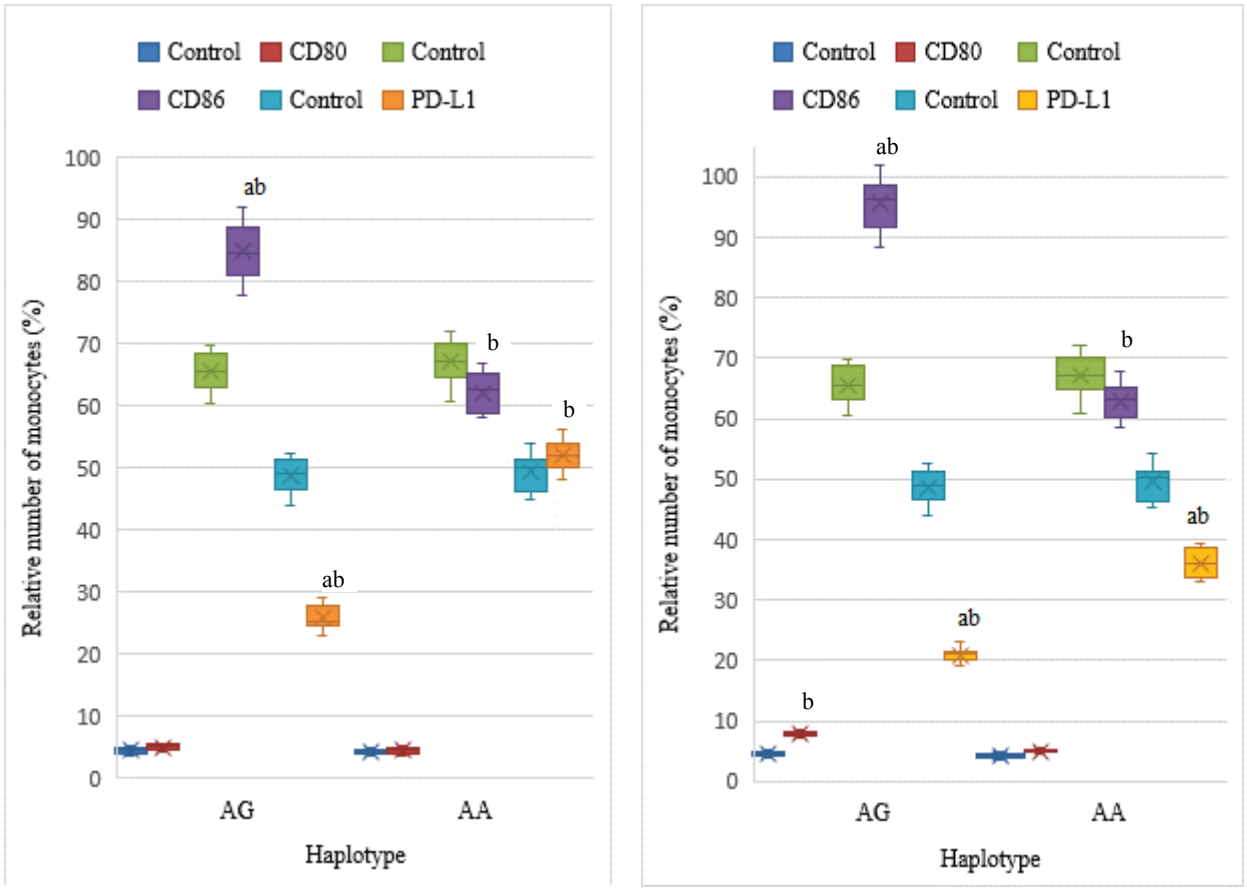
To determine the relationship between the HLA-DRB1*1501-DQB1*0602 haplotype (disease-associated HLA-DR polymorphism), (haplotype AG) and MS, the Pearson test, Spearman correlation coefficient (rs) were used, which defines a weak correlation from 0 to 0.4; moderate correlation from 0.4 to 0.5; a strong association of 0.6 to 1.00 and a diagnostic odds ratio (DOR).

Regarding the monitoring of immunological parameters, the study determined the relative number of CD80, CD86 and PD-L1-positive peripheral blood monocytes in patients with MS. Based on the assessment of the Shapiro-Wilk W-test for the parameters studied in the study, it was determined that the distribution of the sample does not correspond to a normal (Gaussian) distribution, since the $P < 0.05$.

The relative number of CD80-expressing monocytes in RRMS patients carrying the disease-associated HLA-DR polymorphism (haplotype AG) did not differ from those in patients with the AA haplotype ($P = 0.09$) and controls ($P = 0.10$). In PMS patients with the AG haplotype, the study value was increased relative to those in patients with the AA haplotype ($P < 0.0001$; r = 0.91) and controls ($P < 0.0001$, Fig. 1a).

The studied parameter in the blood of PMS patients with haplotype AA did not differ from the control group ($P = 0.05$), and did not differ from the results obtained in RRMS patients of the correspondding group ($P = 0.05$). In addition, the relative number of monocytes expressing CD80 in PMS patients with haplotype AG increased 1.59-fold compared with the studied parameters in the corresponding group of RRMS patients ($P < 0.0001$, Fig. 1b).

