SYSTEMIC INFLAMMATION BIOMARKERS IN 6-OHDA- AND LPS-INDUCED PARKINSON'S DISEASE IN RATS

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Hematological and immunological markers of systemic inflammation were studied in 6-hydroxydopamine (6-OHDA)- and lipopolysaccharide (LPS)-induced models of Parkinson's disease (PD). Experiments were carried out on adult male Wistar rats: 1 – intact animals; 2 – sham-operated animals and 3 - 6-OHDA- and LPS-lesioned animals. PD development was confirmed by the results of behavioral testing (apomorphine test, open field test) and immunohistochemical detection of the loss of dopaminergic neurons. Hematological indices (complete blood count and differential leukocyte count (DLC)) were examined using hematological analyser. Immunological indices included phenotypic (CD206 and CD80/86) and metabolic (oxidative metabolism and phagocytic activity) characteristics of circulating monocytes (Mo) and granulocytes (Gr), which were determined by flow cytometry, as well as plasma levels of C-reactive protein, which were determined by ELISA. LPS-induced PD was associated with neutrophilia, 1.9 times increased neutrophil-to-lymphocyte ratio, 3 times increased platelet-to-lymphocyte ratio, and 3 times increased systemic immune inflammation index as compared to intact animals. Functional profile of circulating phagocytes from LPS-lesioned animals was characterized by the pro-inflammtory metabolic shift, as was indicated by 5 times increased oxidative metabolism indices and up-regulated CD80/86 expression along with decreased phagocytic activity and CD206 expression. 6-OHDA-lesioned rats demonstrated decreased DLC indices as compared to intact and sham-operated rats. Functional profile of circulating phagocytes in this model was characterized by anti-inflammatory shift. The results obtained from this study demonstrated that stereotaxic LPS-induced PD is appropriate rodent model for the study of systemic inflammation which is inherent for the disease pathophysiology.

K e y w o r d s: Parkinson's disease, 6-OHDA, lipopolysaccharide, systemic inflammation, differential leukocyte count, peripheral blood phagocytes, reactive oxygen species, phagocytic activity, phagocyte phenotypic markers.

Parkinson's disease (PD) is one of two most common neurodegenerative disorders affecting about 2% of aged population worldwide. It is characterized by the degeneration of dopaminergic neurons in the substantia nigra with the accumulation of protein aggregates in the cytoplasm of neurons, and clinically, it is associated with the bradykinesia, a resting tremor, and rigidity [1]. The rapid ageing of the population is a permanent worldwide phenomenon. According to current prognoses, the rise in aged people will be accompanied with an increase in the number of patients afflicted by a neurological diseases including PD [2]. The supposed increase in PD affected patients emphasizes the necessity for deep insight into the mechanisms that may contribute to disease onset and aggravation in order to develop new prophylactic and therapeutic options, and to identify potential drug candidates.

Neuroinflammation is a well-known and widely investigated hallmark of PD and one of the pivotal mechanisms, which is involved in the neuronal degeneration [3]. In addition, durable association is established between PD pathogenesis and systemic inflammation (SI) [4]. Nevertheless, the precise role

of SI in PD pathophysiology is yet to be completely elucidated. All PD patients exhibit an elevation of serum pro-inflammatory cytokine levels and reduced CD4+ to CD8+ lymphocyte ratio irrelevant of the presence or absence of concomitant organ-specific or non-specific inflammatory pathologies. This, according to experts, may occur from the diffusion of inflammatory mediators, produced by activated micro- and astroglia cells, to the periphery with activation of immune cells and consequential appearance of SI markers in the blood [5]. The lack of dopamine (as a result of the neurodegeneration in substantia nigra) which is known to inhibit TRAF6-mediated NF-κB activation and inflammation via the D5 dopamine receptor in innate immunity cells, is another reason of SI development in PD [6]. On top of that, local neuroinflammation induces early hepatic acute phase response, which is responsible for the leukocyte recruitment into the blood and their pro-inflammatory metabolic shift [7]. Pro-inflammatory mediators produced by activated peripheral immunocytes can come from the periphery to the brain, either through neural (transfer through the autonomic nervous system) or humoral (transporting with blood through the disturbed blood-brain barrier) pathways [8]. Pro-inflammatory mediators, produced by peripheral immunocytes, exacerbate neuroinflammation and neurodegeneration. In addition, peripheral immunocytes, mainly monocytes and neutrophils, are recruited to the site of neuroinflammation and promote the transition of primed microglia to the activated state [9]. In such a way, local and systemic inflammatory processes form a vicious cycle (*circulus vitiosus)* which lends chronic nature to the PD [10]. That is why inflammatory components including SI are considered as targets for PD treatment.

