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Donor-specific production of cytokines by blood cells under the influence of immunomodulators: New aspects of a personalized approach in medicine

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Abstract
Aim – to study the immunomodulator-induced individual cytokine production by peripheral blood mononuclear cells (PBMCs) and to evaluate the potential of using this approach as a universal cellular test system in personalized medicine.
Material and methods. The peripheral blood mononuclear cells given by donors were cultured *in vitro* in the presence of immunomodulators Imunofan and Polyoxidonium. After incubation, the enzyme-linked immunosorbent assay (ELISA) of the culture medium for the presence of pro-inflammatory cytokines IL-6, IL-8/CXCL8, MCP-1/CCL2, and IFN-α cytokines was performed.
Results. The results of the experiment have demonstrated the absence of spontaneous or immunomodulator-induced production of IFN-α by PBMCs. These data correspond to the information presented earlier in the scientific literature. We also observed a pronounced inhibitory effect of both immunomodulators on the production of cytokines MCP-1/CCL2, IL-6, IL-8/CXCL8 by PBMCs, along with the individual variability of their production and the cumulative effect of production over time.
Conclusion. The features of the production of pro-inflammatory cytokines by PBMCs into the medium in the presence of immunomodulators, revealed by the *in vitro* screening, can be used to develop universal *in vitro* cellular test systems for personalized diagnosis of a number of socially significant inflammatory and autoimmune diseases.
Keywords: cell biology, peripheral blood mononuclear cells, cytokines, immunomodulators, personalized medicine.
Conflict of interest: nothing to disclose.

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Донор-специфичная продукция цитокинов клетками крови под влиянием иммуномодуляторов: новые аспекты персонифицированного подхода в медицине

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Аннотация
Цель – изучить индуцированную иммуномодуляторами индивидуальную продукцию цитокинов клетками – мононуклеарами периферической крови и оценить потенциал использования данного подхода в качестве универсальной клеточной тест-системы в персонифицированной медицине.
Материал и методы. Мононуклеары, изолированные из периферической крови доноров, культивировали *in vitro* в присутствии иммуномодуляторов имунофан и полиоксидоний. После инкубации был проведен иммуноферментный анализ культуральной среды на присутствие провоспалительных цитокинов IL-6, IL-8/CXCL8, MCP-1/CCL2 и IFN-α.
Результаты. По итогам проведенного эксперимента было продемонстрировано отсутствие спонтанной или индуцированной иммуномодуляторами продукции IFN-α PBMC-клетками. Эти данные соответствуют сведениям, представленным ранее в медицинской литературе. Также наблюдали четко выраженный ингибирующий эффект обоих иммуномодуляторов на продукцию PBMC-клетками цитокинов MCP-1/CCL2, IL-6, IL-8/CXCL8, наряду с индивидуальной вариабельностью их продукции и накопительным эффектом продукции во времени.

Выводы. Выявленные посредством *in vitro* скрининга особенности продукции провоспалительных цитокинов в питательную среду PBMC-клетками в присутствии иммуномодуляторов могут быть использованы при создании универсальных клеточных тест-систем *in vitro* для персонифицированной диагностики ряда социально значимых заболеваний воспалительного и аутоиммунного характера.

Ключевые слова: клеточная биология, мононуклеарные клетки периферической крови, цитокины, иммуномодуляторы, персонифицированная медицина.

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PBMC – Peripheral Blood Mononuclear Cells – мононуклеарные клетки периферической крови; NK-клетки – клетки натуральные киллеры; E. coli LPS / LPS – бактериальный липосахарид кишечной палочки; IL-8/CXCL8 – интерлейкин 8; IL-6 – интерлейкин 6; TNF-α – фактор некроза опухоли-альфа; IFN-α – интерферон-альфа; СОКСПК – Самарская областная клиническая станция переливания крови; MCP-1/CCL2 – моноцитарный хемоаттрактантный протеин-1.

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BACKGROUND

The immune system plays a crucial role in recognizing both foreign agents and pathological changes in its cells. Control by the immune system is expressed, among other things, to changes in the balance of blood cytokines, which are widely and actively used in diagnosing autoimmune and inflammatory socially significant diseases. However, the individual level of cytokines in the blood depends on various factors not directly related to the disease. This may include hypo (including genetics) and hyperimmune status, age, and sex of the patient, time of year, environment, and other factors [1].