In patients with RRMS and PMS with the AG haplotype, the relative number of CD86 cells in peripheral blood monocytes was significantly higher than in patients of these groups homozygous for the A allele ($P < 0.0001$; r = 0.95) and ($P < 0.0001$; r = 0.87), respectively. In addition, the level of CD86 expression on peripheral blood monocytes in patients heterozygous for the disease-associated polymorphism with RRMS and PMS was significantly higher than in healthy individuals heterozygous for this allele $P < 0.0001$. However, the relative number of monocytes expressing CD86 in patients with RRMS and PMS without the disease-associated HLA-DR polymorphism did not differ significantly from that in healthy individuals with the AA haplotype ($P = 0.06$), respectively. The relative number of monocytes expressing CD86 in PMS patients carrying the disease-associated HLA-DR polymorphism was increased compared with the studied values in the corresponding group of patients with RRMS ($P < 0.0001$). Whereas the studied value in PMS patients with the AA haplotype did not differ from the results obtained in RRMS patients of the corresponding group ($P = 0.06$, Fig. 1).



a *b*

Fig. 1. Relative number of monocytes (%) expressing co-signaling molecules of patients with MS ; relapsing-remitting multiple sclerosis-RRMS-(A) and progressive multiple sclerosis-PMS-(B) depending on SNP rs9271366 (single nucleotide polymorphism – minor G-allele), haplotypes (AG & AA), compared to control (*a*), compared to haplotype AA (*b*); Me (Q25 %; Q75 %); Control (AA) n = 350; Control (AG) n = 14; PMS (AA) n = 17; PMS (AG) n = 141; RRMS (AA) n = 47; RRMS (AG) n = 137

Similar dynamics were observed in the expression of PD-L1 molecules in peripheral blood monocytes of MS patients carrying the disease-associated HLA-DR polymorphism (Fig. 1a).

The work showed that in patients with MS (RRMS and PMS) and the presence of the disease-associated HLA-DR polymorphism, the level of expression of PD-L1 molecules on peripheral blood monocytes was significantly lower than in patients with RRMS and PMS homozygous for the A-allele ($P < 0.0001$; $r = 0.96$) and ($P < 0.0001$; $r = 0.85$), respectively. In addition, the studied indicator in patients with the AG haplotype with different MS courses was significantly lower compared to the control group ($P < 0.0001$). The level of PD-L1 expression on peripheral blood monocytes of patients with PMS, carriers of the disease-associated HLA-DR polymorphism, was reduced compared to the studied indicators of the corresponding group of patients with RRMS ($P < 0.0001$). Similar dynamics were demonstrated by the studied indicator in patients with PMS with the AA haplotype, which decreased by 1.43 times compared to the results obtained in patients with RRMS of the corresponding group ($P < 0.0001$, Fig. 1b).

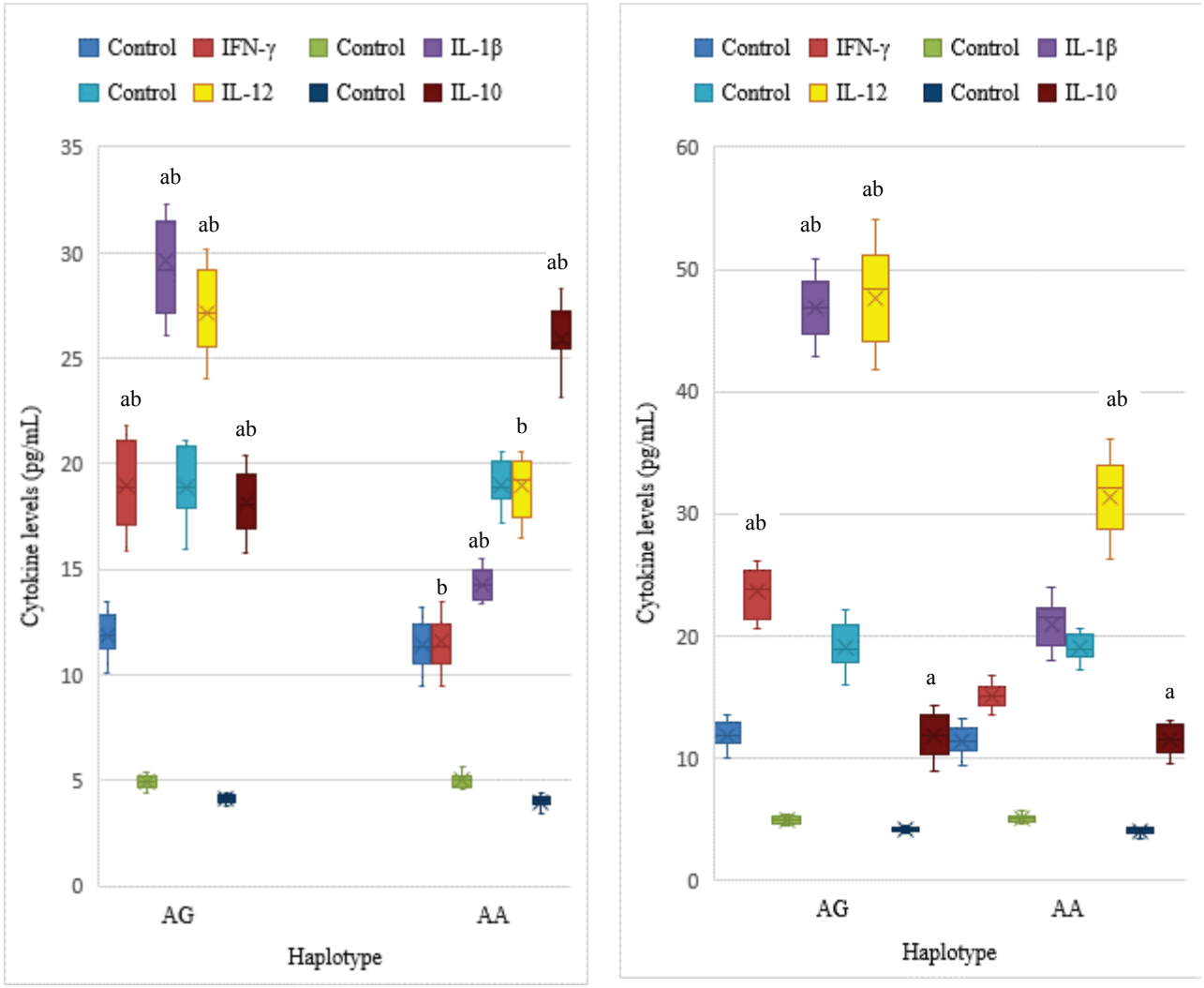
Based on the results obtained (Fig. 2), in the control group of healthy individuals, no significant differences were observed between the levels of cytokines IFN-γ, IL-1β, IL-12, IL-10 depending on the presence of SNP rs9271366 G (AG & AA). Exactly: $P = 0.76$, $P = 0.15$, $P = 0.50$, $P = 0.13$, respectively.