To mimic PD in humans, numerous animal models have been developed, which reproduce many aspects of idiopathic disease including neuroinflammation [11]. Among the neurotoxic models of PD, the 6-hydroxydopamine (6-OHDA) rodent model has got greatest attention. This model is usually exploited for studying the disease pathophysiology and for testing neuroprotective potential of pharmaceutic compounds [12]. Lipopolysaccharide (LPS)-induced model is used for studying the inflammatory processes in PD pathogenesis and for the development anti-inflammatory treatments for the disease [13]. Nevertheless, data on reproducing SI in these models are scarce, and mainly concern peripheral blood cy-tokine profile [13, 14]. Considering aforementioned, we aimed to conduct comprehensive comparative evaluation of these most widely used PD models with respect to the manifestation of SI.

Materials and Methods

Animal models and study design. The study was performed on adult male Wistar rats (220-250 g) bred in the vivarium of the Educational and Scientific Centre "Institute of Biology" of Taras Shevchenko National University of Kyiv, Ukraine. The animals were kept in standard conditions with *ad libitum* access to water and food (standard diet). Animal protocol was approved by the University Ethics Commitee according to Animal Welfare Act guidelines. All procedures with animals were conducted in conformity with the principles of humanity as it was written in "General principles of animal experimentation" approved by the National Congress on bioethics (Kyiv, 2001–2007) and in conformity with Council directive of November 24, 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (86/609/EEC).

6-OHDA-induced hemiparkinsonism. Thirty rats were used in this experiment. Before the experiment, animals were randomized by weight and divided into three groups ($n = 10$): I – intact, II – sham-operated, III – lesioned with 6-OHDA. At the day of surgery, rats from groups II and III were anesthetized with nembutal (50 mg/kg, i.p., Sigma, USA) and placed in a stereotaxic instrument (SEJ-4, Ukraine). Animals were then injected i.p. with 40 mg/kg pargyline (Sigma, USA), which inhibits the metabolic conversion of 6-OHDA by monoamine oxidase, and 25 mg/kg desipramine (Sigma, USA), which blocks the uptake of neurotoxin by noradrenergic neurons [15]. Lesions in group III were made by the unilateral injection of 12 μg 6-OHDA dissolved in 4 μl of 0.1% ascorbic acid in isotonic saline solution into the left lateral ascending bundle (2.2 mm caudal and 1.5 mm lateral from the bregma in accordance with the atlas coordinates) [16]. Neurotoxin was injected into the brain tissue at a rate of 1 μl/min (every 15 s). The injector was left in place for 5 min before slowly withdrawing it to allow for toxin diffusion and prevent the toxin reflux. The sham-operated rats received vehicle only (0.1% ascorbate in 0.9% sodium chloride) at the same coordinates.

LPS-induced hemiparkinsonism. Thirty rats were used in this experiment. Before the experiment, animals were randomized by weight and divided into three groups (*n* = 10): I – intact, II – sham-operated, III – lesioned with LPS. At the day of surgery, rats from groups II and III were anesthetized with a mixture of ketamine (75 mg/kg diluted in sterile water for injection, Sigma, USA) and 2% Xylazine (400 µl /kg, Alfasan International BV, Netherlands) i.p. Lesions in group III were made by the unilateral injection of 10 µg LPS (Sigma, USA) in the volume of 2 µl into the substantia nigra (AP = -5.3; ML = ± 2.0; DV = -7.2) according to Hoban et al., 2013 [17]. LPS was injected into the brain tissue at a rate of 1 µl/ min (every 15 s). The injector was left in place for 5 min before slowly withdrawing it to allow for LPS diffusion and prevent the endotoxin reflux. The sham-operated rats received vehicle only (0.9% sodium chloride) at the same coordinates. PD development was ascertained by the results of behavioral testing and post-mortem assessment of nigrostriatal neurodegeneration using semi-quantitative tyrosine hydroxylase (TH) immunohistochemistry. SI biomarkers (leukocyte, neutrophil and monocyte count, neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR), plasma level of C-reactive protein (CRP), metabolic profile of circulating phagocytes) were examined at the time point of the experiment cessation (day 29 after the surgery).