Therefore, when diagnosing inflammatory and autoimmune diseases and monitoring their course and response to treatment, it is more appropriate to use a model based on studying the characteristics of cytokine secretion by stimulated peripheral blood mononuclear cells (PBMCs). PBMCs can be easily isolated from the peripheral blood using Ficoll, and they are mononuclear cells consisting mainly of lymphocytes (T, B, and NK cells) and monocytes. Gradient centrifugation in Ficoll enables the separation of nonnucleated (erythrocytes and platelets) and polymorphonuclear (neutrophils, basophils, and eosinophils) cells.

In recent years, certain studies have demonstrated that stimulated PBMCs from donors with pathology produce a different profile of cytokines/chemokines than stimulated PBMCs from healthy donors [2–4]. Specifically, PBMCs from healthy donors stimulated with bacterial oligosaccharide (*Escherichia coli* LPS/LPS) produced more interleukin 8 (IL-8) and less interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α) than LPS-stimulated PBMCs from patients with chronic periodontitis [2].

As part of the development of personalized approaches to the diagnosis and prognosis of various pathologies, a study (n = 500) was conducted to analyze the possible influence of intestinal microflora on the production of cytokines by PBMCs obtained from healthy donors. The study results showed a correlation between donor-specific variabilities in the induction of cytokine production when PBMCs were exposed to various pathogen representatives of the intestinal microflora. High individual variability in the intensity of cytokine production upon the induction of PBMCs by the same pathogen has also been demonstrated. Moreover, no pathogen-specific stimulation of the production of different types of inflammatory cytokines by PBMCs was detected [5].

Researchers also revealed a correlation between the severity of sepsis and the development of a poor outcome and reduced production of TNF-α by LPS-stimulated PBMCs in these patients [6]. Considering the results of this study, a personalized approach to predicting sepsis was proposed, namely, the course of the pathological process could be more favorable if the PBMCs of such patients, in response to LPS stimulation, produce >250 pg/mL of TNF-α [3]. Such a predictive model can be used by doctors to select the most effective personalized treatment regimen.

As part of our research to develop new methods for predicting the development and course of socially significant inflammatory and autoimmune diseases, we suggest that PBMCs can also be used as a personalized cellular test system to identify the individual pattern of cytokine production under the influence of various immunomodulators. Immunomodulators are drugs that can stimulate or inhibit humoral and/or cellular immunity, depending on their current functional state. To test this hypothesis, we analyzed the effect of the immunomodulators Polyoxidonium and Imunofan on the production of inflammatory cytokines by PBMCs cultured in vitro.

Polyoxidonium (copolymer of 1,4-ethylenepiperazine N-oxide and (N-carboxyethyl)-1,4-ethylenepiperazinium bromide) with a molecular

weight of 80 kDa is a representative drug with an immunomodulatory effect caused by stimulation of antibody formation, as well as effects on phagocytic and NK cells. In certain doses, Polyoxidonium can modulate the synthesis of cytokines IL-1β, IL-6, and TNF-α. Moreover, it behaves as a true immunomodulator, enhancing the formation of TNF-α in individuals with an initially reduced synthesis of cytokines, and has no effect or even slightly reduces TNF-α production in individuals with an initially increased intensity of cytokine synthesis [7]. Polyoxidonium is used as an adjuvant in influenza vaccines. An adjuvant vaccine containing the drug stimulates more actively the production of Th-1 cytokines (IL-12, IFN-γ, IL-2, IL-6, IL-1β, and TNF-α) [8]. As an adjuvant for breast cancer immunotherapy, Polyoxidonium promotes the regression of pathological changes in 6 of 20 patients, including episodes with a triple-negative variant of the disease [9].

Polyoxidonium also has antioxidant, detoxifying, membrane-protective, and chelating effects [10]. Its antioxidant properties are associated with the ability to intercept reactive oxygen species, superoxide anion, hydrogen peroxide, and hydroxyl radicals in an aqueous environment, decrease the concentration of catalytically active divalent iron, inhibit lipid peroxidation, and suppress spontaneous and induced luminol and lucigenin-dependent chemiluminescence. In an experiment, Polyoxidonium, and copper sulfate were administered together, where the former provided 100% protection of animals from the toxic effects of the latter, with 100% death of control animals [7]. The membrane-protective properties of the drug protect cells from the damaging effects of some toxic substances. The combination of immunomodulatory, antioxidant, and detoxifying properties makes Polyoxidonium an effective immunomodulatory agent with anti-inflammatory activity [11–15].