In our work, the level of cytokines IFN-γ, IL-1β, IL-12, IL-10 was determined in the supernatant of mononuclear macrophages of patients with MS. Thus, in patients with RRMS with the presence of the disease-associated polymorphism HLA-DR (haplotype AG), the levels of IFN-γ IL-1β and IL-12 were increased. The level of IFN-γ in the supernatant of mononuclear macrophages of RRMS patients with the presence of the disease-associated HLA-DR polymorphism (haplotype AG) was 1.63 times higher compared to the results obtained in patients of this group with the absence of the disease-associated HLA-DR polymorphism (haplotype AA) ($P < 0.0001$; $r = 0.88$) and 1.59 times higher compared to the control group ($P < 0.0001$), respectively. At the same time, in RRMS patients with the absence of the disease-associated HLA-DR polymorphism (haplotype AA) the studied indicator did not differ from the control group ($P = 0.76$, Fig. 2a).

The IL-1β content in the supernatant of mononuclear macrophages of RRMS patients with haplotype AG was increased 2.02-fold compared with the corresponding values in patients with haplotype AA ($P < 0.0001$; $r = 0.85$) and 5.96-fold compared with the studied results in the control group ($P < 0.0001$), respectively. Patients of this group with the absence of the disease-associated HLA-DR polymorphism (haplotype AA) also had a significant difference (2.83-fold) in terms of increased IL-1β levels compared with the control group ($P < 0.0001$).

The IL-12 content in the supernatant of mononuclear macrophages of RRMS patients with haplotype AG was 1.47-fold increased compared with the corresponding values of patients with haplotype AA ($P < 0.0001$; $r = 0.82$) and 1.46-fold higher than the studied values in the control group ($P < 0.0001$), respectively. RRMS patients without the disease-associated HLA-DR polymorphism (haplotype AA) did not have a significant difference in IL-12 levels compared with the control group ($P = 0.48$) (Fig. 2A).

In PMS patients - carriers of the disease-associated HLA-DR polymorphism, the cytokine profile had significant differences compared to the results of the control group and the indicators obtained in PMS patients with the absence of the disease-associated HLA-DR polymorphism, with the exception of IL-10 indicators.



a *b*

Fig. 2. Cytokine levels (pg/mL) determination in the macrophages mononuclear supernatant of patients with MS ; relapsing-remitting multiple sclerosis-RRMS-(A) and progressive multiple sclerosis-PMS-(B) depending on SNP rs9271366 (single nucleotide polymorphism-minor allele G), haplotypes (AG & AA), compared to control (*a*), compared to haplotype AA (*b*); Me (Q25 %; Q75 %); Control (AA) n = 350; Control (AG) n = 14; PMS (AA) n = 17; PMS (AG) n = 141; RRMS (AA) n = 47; RRMS (AG) n = 137

Specifically: the level of IFN-γ in the supernatant of mononuclear macrophages of PMS patients with the presence of the disease-associated HLA-DR polymorphism was 1.55 times higher compared to the results obtained in PMS patients with the absence of the disease-associated polymorphism ($P < 0.0001$; $r = 0.83$) and 1.98 times higher compared to the indicators of the control group ($P < 0.0001$), respectively. At the same time, in PMS patients with the absence of the disease-associated HLA-DR polymorphism, the studied indicator was 1.34 times higher in terms of IFN-γ content compared to controls ($P < 0.0001$). The level of IFN-γ in the supernatant of mononuclear macrophages of PMS patients with the AG haplotype was increased compared to the studied indicators of RRMS patients of the corresponding group ($P < 0.0001$). The studied indicator in PMS patients with the AA haplotype was also increased compared to the obtained indicators of RRMS patients with the same haplotype ($P < 0.0001$, Fig. 2b).

The IL-1β content in the supernatant of mononuclear macrophages of PMS patients with the AG haplotype was increased 2.20 times compared with the corresponding indicators of patients with the AA haplotype ($P < 0.0001$; $r = 0.84$) and 9.55 times compared with the studied results in the control group ($P < 0.0001$), respectively. PMS patients with the absence of the disease-associated HLA-DR polymorphism also had a significant difference (4.22 times) in the increased level of IL-1β compared with the control group ($P < 0.0001$). The IL-1β level in the supernatant of mononuclear macrophages of PMS patients with the presence of the disease-associated HLA-DR polymorphism was increased 1.60 times compared with the studied indicators of RRMS patients of the corresponding group ($P < 0.0001$). The studied indicator in PMS patients with the AA haplotype was also increased by 1.49 times compared with the obtained indicators in RRMS patients with the same haplotype ($P < 0.0001$, Fig. 2b).