Behavioral testing. Open field test was performed 28 day after the surgery as described previously [18]. To assess voluntary movement, anxiety, and exploratory behavior in new environment, the animals were tested in the Open Field arena (a square field with sides of 100 cm and a wall height of 30 cm, which was illuminated by two LED lamps (each with 60W) located at a height of 2 m) for 5 min. Ambulatory activity was recorded using an IP camera followed by the analysis using MATLAB.

Apomorphine test. Apomorphine administration induces abnormal contralateral rotations in hemi-PD model rats [19], therefore apomorphine test is commonly used to judge the success of the model, and the degree of neuron damage in the study of PD. The test was performed 7 and 14 days after the surgery. Apomorphine (Sigma, USA) was i.p. administered to the lesioned rats at a dose of 0.5 mg/kg. Five minutes after the injection, the animals were individually placed into a 40 cm-diameter cylinder and the counterclockwise (contralateral) rotations

were monitored and recorded for 30 min. Lesioned animals scoring over 6 rpm were considered as successfully lesioned with 86.6% dopaminergic neuron (DN) loss. Rats scoring less than 6 rpm (0-2 rpm) were considered as successfully lesioned with 44% DN loss [15].

Immunohistochemistry. Immunohistochemical staining of rat brain sections with antibodies to tyrosine hydroxylase (TH) was performed on day 29 after the surgery. Rats were deeply anesthetized and transcardially perfused with heparinised 0.9% sodium chloride followed by 4% paraformaldehyde. The brains were removed, frozen and then sectioned. Sections (50 µm) were processed for TH immunohistochemical detection using ABC–peroxidase method as described previously [20]. Endogenous peroxidase activity was blocked using blocking solution (Dako, EnVision Flex, DM821), and sections were incubated with anti-TH antibody, dilution 1:200 (Millipore, AB152) overnight (4°C) followed by immunostaining. Nonspecific antibody binding was blocked with 4% milk powder in Tris buffered saline (TBS) with 0.2% Triton X-100. The sections were then rinsed and incubated with biotinylated horse anti-rabbit IgG (1:200) for 60 min. After this, sections were incubated with an avidin-biotin complex solution followed by diaminobenzidine (Dako, EnVision), and were assessed using a Primo Star microscope, Zeizz. Each sample was scored semi-quantitatively as to the intensity of immunostaining on a four-point scale, with 0 indicating absence of staining, 1+ indicating the lowest level of detectable staining and/or nonhomogeneous weak staining, 2+ indicating moderate homogeneous staining, and 3+ indicating intense homogeneous staining [21]. We don't state percentage of stained cells because relatively homogeneous immunostaining was observed in all positive brain samples using these antibodies.

Hemogram analysis. The study of hematological parameters was carried out on day 29 after the surgery. Hematological parameters were determined using an analyzer "Particle counter model PCE 210" (ERMA, Japan), adapted for the study of blood cells in rats and mice. NLR, MLR, and PLR were calculated as the ratio of the absolute number of cells in the respective populations. Systemic immune inflammation index was calculated according to Erdoğan, 2020 [22].

Plasma CRP level examination. Plasma was measured for C-reactive protein (CRP) using ELISA Kit (Labcare Diagnostics India Pvt Ltd) according to the manufacturer recommendations.