Imunofan (synthetic hexapeptide arginyl-alpha-aspartyl-lysyl-valine-tyrosyl-arginine) with a molecular weight of 836 Da has immunoregulatory, detoxifying, and hepatoprotective effects and is used for treating several diseases [16]. It stimulates the maturation processes of T lymphocytes, cooperative interactions of CD4+ cells, and bone marrow cells; increases the activity of NK cells and the oxygen-dependent bactericidal system of neutrophils; activates the early stages of antibody genesis and synthesis of immunoglobulins M, G, and A; and stimulates impaired production of thymic hormones, including serum thymic factor, interleukin-2 (IL-2) and interferon-α (IFN-α) [17]. Imunofan is used as an adjuvant in baseline antiulcer therapy [18] as an immunocorrection in the complex treatment of patients with cancer, which reduces the probability of local and systemic complications during radiation therapy and thereby helps ensure treatment continuity [16, 19]. Imunofan was indicated to be effective in treating immune disorders in patients with chronic intoxication with organophosphorus compounds; therefore, the drug, administered for 5 days, contributed to the restoration of cellular and humoral immunity and regulated the blood content of cytokines in patients [20].

By influencing PBMCs with immunomodulators and assessing the response-induced production of cytokines, which varies depending on the characteristics of the patient's body, the most effective individual treatment plan can be selected, which fully meets the criterion of a personalized approach in medicine.

AIM

This study aimed to examine the individual production of cytokines by PBMCs induced by immunomodulators and evaluate the potential of using this approach as a universal cell test system in personalized medicine.

MATERIAL AND METHODS

Blood samples from potentially healthy individuals, namely, blood donors from the Samara Regional Clinical Blood Transfusion Station (SOKSPK), were analyzed. As part of the study, approval documentation was issued by the Bioethical Committee of Samara State Medical University (Protocol No. 215 of January 20, 2021). The study has seven participants. In 100% of the episodes, male patients aged 31.4 ± 4.0 years were involved. The selection of the study participants from the SOKSPK donor registry was performed randomly. Each participant signed a voluntary informed consent form for the processing of personal data and transfer of private health information and biological material (venous blood). Venous blood was collected into vacuum tubes containing sodium heparin (Welhai Hongyu, Medical Devices Co., Ltd., China) from the veins in the cubital fossa. The procedure was performed in the morning at 09.00 on an empty stomach.

Study design. During this study, the production of several cytokines (MCP-1/CCL2, IL-6, IL-8/CXCL8, and IFN-α) by PBMCs obtained from healthy donors and those stimulated with the immunomodulators Imunofan and Polyoxidonium was evaluated. For this purpose, PBMCs were isolated and cultivated, immunomodulators were added, and cytokine production was evaluated by enzyme-linked immunoassay (ELISA). Three series of experiments were performed.

Series 1 of experiments enabled us to assess which of the cytokines analyzed (MCP-1/CCL2, IL-6, IL-8/

CXCL8, and IFN-α) are produced by PBMCs in vitro. Thus, whole blood cells from five donors were combined into one lot. In series 2 of experiments, the individual pattern of cytokine expression induced by immunomodulators was assessed; therefore, PBMCs isolated from individual donors (No. 72219 (30 years old) and No. 16427 (32 years old)) were used, which were not combined into one lot. Series 3 of experiments was necessary to confirm or refute the phenomenon of the cumulative effect of cytokine production over time.

The data obtained from the study were tabulated, and the arithmetic mean values and standard errors of the arithmetic mean values were calculated. The significance of differences between the two arithmetic means was calculated using Student's t-test. Differences were considered significant at $p \leq 0.05$.

Isolation of PBMCs from the donor blood. PBMCs from peripheral heparinized venous blood of donors were isolated sedimentation in a density gradient (ρ = 1.077 g/cm3) Ficoll–Verografin (BioloT, Russia), according to the method of Boyum [21]. After gradient centrifugation, the cells were washed twice in a sterile RPMI-1640 nutrient medium with L-glutamine (Sigma-Aldrich, USA). To conduct experiments, PBMCs were maintained in RPMI-1640 medium with L-glutamine supplemented with 80 μg/mL gentamicin (Dalkhimpharm, Russia), 50 U/mL penicillin (BioloT, Russia), and 50 μg/mL streptomycin (BioloT, Russia). Cell viability was determined using trypan blue staining (BioloT, Russia).