The IL-12 content in the supernatant of mononuclear macrophages of PMS patients with the AG haplotype was 1.51 times higher than the corresponding indicators of patients with the AA haplotype ($P < 0.0001$; $r = 0.81$) and 2.61 times higher than the studied indicators in the control group ($P < 0.0001$), respectively. PMS patients with the AA haplotype had a significant difference in terms of an increased, 1.70 times, level of IL-12 compared to the control group ($P < 0.0001$).

The level of IL-12 in the supernatant of mononuclear macrophages of PMS patients with the presence of the disease-associated HLA-DR polymorphism was increased 1.78 times compared to the studied indicators of RRMS patients of the corresponding group ($P < 0.0001$). The studied indicator in PMS patients with the AA haplotype was also increased by 1.74 times compared to the obtained indicators in RRMS patients with the same haplotype ($P < 0.0001$, Fig. 2b).

The IL-10 level in the supernatant of mononuclear macrophages of RRMS patients with the disease-associated HLA-DR polymorphism (haplotype AG) was increased by 4.38 times compared to controls ($P < 0.0001$), but decreased by 1.42 times compared to the values of the study group of patients with haplotype AA ($P < 0.0001$; $r =$

−0.91). RRMS patients without the disease-associated HLA-DR polymorphism (haplotype AA) also had a significant difference in terms of increased, 6.58-fold, IL-10 level compared to controls ($P < 0.0001$).

The IL-10 level in the supernatant of mononuclear macrophages of PMS patients with the AG haplotype was increased 3.03 times compared to the control ($P < 0.0001$), but did not differ from the indicators of the studied group of patients with the AA haplotype ($P = 0.23$; $r = 0.12$). PMS patients with the AA haplotype also had a significant difference in terms of an increased, 2.90 times, level of IL-10 compared to the control group ($P < 0.0001$). The IL-10 level in the supernatant of mononuclear macrophages of PMS patients with the presence of the disease-associated HLA-DR polymorphism was reduced 1.49 times compared to the studied indicators of RRMS patients of the corresponding group ($P < 0.0001$). The studied indicator in PMS patients with haplotype AA was also reduced by 2.27 times compared to the obtained indicators in RRMS patients with the same haplotype ($P < 0.0001$).

Therefore, based on the obtained results, the indicators of the antigen-stimulating function of the costimulatory molecules CD80, CD86 and PD-L1 expressed on peripheral blood monocytes and the level of monocyte secretion of cytokines IFN-γ, IL-1β, IL-12, IL-10 in patients with MS are mediated by the influence of a hereditary factor, the presence of a disease-associated HLA-DR polymorphism.

Discussion

It is known that susceptibility to multiple sclerosis (MS) is consistently associated with the genotype of the human leukocyte antigen HLA-DRB501-DRB1*1501, but the impact on disease severity and clinical outcome varies in different populations (De Silvestri et al., 2019; Delfan et al., 2021; Prapas & Anagnostouli, 2024). Thus, in countries in Eastern Europe, a pronounced association of this disease with the presence of the HLA-DRB1*1501-DQB1*0602 haplotype in patients, one of the specific markers (tag SNP), has been found. The tag SNP is a representative single nucleotide polymorphism in a region of the genome with high linkage disequilibrium (Zivadinov et al., 2007).

It is possible to identify genetic variability and association with phenotypes of each SNP in a chromosomal region, which is the G allele of SNP rs9271366, located on chromosome 6p21.3 at the HLA locus with localization 32619080 (Al Jumah et al., 2018; De Silvestri et al., 2019). SNP rs9271366 is characterized by the presence of two alleles A and G. Detection of HLA-DRB1*1501 by typing SNP rs9271366 has a high level of sensitivity and specificity (Zivadinov et al., 2007). According to the literature (Wu et al., 2010), studies of the correlation of the HLA-DRB5*01-DRB1*1501 genotype and phenotype in a large cohort of MS patients in Western Australia show that in this population, which is mainly of Anglo-Celtic and Northern European origin, it is relevant from the point of view of population genetics to study the molecular genetic component of the disease. HLA-DRB5*01-DRB1*1501 is not only a strong factor determining the risk of the disease, but also can. be associated with the severity of the disease. This was also the basis for the identification of this haplotype in Ukraine.

Based on the developed methodology, by typing SNP rs9271366, we identified the carrier of this disease-associated HLA-DR polymerphism (G allele) among the population of the northeastern region of Ukraine with MS, which was determined in patients with PMS and RRMS who were heterozygous for the G-allele of SNP rs9271366, which indicates the presence of a powerful genetic risk factor for the development of MS. Most often, the minor allele was determined among patients with a progressive form of multiple sclerosis (89.2% vs 10.8%, $P < 0.05$), which is 23.5 times more often than in healthy individuals. Among patients with a relapsing-remitting form of the disease, the G-allele was determined in 74.5% vs 25.5%, $P < 0.05$.