Circulating phagocyte metabolic profile assessment. Metabolic profile of circulating phagocytes (monocytes (Mo) and granulocytes (Gr)) was characterized by their phagocytic activity, oxidative metabolism, and phenotypic marker expression, which were examined by flow cytometry. Phagocytic activity was detected as described earlier [23]. FITC-labeled thermally inactivated cells of *Staphylococcus aureus* Cowan I (collection of the Department of Microbiology and Immunology, ESC "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv) were used as an phagocytosis object. Blood samples were incubated at 37°C for 30 min with bacterial cells. Phagocytosis was stopped by adding a 'stop' solution (PBS with 0.02% EDTA and 0.04% paraformaldehyde). Data are presented as the phagocytosis index (PhI) (the average fluorescence per phagocytic cell). The oxidative metabolism of phagocytes was examined using 2′7′-dichlorodihydrofluorescein diacetate (H_2DCFDA, Invitrogen) as described previously [23]. Reactivity reserve of the oxidative metabolism was characterized by the modulation coefficient (MC). MC was defined after the treatment of blood samples with phorbol 12-myristate 13-acetate (PMA) *in vitro* and was calculated by the formula:

$$MC = ((S - B)/B) \times 100,$$

where S − index value after treatment with PMA *in vitro*; B − index value of untreated cells (basal value).

For phagocyte phenotyping, FITC-labeled anti-CD80/86, and phycoerythrin (PE)-labeled anti-CD206 antibodies (Becton Dickinson, Pharmingen, USA) were used. Results were assessed using FACSCalibur flow cytometer and CellQuest software (Becton Dickinson, USA). Granulocytes and monocytes were gated according to forward and side scatter.

Statistical analysis. All data are presented as mean ± SD. Statistical differences were calculated using ANOVA with Tukey's post-hoc test for multiple comparisons, and a two tailed *t*-test and non-parametric Mann-Whitney U test for single comparisons. χ^2 test was used for qualitative data. Differences were considered significant at $P \leq 0.05$.

Results and Discussion

Among numerous PD animal models, 6-OHDA- and LPS-induced are most extensively used to explore disease pathophysiology, and to search for the therapeutic targets. These models differ by the mechanisms of PD inducing, and each of them has advantages and drawbacks. Both these models are usually referred to as the pathogenic models (in contrast to so-called ethologic or disease gene-based models) Neuroinflammation along with motor disturbances are common features of these models. Intracerebral injection with the 6-OHDA toxin leads to dopaminergic neuron death and depletion of dopamine in the striatum, as well as motor dysfunction akin to that of PD patients. Common problem with most of the toxin-based PD models is their acute nature, which is entirely dissimilar from the slow progression of PD observed in patients [24]. LPS induces activation of microglial cells, which release pro-inflammatory and neurotoxic mediators causing neuronal damage. Damaged neurons in turn produce injury (damage-associated) signals which activate microglial cells. In such a way, LPS induces slow progressive neurotoxicity, more inherent to clinical picture of the disease [25]. These facts allowed us to suggest that the systemic manifestation of the inflammatory process may differ in these two models.

PD development in 6-OHDA- and LPS-lesioned rats was accompanied by behavioral disturbances (Table 1). In apomorphine test, rotation rate in 6-OHDA-lesioned rats was 7.6 rpm on day 8 after the surgery. It indicates ≥80% neuron damage and reflects acute severe unilateral neurodegeneration. One week later, rotation rate in this group reached 8.5 rpm, that indicate progressive DN death. Rotation rate in LPS-lesioned group was 0.6 rpm 7 days after, and 0.7 rpm 14 days after the PD initiation indicating moderate but progressive DN loss. Our results accord with the data of Eidson et al., 2017, which reported about moderate progressive decrease in dopamine levels (≤50%) in rats after intracerebral administration of LPS. Consequently, apomorphine causes only weak contralateral rotational activity (0-2 rpm) in lesioned animals [26]. DN loss in LPS-lesioned rats was additionally confirmed by the TH immunostaining, which indicated ~60% decrease of TH-positive neurons in affected animals.

Our results of Open Field Test showed that ambulatory activity in animals lesioned with 6-OHDA was similar to those observed in the LPS-lesioned group, excluding a potential effect on grooming activity, which was decreased only in 6-OHDA group. Significant decreases in total movement distance and the number of rearing were observed in both model groups compared with the sham-operated animals.