Stimulation of PBMCs by immunomodulators. To evaluate the induced production of cytokines by PBMCs, we used official dosage forms of Polyoxidonium in the form of a solution containing 6 mg/mL of the active substance (Petrovax Pharm, Russia) and Imunofan in the form of a solution with an active substance content of 45 μg/mL (Bionoks, Russia).

The official forms of both immunomodulators were diluted into extemporaneous solutions with concentrations (based on the active substance) of 50, 25, and 12.5 ng/mL for series 1 and 200 ng/mL for series 2 and 3.

In each series of experiments, a nutrient medium containing PBMCs 2 × 106 cells/mL was placed into a 96-well plate (TPP, Switzerland) at a volume of 100 μL per well. The total PBMC content in the wells was 2 × 105 cells/well. In series 1 of experiments, PBMCs from the combined lot were used to fill two rows of wells, with three wells in each row. Moreover, 100 μL of an extemporaneous solution of Polyoxidonium at concentrations of 50, 25, and 12.5 ng/mL was added to the wells of row 1, and 100 μL of an extemporaneous solution of Imunofan at concentrations of 50, 25, and 12.5 ng/mL was added to the wells of row 2. The final contents of immunomodulators in the wells of each row were 25, 12.5, and 6.25 ng/well.

In series 2 and 3, PBMCs from individual donors were used. For each donor, two rows of wells were filled, with one well in each row. In the well of row 1, 100 μL of an extemporaneous solution of Polyoxidonium at a concentration of 200 ng/mL was added to the PBMC; 100 μL of an extemporaneous solution of Imunofan at a concentration of 200 ng/mL was added in the well of row 2. The final content of immunomodulators in the wells was 100 ng/well.

In series 1, immunomodulators were added to the wells of a plate containing freshly isolated PBMCs in a nutrient medium. Then, the plate was incubated for 20 h in a CO_2 incubator (Binder, Germany) at 37°C and 5% CO_2. In series 2 of experiments, a plate with PBMCs in a nutrient medium was initially incubated in a CO_2 incubator for 18 h at 37°C and 5% CO_2. The nutrient medium was then changed to a fresh one, immunomodulators were added to the wells, and the plates were again placed in a CO_2 incubator for 20 h at 37°C and 5% CO_2. In series 3 of experiments, as in series 2, the plate with PBMCs was initially incubated in a nutrient medium in a CO_2 incubator for 18 h at 37°C and 5% CO_2. Then, immunomodulators were added to the wells without replacing the nutrient medium with a fresh one, and they were again placed in a CO_2 incubator for 48 h at 37°C and 5% CO_2.

ELISA. After adding immunomodulators to the wells of the plate with PBMCs with a nutrient medium and completing the incubation, the contents of the wells were subjected to ELISA in relation to all series of experiments. Moreover, the ELISA process fully complied with the instructions attached to the diagnostic kit (Vector, Russian Federation). The ELISA sensitivity was 0.5, 2, 15, and 5 pg/mL for IL-6, IL-8, MCP-1, and IFN-α, respectively.

Methods for statistical processing of the research results. The data from the study results were compiled into tables and graphs using Microsoft Excel. Moreover, the mean values (M) and errors of average values (m) were calculated. Then, we assessed the significance of the mean values and the differences between the mean values. For this purpose, an experimental criterion for the normalized deviation texp was determined for the difference between the average sample size and the mean values of the two samples. Then, the resulting experimental criterion texp was compared with ttable for a confidence level P of 0.95. If texp was greater than or equal to ttable, then the average sample size significantly indicated the general average size, or the two samples differed with a probability ≥0.95 (significance 95%, error probability 5%).