High levels of HLA class II gene expression may directly influence the concentration of MHC-antigen peptide complexes and the expression of antigen-presenting molecules CD80, CD86 and PD-L1 on monocytes in patients with MS through the interaction of genetic, molecular and cellular processes that regulate the function of these cells (Prapas & Anagnostouli, 2024). Specifically, increased expression and presentation of HLA class II antigens (in particular, HLA-DRB1*1501) encodes major histocompatibility complex class II molecules that present antigenic peptides (MHC-II-antigen peptide) on the surface of monocytes to $CD4^+$ T - cells, enhancing their activation through the TCR (T-cell receptor), which triggers an immune response. The presence of HLA-DRB1*1501 alleles enhances the presentation of self-antigens and alters the expression of co-signaling molecules CD80, CD86 and PD-L1 on monocytes. These changes contribute to T-cell hyperactivation and chronic inflammation, which plays a key role in the pathogenesis of MS (Prapas & Anagnostouli, 2024). The co-signaling molecules CD80, CD86 and PD-L1 are key regulators of the immune response, ensuring efficient activation or inhibition of T-cells by interacting with their receptors. In the context of MS, these molecules play an important role in maintaining or breaking immune tolerance. CD80 and CD86 molecules are expressed on the surface of antigen-presenting cells (APCs), such as monocytes, dendritic cells and macrophages, and are involved in T-cell activation. Monocytes, macrophages and microglia express a variety of signaling molecules and receptors specific for innate immunity. The expression of CD80 and CD86 is induced by pro-inflammatory cytokines, such as IFN-γ and TNF-α. Numerous studies on APC have shown that the expression of co-signaling molecules is a link between innate and adaptive immunity (Chastain et al., 2011; Wagner et al., 2015; Sospedra & Martin, 2016; Ogawa et al., 2019). In the normal state of the CNS, microglia cells are characterized by a branched morphology and constantly "scan" brain tissue for minor damage. During damage, inflammation, and infectious processes in the CNS, activated microglia, through the expression of co-signaling molecules CD80, CD86, and many others, are responsible for the elimination of cell debris and pathogens mediated by cytokines, which can also be correlated with the processing and presentation of antigen to T-cells infiltrating the CNS. Acting as direct orthologs of microglia, peripheral blood monocytes represent a valuable model system for studying the role of microglia in neurodegeneration. In addition, monocytes are among the most sensitive cells of the immune system, capable of responding to even minor pathological processes in the body at the earliest stages. All this makes them a unique and promising object for determining biomarkers of the course of MS (Koliada et al., 2020).

In our work, it was shown that increased expression of CD80+ on peripheral blood monocytic cells was detected only in patients with PMS with the presence of the disease-associated HLA-DR polymorphism (haplotype AG). Similar dynamics regarding the expression of these molecules in patients with PMS were observed in the works of Wiesemann et al. (2008). In patients with both progressive types of the disease and in patients with relapsing-remitting multiple sclerosis, the relative number of CD86+ cells was significantly higher in patients with the presence of the AG haplotype, which may indicate a probable dependence on the presence of the G-allele. This may be due to excessive activation of T-helper cells (in particular, Th1 and Th17), which produce pro-inflammatory cytokines (IFN-γ, IL-17), contributing to inflammation and demyelination in MS in patients with the presence of specific HLA-II class alleles, in particular HLA-DRB1*1501 (Hollenbach & Oksenberg, 2015; Wagner et al., 2015; Zadeh et al., 2017). The expression of CD80 and CD86 on blood monocytes in MS patients is determined by the influence of pro-inflammatory cytokines, genetic predisposition, activation of Toll-like receptors and impaired regulatory mechanisms. This leads to chronic inflammation and activation of autoaggressive immune cells, which underlies the development and progression of MS (Koliada et al., 2022).

In patients with a different course of MS, carriers of disease-associated HLA-DR polymorphism, expression of co-signal CD86 on peripheral blood monocytes was higher than the studied indicators of MS patients homozygous for the A-allele. In addition, the relative number of monocytes expressing CD86 in patients with PMS and RRMS with the absence of disease-associated HLA-DR polymorphism (haplotype AA) did not differ from the indicators of healthy individuals with haplotype AA. The results obtained in our work indicate a correlation between the presence of the G-allele and increased expression of HLA-II class molecules on the surface of mo-

nocytes and, accordingly, co-signal CD80 and CD86, which activate T-cells in patients with MS, carriers of disease-associated polymorphism. Studies (Wagner et al., 2015; Zadeh et al., 2017) indicate that increased expression of CD80/CD86 enhances the activation of autoaggressive T-cells (especially Th1 and Th17), contributing to inflammation and demyelination in MS in patients with the presence of specific HLA-II class alleles, in particular HLA-DRB1*1501. The expression of CD80 and CD86 on blood monocytes in MS patients is determined by the influence of proinflammatory cytokines, genetic predisposition, activation of Toll-like receptors and disruption of regulatory mechanisms. This leads to chronic inflammation and activation of autoaggressive immune cells, which underlies the development and progression of MS (Wagner et al., 2015; Zadeh et al., 2017).

Increasing evidence (Li et al., 2021; Sadeghnejad et al., 2024) suggests that Programmed Death-Ligand 1 (PD-L1) as an inhibitor of T-cell activation is involved in the pathogenesis of autoimmune diseases, including MS. PD-L1 is predominantly expressed on activated T-cells, B-cells, dendritic cells (DCs), macrophages, mesenchymal stem cells, and cultured mast cells of bone marrow origin. PD-1 expression and its binding affinity to ligands regulate the threshold for induction and maintenance of T-cell tolerance, as well as immune cell activation and cytokine secretion (Francisco et al., 2010; Sambucci et al., 2018). It is known that the programmed cell death inhibitory receptor (PD-1) and its programmed cell death ligand (PD-L1) are negative regulators of immune responses that maintain immune tolerance by regulating the expansion, differentiation, and activation of immune cells (Francisco et al., 2010; Sambucci et al., 2018). PD-1 and its ligands (PD-L1 and PD-L2) – transmembrane proteins that have different expression patterns and differ in their affinity - play an important role in the regulation of immune homeostasis and in the maintenance of peripheral tolerance through secondary co-signaling in activated monocytes. PD-1 and its ligands protect against potentially pathogenic autoreactive effector T-cells by simultaneously influencing two mechanisms of peripheral tolerance: by stimulating the development and function of Regulatory T cells (Tregs) and by directly inhibiting potentially pathogenic autoreactive T-cells in the periphery, maintaining a threshold for T-cell activation high enough to protect against autoimmune processes (Sambucci et al., 2018; Sun et al., 2019; Sadeghnejad et al., 2024).