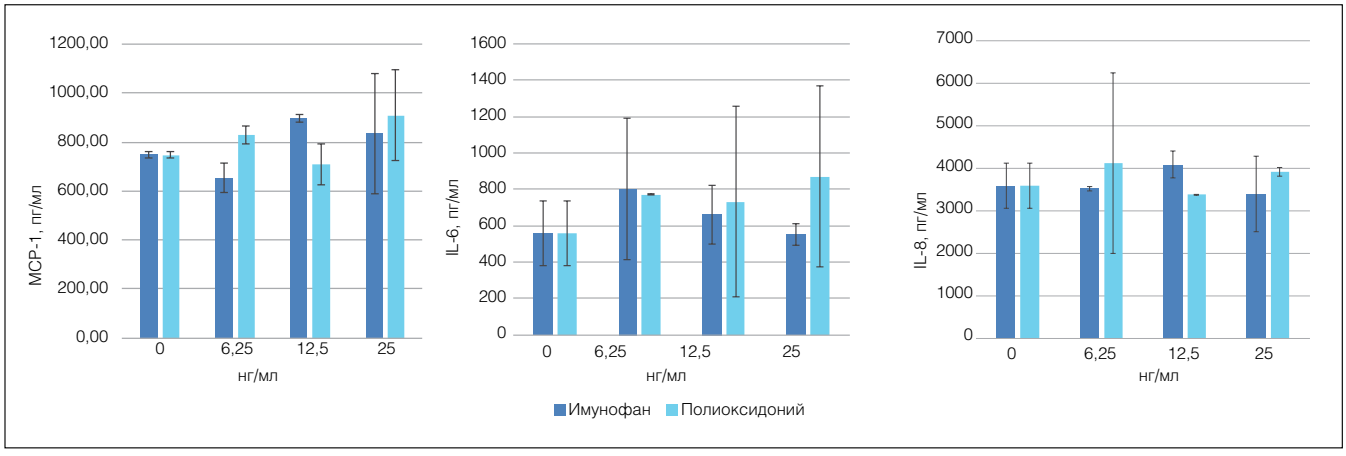

Figure 1. *In vitro screening of cytokines MCP-1/CCL2 (A), IL-6 (B), IL-8/CXCL8 (C) production by PBMCs obtained from 5 healthy donors (joined lot).*

Рисунок 1. *In vitro скрининг продукции цитокинов MCP-1/CCL2 (A), IL-6 (B), IL-8/CXCL8 (C) PBMC-клетками от 5 здоровых доноров (объединенный лот).*

RESULTS

In series 1 of experiments, analysis of the expression of cytokines (MCP-1/CCL2, IL-6, IL-8/CXCL8, and IFN-α) by PBMCs in the nutrient medium showed the following results. Spontaneous production of cytokines (i.e., without the addition of immunomodulators) by PBMCs was quite high at 500–3500 pg/mL (**Fig. 1**). Moreover, in quantitative terms, the expression of IL-8/CXCL8 was significantly higher than that of IL-6 and MCP-1/CCL2. Differences were statistically significant ($p \leq 0.05$) in both cases. With the addition of immunomodulators, the levels of production of IL-6, IL-8/CXCL8, and MCP-1/CCL2 changed according to the dynamics of the diagrams in Figure 1, indicating the significance of the differences between spontaneous (initial) and induced levels of cytokine secretion by PBMCs.

Moreover, ELISA results showed the absence of IFN-α expression by PBMCs both before and after the addition of immunomodulators. Optical density (OD) indicators between samples in wells containing a nutrient medium and PBMCs stimulated with the immunomodulators Imunofan and Polyoxidonium (in various concentrations) did not significantly differ from the OD indicators in wells with control RPMI medium (blank) or standard sample with zero concentration of IFN-α (0 pg/mL) (**Table 1**). Differences were not statistically significant ($p \geq 0.05$). Therefore, IFN-α expression was not assessed in experiment series 2 and 3.

In series 2 of experiments, the levels of spontaneous production of cytokines (MCP-1/CCL2, IL-6, and IL-8/CXCL8) were comparable to those in series 1 (**Figs. 1–4**). The differences between the results of series 1 and 2 of experiments for this parameter were not statistically significant ($p \geq 0.05$) for all three cytokines analyzed. The quantitative ratio of cytokines during their spontaneous production in series 2 of experiments was similar to that in series 1. The expression of IL-8/CXCL8 (**Fig. 3**) significantly exceeded those of of IL-6 (**Fig. 4**) and MCP-1/CCL2 (**Fig. 2**). Differences were statistically significant ($p \leq 0.05$) in both cases.

In general, in series 2 of experiments, when comparing spontaneous and induced expression of cytokines by PBMCs, a significant inhibitory effect of immunomodulators was revealed (**Fig. 4**). The differences between spontaneous and induced expression in this series of experiments were statistically significant for all three cytokines analyzed ($p \leq 0.05$).

We also recorded individual differences in the production of cytokines by PBMCs of donors against exposure to immunomodulators (**Figs. 2–4**). Under equal experimental conditions, MCP-1 production by donor 116427 cells remained unaltered after the addition of immunomodulators (**Fig. 2B**). The differences between spontaneous and induced expression of MCP-1 in this donor group were not statistically significant ($p \geq 0.05$). Similarly, a significant inhibitory effect of immunomodulators was noted in donor 72219 (**Fig. 2A**). Differences in the spontaneous secretion of MCP-1 in donor 72219 were statistically significant ($p \leq 0.05$).

Regarding IL-8/CXCL8 production, an inhibitory effect was registered in both donors from the use of

No.	Sample description	OD
1	RPMI medium only (blank)	0,0149
2	0 ng/mL immunomodulators	0,0117
3	Imunofan 6.25 ng/mL	0,0121
4	Imunofan 12.5 ng/mL	0,0154
5	Imunofan 25 ng/mL	0,0116
6	Polyoxidonium 6.25 ng/mL	0,0136
7	Polyoxidonium 12.5 ng/mL	0,0166
8	Polyoxidonium 25 ng/mL	0,0114

Table 1. *In vitro screening of cytokine IFN-α production by PBMCs*

Таблица 1. *In vitro скрининг продукции цитокина IFN-α PBMC-клетками*

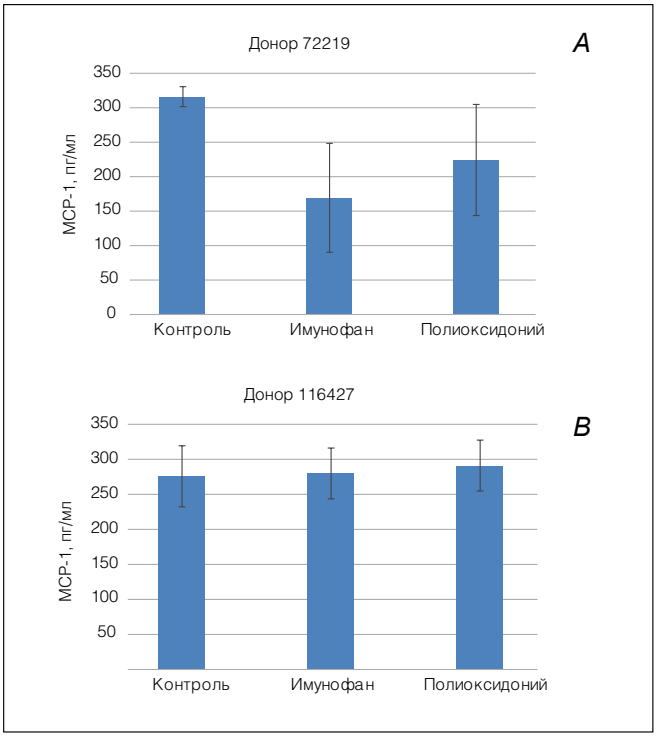

Figure 2. *Comparison of MCP-1 production by PBMCs from individual donors No. 72219 (A) and No. 116427 (B) in response to stimulation with immunomodulators*

Рисунок 2. *Сравнение продукции MCP-1 PBMC-клетками от индивидуальных доноров №72219 (A) и №116427 (B) в ответ на стимуляцию иммуномодуляторами. .*

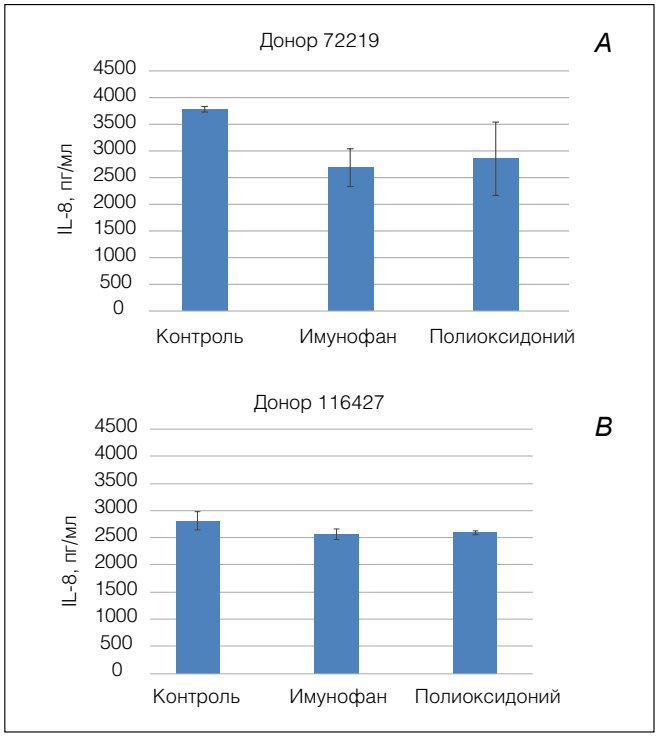

Figure 3. *Comparison of IL-8 production by PBMCs from individual donors No. 72219 (A) and No. 116427 (B) in response to stimulation with immunomodulators.*