Our work showed that in patients with a different course of MS, carriers of disease-associated polymorphism, the level of expression of PD-L1 molecules on peripheral blood monocytes was lower than in patients homozygous for the A-allele, respectively. In addition, the level of expression of PD-L1 molecules on blood monocytes of patients with PMS and RRMS with haplotype AG was reduced compared to the control group. This may indicate a significant activation of autoimmune processes and dysregulation of apoptosis mechanisms, which may be mediated by a hereditary factor in the presence of disease-associated HLA-DR polymorphism. And at the same time, the expression of PD-L1 molecules on blood monocytes of patients with progressive type of MS with haplotype AA is reduced compared to the control group, while the corresponding studied indicator in the blood of patients with RRMS does not differ from the control, which may indicate an indirect effect of the disease-associated HLA-DR polymorphism on the activation of autoimmune processes. Obviously, a decrease in the level of PD-L1 expression in patients with MS may occur due to peripheral tolerance of T-cells, which may be associated with the influence of the disease-associated HLA-DR polymorphism, as evidenced by the correlation dependence of the reduced level of expression of PD-L1 molecules on monocytes of patients with haplotype AG, but this requires additional studies.

MS is promoted by genetic factors that result in abnormalities in peripheral immune cells migrating to the CNS, which then leads to an autoimmune response directed against the oligodendroglia-derived myelin sheath (OLG) of nerve fibers. Oligodendrocyte progenitor cells (OPCs) in the adult CNS are recruited into MS cells and are thought to promote remyelination during remission, although this ability is impaired as the disease progresses (Dobson & Giovannoni, 2019; Meijer et al., 2021; Kimoff et al., 2022). While APCs provide T-cells with their cognate antigen and create a specific cytokine environment, T-cells use cytokines to maintain their function and polarization in an autocrine manner and regulate immune responses across cell types. This process ultimately results in specific cytokine signatures associated with specific T-cell subsets that disrupt the integrity of the blood-brain barrier, leading to inflammation, demyelination and neuronal damage. In MS, cytokines exhibit redundancy in their activity, with the result that identical functions can be elicited by different cytokines when they are released in a cascade and one cytokine induces target cells to produce other cytokines (Hollenbach & Oksenberg, 2015; Kallaur et al., 2017; Göbel et al., 2018).

The study of the relationship between the state of cytokineogenesis and MS activity is one of the current areas of research into the functional state of peripheral blood mononuclear phagocytes in patients with MS. Thus, in our work, based on the determination of the level of cytokines IFN-γ, IL-1β, IL-12, IL-10 in the supernatant of mononuclear macrophages of patients with different courses of MS, a cytokine pro-inflammatory imbalance was detected in patients with MS, mainly carriers of the disease-associated HLA-DR polymorphism (haplotype AG), mediated by the presence of the disease-associated G-allele (Fig. 2), which affects the course, nature of exacerbations, progression and clinical manifestations. RRMS is the most common form of MS, which occurs in 85-90% of patients in the early stages of the disease (Dobson & Giovannoni, 2019; Kimoff et al., 2022).

Our data indicate that the cytokine profile of RRMS patients with the disease-associated G-allele (haplotype AG) was increased for IFN-γ IL-1β and IL-12 levels. Thus, the level of IFN-γ in the supernatant of mononuclear macrophages of RRMS patients with haplotype AG was 1.63 times higher compared to the results obtained in patients of this group with haplotype AA. The studied indicator was also 1.59 times higher compared to the indicators of the control group. And at the same time, in PMS patients with haplotype AA the studied indicator did not differ from the indicators of the control group, which indicates an indirect effect of the hereditary factor, the disease-associated HLA-DR polymorphism on the course of MS in patients of the studied group.

In PMS patients carrying the disease-associated HLA-DR polymorphism (haplotype AG), the cytokine profile showed an imbalance towards an increase in the level of pro-inflammatory cytokines based on significant differences compared to the results of the control group and the indicators obtained in PMS patients with haplotype AA, with the exception of IL-10 indicators. Precisely: the level of IFN-γ in the supernatant of mononuclear macrophages of PMS patients with haplotype AG was higher compared to the results obtained in PMS patients with haplotype AA. The content of IFN-γ in the supernatant of mononuclear macrophages of PMS patients with haplotype AG was also increased compared to the indicators of the control group. In PMS patients with haplotype AA, the studied indicator was increased in terms of IFN-γ content compared to controls, which is consistent with the results obtained in the works of Tupotilov & Kolyada (2018) and Vdovichenko et al. (2020) regarding increased IFN-γ content in patients with PMS. In addition, increased IFN-γ content in patients with PRS is positively correlated with the presence of the disease-associated HLA-DR polymorphism (haplotype AG).

It is known that IFN-γ plays a dual role in the immunopathogenesis of MS, precisely: IFN-γ has a pro-inflammatory effect on the activation of microglia and macrophages and is able to enhance the expression of CD80/CD86 on monocytes, suppressing the activation of T-cells, and enhances the activation of microglia, which produces toxic molecules, including reactive oxygen and nitrogen species (Sun et al., 2019; Sadeghnejad et al., 2024). This increases myelin damage, leading to neuroinflammation and progressive neurological deficits characteristic of MS (Meijer et al., 2021). On the other hand, IFN-γ stimulates the expression of MHC-II molecules on antigen-presenting cells, such as microglia and macrophages, which enhances the presentation of self-antigens to T lymphocytes and supports the autoimmune response (Kimoff et al., 2022). IFN-γ also increases the permeability of the blood-brain barrier (BBB), allowing immune cells to enter the CNS, where they cause inflammation and demyelination. IFN-γ can also affect T-cell differentiation and activation, promoting the polarization of T-helper cells into Th1-cells, which play a central role in

autoimmune inflammation. At the same time, Th1- cells secrete IFN-γ, creating a positive cycle of inflammation in MS (Hollenbach & Oksenberg, 2015; Li et al., 2021; Kimoff et al., 2022). IFN-γ also stimulates the production of pro-inflammatory cytokines, such as IL-1β, TNF-α, and IL-6, which increase inflammation and tissue damage in the CNS in MS (Dendrou et al., 2015; Meijer et al., 2021).

IL-1 has been shown to expand encephalitogenic GM-CSF producers, as demonstrated by the excess of various cytokines in animal experiments. Increased GM-CSF production has been found in MS patients, which is reduced as a result of immunotherapy with specific monoclonal antibodies (Göbel et al., 2018). IL-1β promotes the recruitment of immune cells to the site of inflammation, which increases CNS tissue damage due to a negative effect on oligodendrocytes. Monocytes, dendritic cells, microglia, as well as B cells and natural killer (NK) cells are able to synthesize IL1β. IL-1β supports the differentiation of T- helper cells of the 17-th type (Th17), which play a central role in the autoimmune processes in MS. Th17 cells increase inflammation and cause damage to nervous tissue. (Göbel et al., 2018; Meijer et al., 2021).

The results of our study showed that the content of IL-1β in the supernatant of mononuclear macrophages of RRMS patients with haplotype AG was increased by 2.02 times compared with the corresponding indicators of patients with haplotype AA and by 5.96 times compared with the studied results in the control group, respectively, which indicates a positive correlation with the presence of the disease-associated HLA-DR polymorphism in patients of the study group. Patients of this group with haplotype AA also had an increased, by 2.83 times, level of IL-1β compared with the control group.

The content of IL-1β in the supernatant of mononuclear macrophages of PMS patients with haplotype AG was increased compared to the corresponding indicators of patients with haplotype AA and compared to the studied results in the control group. At the same time, the increased content of IL-1β in PMS patients was positively correlated with the presence of a disease-associated polymorphism in patients of this group. PMS patients with haplotype AA also had an increased level of IL-1β content compared to the control group, which indicates a pronounced uncompensated proinflammatory activation, especially taking into account the indicators of IL-10 in this group. It is known that in MS patients, elevated levels of IL-1β are often observed even in cerebrospinal fluid and peripheral blood, especially during exacerbations (Kallaur et al., 2017). Studies have shown an association of IL-1β with disease severity, where higher levels of IL-1β were associated with more pronounced inflammatory processes and a greater number of lesions in the brain (according to MRI data), although direct studies that would establish a correlation between IL-1β levels and MS severity are limited, the available literature data indicate an important role of this cytokine in the development and progression of the disease (Mendiola & Cardona, 2018; Tupotilov & Kolyada, 2018; Brown et al., 2019; Vdovichenko et al., 2020).

It is known that IL-12 is able to potentiate the secretion of IFN-γ, increasing the penetration of immune cells through the blood brain barrier (BBB) and enhancing inflammation in the CNS by stimulating the activity of microglia and macrophages (Göbel et al., 2018; Meijer et al., 2021). The results of our work showed that the content of IL-12 in the supernatant of mononuclear macrophages of RRMS patients with haplotype AG was 1.47 times higher compared to the corresponding indicators of patients with haplotype AA and 1.46 times higher than the studied indicators in the control group, respectively, which indicates the influence of the disease-associated HLA-DR polymorphism in patients of this group on the increased level of IL-12. At the same time, in RRMS patients with haplotype AA, the studied indicator of the level of IL-12 did not differ from the results obtained in the control group. Thus, the obtained results confirm the literature data that IL-12 is a key regulator in the development of autoimmune diseases, which is emphasized by the data that its increased levels were found in the cerebrospinal fluid and lesions of patients with MS (Göbel et al., 2018).

In PMS patients heterozygous for the G-allele, the level of IL-12 in the supernatant of mononuclear macrophages was increased compared to the corresponding indicators in patients with haplotype AA and the studied indicators in the control group. In PMS patients with haplotype AA, the level of IL-12 was increased compared to the control group. Such a cytokine pro-inflammatory imbalance in PMS patients is more pronounced in patients of this group with the presence of the disease-associated HLA-DR polymorphism (haplotype AG), which indicates a decrease in the reserve capabilities of the regulatory link of humoral immunity in PMS patients (Tupotilov & Kolyada, 2018; Koliada et al., 2020; Vdovichenko et al., 2020).