Рисунок 3. *Сравнение продукции IL-8 PBMC-клетками от индивидуальных доноров №72219 (A) и №116427 (B) в ответ на стимуляцию иммуномодуляторами.*

both Polyoxidonium and Imunofan. The differences in spontaneous expression in this series were statistically significant ($p \leq 0.05$). However, in donor 72219, the differences between the induced and spontaneous expression of IL-8/CXCL8 were significantly ($p \leq 0.05$) more pronounced than those in donor 116427 (**Fig. 3**). Regarding the level of IL-6 production, a significant inhibitory effect was also noted in both donors from the use of both Polyoxidonium and Imunofan. Differences in spontaneous expression levels were statistically significant in both donors ($p \leq 0.05$). However, in this series, the degree of differences identified was significantly ($p \leq 0.05$) more significant in donor 116427 (**Fig. 4**).

As shown in **Figure 5**, the concentration of MCP-1 in the nutrient medium containing PBMCs was significantly higher when incubated with immunomodulators for 48 h. The difference in the same parameter during the 20-h incubation was statistically significant.

DISCUSSION

The development of biotechnologies in the context of accelerating a personalized approach in medicine requires special attention to the use of stimulated blood cells. In response to external stimulation, these cells produce cytokines in a donor-specific manner and are promising materials for the development of universal cell test systems for the diagnosis of socially significant inflammatory and autoimmune diseases. Accordingly, we conducted pilot experiments to assess the production of several cytokines (MCP-1/CCL2, IL-6, IL-8/CXCL8, and IFN-α) by PBMCs obtained from healthy donors and stimulated with the immunomodulators Imunofan and Polyoxidonium.

According to the results of series 1 of experiments, the absence of spontaneous or immunomodulator-induced IFN-α production was most clearly demonstrated. These data correspond to information previously published in the literature, particularly that Polyoxidonium-stimulated blood cells cannot induce the synthesis of IFN-α but have modulating activity in terms of the production of IL-1β, IL-6, and TNFα [7]. Imunofan does not stimulate IFN-α production by PBMCs from healthy donors. For MCP-1/CCL2, IL-6, and IL-8/CXCL8, the assessment of their production by PBMCs from five healthy donors demonstrated quantitatively detectable (significantly above the ELISA sensitivity barrier) secretion of these cytokines into the nutrient medium.

In experiments with cells from individual donors, in most cases, a clearly expressed inhibitory effect of both immunomodulators on the production of MCP-1/CCL2, IL-6, and IL-8/CXCL8 was noted. These cytokines are involved in the pathogenesis of various diseases. IL-6 is a multifunctional cytokine, and its blood level is increased in cases of acute and chronic forms of inflammation and autoimmune processes. The latter occurs in socially

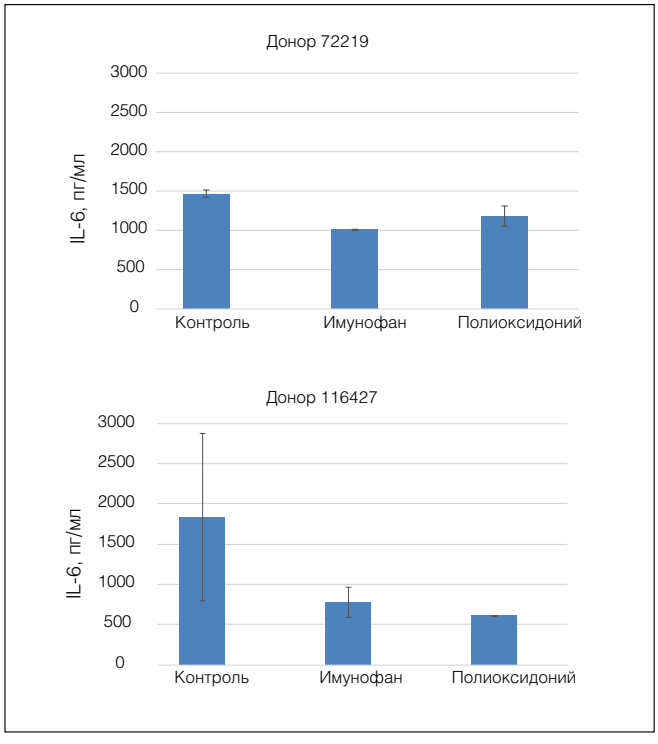

Figure 4. *Comparison of the IL-6 production by PBMCs from individual donors No. 72219 (A) and No. 116427 (B) in response to stimulation with immunomodulators.*

Рисунок 4. *Сравнение продукции IL-6 PBMC-клетками от индивидуальных доноров №72219 (A) и №116427 (B) в ответ на стимуляцию иммуномодуляторами.*

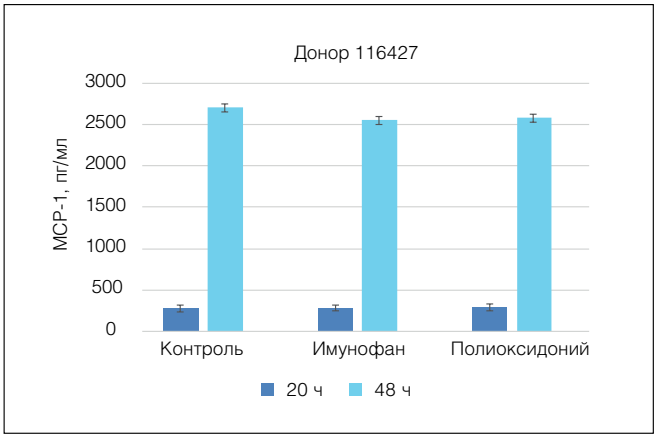

Figure 5. *Comparison of MCP-1 production into the breeding ground by PBMCs obtained from donor No. 116427 after incubation with immunomodulators during 20 and 48 hours.*

Рисунок 5. *Сравнение продукции MCP-1 в питательную среду PBMC-клетками донора №116427 с инкубацией в присутствии иммуномодуляторов в течение 20 и 48 часов.*

significant pathologies such as rheumatoid arthritis, psoriasis, and Castleman's disease [22–24]. MCP-1/CCL2 and IL-8/CXCL8 are powerful inflammatory mediators belonging to the group of chemokines whose main function is to stimulate the movement of immune cells to the inflammation site [25–27]. MCP-1/CCL2, IL-6, and IL-8/CXCL8 presented in this study already serve as diagnostic markers of some nosologies, and their use in the diagnostics of the above socially significant diseases is undoubtedly increasing annually [28–29]. The detection of individual variability in the production of MCP-1/CCL2, IL-6, and IL-8/CXCL8 by blood PBMCs from different donors in response to stimulation with immunomodulators is an important prerequisite for the development of personalized diagnostic tests using patient blood cells.

CONCLUSION

Series 1 of experiments was used to assess which of the analyzed cytokines (MCP-1/CCL2, IL-6, IL-8/CXCL8, and IFN-α) are produced by PBMCs in the nutrient medium under in vitro conditions. The results of this series indicate the absence of IFN-α production by blood PBMCs and their ability to produce IL-6, IL-8/CXCL8, and MCP-1/CCL2 under these experimental conditions. The significant spread in standard deviations of values in series 1 of experiments, when cells from five donors were combined into one lot, is probably due to the donor-specific variability in the production of individual cytokines. To confirm this hypothesis, we conducted series 2 of experiments.

Series 2 experiments was performed using PBMCs isolated from individual donors. In this series of experiments, PBMCs were preincubated in serum-free medium overnight, and the next day, before adding immunomodulators, they were washed from the culture medium. Thus, the cytokines accumulated overnight were removed, which enabled, after incubation for 20 h, a more accurate assessment of the effect of immunomodulators on the synthesis of cytokines. This series of experiments reliably demonstrated individual variability in the production of individual cytokines and confirmed our hypothesis.

To ensure the cumulative effect of cytokine production over time, series 3 of experiments was performed, in which immunomodulators were added to preincubated PBMCs from donor 16427 without changing the nutrient medium, followed by incubation for 48 h. The content of MCP-1/CCL2 in this series was significantly (an order of magnitude) higher than that in series 2, which confirmed the presence of a cumulative effect.

In general, in vitro screening of cytokine production into the culture medium by PBMCs in the presence of Imunofan and Polyoxidonium demonstrated the absence of IFN-α production along with individual variability in the production of IL-6, IL-8/CXCL8, and MCP-1/CCL2, which may be used to create universal in vitro cell test systems for personalized diagnostics of some socially significant inflammatory and autoimmune diseases.

Conflict of *interest. The authors declare no conflict of interest.*

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