According to the results of the analysis of literature data, it is known that IL-10 is a potent anti-inflammatory cytokine, the action of which is aimed at reducing the release of IL-1β, IL-12, Th1 and Th2 proliferation, antigen-presenting activity of monocytes and macrophages, and also has neuroprotective properties (Göbel et al., 2018). In addition, it is known that IL-10 production increases in MS patients during remission (Göbel et al., 2018; Meijer et al., 2021). The results of our work showed that the level of IL-10 in the supernatant of mononuclear macrophages of RRMS patients with the AG haplotype was increased by 4.38 times compared to the control, but decreased by 1.42 times compared to the indicators of the study group of patients with the AA haplotype. In addition, such patients also had an increased, 6.58-fold, level of IL-10 compared to the control group, which indicates a compensatory effect of IL-10 on the pro-inflammatory imbalance. In patients with the presence of a disease-associated polymorphism, the effect of IL-10 on the compensation of the pro-inflammatory imbalance in patients with RRMS is manifested to a lesser extent. A decrease in the level of IL-10 in patients with RRMS correlated with the presence of a disease-associated HLA-DR polymorphism in patients in this group.

In PMS patients with haplotype AG, it was increased compared to controls, but did not differ from the indicators of the study group of patients with haplotype AA. In PMS patients with haplotype AA, the level of IL-10 was increased compared to the control group, which indicates an imbalance between pro- and anti-inflammatory cytokines. Such an imbalance can lead to excessive inflammation, myelin damage and progressive neurological deficits in patients with PMS. According to the literature (Li et al., 2021; Kimoff et al., 2022), it is known that IL-10 is able to reduce the expression of MHC-II molecules on APCs and, accordingly, the expression of CD80/CD86 on monocytes, dendritic cells, macrophages, limiting the activation of autoimmune T-cells and supporting the differentiation of T-regulatory cells (Treg), which suppress the activity of inflammatory Th1 and Th17 cells responsible for demyelination. Whereas, IL-12, on the contrary, is able to increase inflammation, activate Th1 cells and promote myelin destruction (Chastain et al., 2011; Sadeghnejad et al., 2024).

Thus, the pro-inflammatory cytokines IFN-γ, IL-12, IL-1β and the anti-inflammatory IL-10 play a key role in the pathogenesis of MS, participating in the activation of inflammation, myelin damage and the enhancement of the immune response (Dendrou et al., 2015; Göbel et al., 2018). The immunopathogenesis of multiple sclerosis (MS) involves the activation and imbalance of components of innate and adaptive immunity. The co-signaling molecules CD80, CD86 and PD-L1, expressed on monocytes and other antigen-presenting cells (APCs), mediated by the influence of a hereditary factor, the presence of a disease-associated HLA-DR polymorphism, play a key role in the regulation of the immune response (Wagner et al., 2015; De Silvestri et al., 2019). Their interaction with receptors on T lymphocytes determines the direction of the immune response, as well as the level of cytokine production, which is critical for the development and progression of MS. Studying these mechanisms opens up the possibility of developing new targeted therapies that could effectively suppress inflammation and slow disease progression.

Conclusions

A method for identifying the risk group for the development of multiple sclerosis is proposed by determining the SNP rs9271366 (AG) of the HLA-DRB1*1501-DQB1*0602 haplotype in the studied groups of individuals using allele-specific PCR using an exclusive primer system and determining the presence of the SNP rs9271366 minor G-allele by allele-specific PCR. The study results show a posi-

tive correlation between this disease-associated allele G and the development of the disease in patients with progressive and relapsing-remitting forms of multiple sclerosis ($r = 0.65$; $r = 0.75$, respectively).

The presence of the minor disease-associated haplotype HLA-DRB1*1501-DQB1*0602 among the population of the North-Eastern region of Ukraine was most often determined among patients with progressive multiple sclerosis (89.2% vs 10.8%, $P < 0.05$). Among patients with relapsing-remitting form of the disease, the G-allele was determined in 74.5% vs 25.5%, $P < 0.05$.

As a result of the study, it was determined that the expression of antigen-presenting molecules CD80, CD86 and PD-L1 on peripheral blood monocytes depends on the carriership of the disease-associated HLA-DR polymorphism. An increased level of CD86 expression was demonstrated by patients carrying the disease-associated allele with both progressive and relapsing-remitting forms of multiple sclerosis. The level of CD80 expression was significantly increased only in patients with a progressive form of multiple sclerosis, carriers of the AG haplotype. The level of expression of PD-L1 molecules on peripheral blood monocytes in patients with the presence of the disease-associated HLA-DR polymorphism was lower than the indicators in both patients homozygous for the A allele and in comparison with the indicators of the control group, which indicates the depletion of producer cells due to prolonged antigenic stimulation mediated by this hereditary factor.

Our study shows that the level of cytokines IFN-γ, IL-1β, IL-12 in the supernatant of mononuclear macrophages of patients with a progressive form of multiple sclerosis with haplotype AG demonstrates pro-inflammatory activation of the cytokine profile, and the cytokine profile of patients with a relapsing-remitting form of multiple sclerosis with a disease-associated HLA-DR polymorphism (haplotype AG) indicates the influence of the genetic hereditary factor of the disease-associated HLA-DR polymorphism on the level of cytokine expression. The level of IL-10 in the supernatant of mononuclear macrophages of patients with a relapsing-remitting form of multiple sclerosis with haplotype AG indicates a less pronounced effect of this interleukin on the compensation of the pro-inflammatory imbalance in patients with a relapsing-remitting form of multiple sclerosis with the presence of a disease-associated HLA-DR polymorphism.

Studies of the features of antigen-presenting and regulatory cytokine-synthesizing functions of peripheral blood mononuclear phagocytes of patients with multiple sclerosis with the presence of the disease-associated HLA-DR polymorphism by determining the SNP rs9271366 (AG) haplotype can be used to monitor groups at risk of developing the disease.

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