T a b l e   1. Criteria of PD development in 6-OHDA and LPS-lesioned rats

Criterium	6-OHDA-induced model	LPS-induced model
Apomorphine-induced rotation on day 8 after the surgery, rpm	7.6 ± 3.8	0.6 ± 0.4
Apomorphine-induced rotation on day 15 after the surgery, rpm	8.5 ± 3.3	0.8 ± 0.6
Rat ambulatory activity assessed through the open field test (% of sham-operated animals)		
• Total movement distance	64.1	61.4
• Number of rearing	41.0	69.8
• Number of grooming	73.0	99
• Number of defecations	65.7	80.9
The number of TH-positive neurons in the midbrain (% of intact animals; % of sham-operated animals)	Not detected	37.8

The number of defecations was also decreased in lesioned rats. It can indicate gastrointestinal dysfunction with reduced colonic contractions, which is inherent for PD including experimental hemiparkinsonism in rodents [27].

Signs of SI were manifested in different ways in animals with different PD models. Plasma level of CRP is a faithful marker of systemic/peripheral inflammation. Elevated plasma CRP levels are reported to be inherent and correlate with disease severity in PD patients [20]. Data concerning CRP level in blood plasma of rodents with experimental hemiparkinsonism are scarce. In our experiments, CRP plasma levels in animals with both PD models were characterized by significant individual variability. In LPS-lesioned animals, a tend to a lightly higher CRP plasma level as compared to the control was observed (189.0 ± 67.0 vs 126.5 ± 48.5 µg/ml in sham-operated rats) (Fig. 1).

Next, we have examined hematological indices of SI. Complete blood count (CBC)-based biomarkers are a groups of inflammatory parameters based on blood cells [28]. Changes of some CBC indices, such as white blood cell (WBC) types during SI in various pathological states are widely reported [29]. Assessment of diagnostic and prognostic values of routine hemogram indices in PD revealed that the increased neutrophil proportion along with lowered lymphocyte (Ly) count has the strongest association with SI in PD and disease severity [30, 31]. Hemogram analysis revealed a decrease in the Ly fractions in animals with both models of PD, more pronounced in rats with 6-OHDA-PD (Fig. 2). At the same time, in animals with 6-OHDA-PD, the relative amount of Mo was twice as high as in intact animals, whereas the Gr proportion did not differ from that of intact animals. As opposed to the rats with 6-OHDA-PD, monocyte fraction in rats with
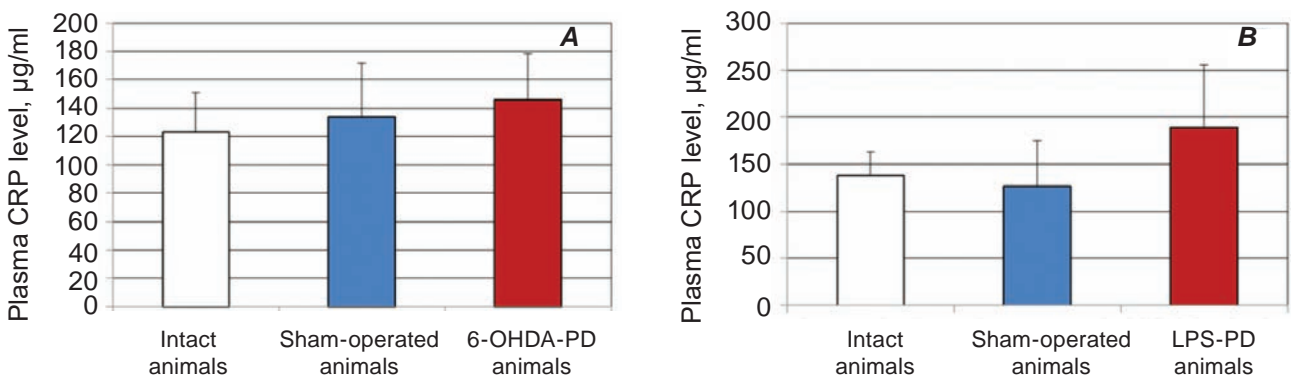

*Fig. 1. Plasma level of C-reactive protein in rats with different Parkinson's disease models. Data are presented as mean ± SD, n = 10. **A** – 6-OHDA induced PD, **B** –LPS-induced PD*
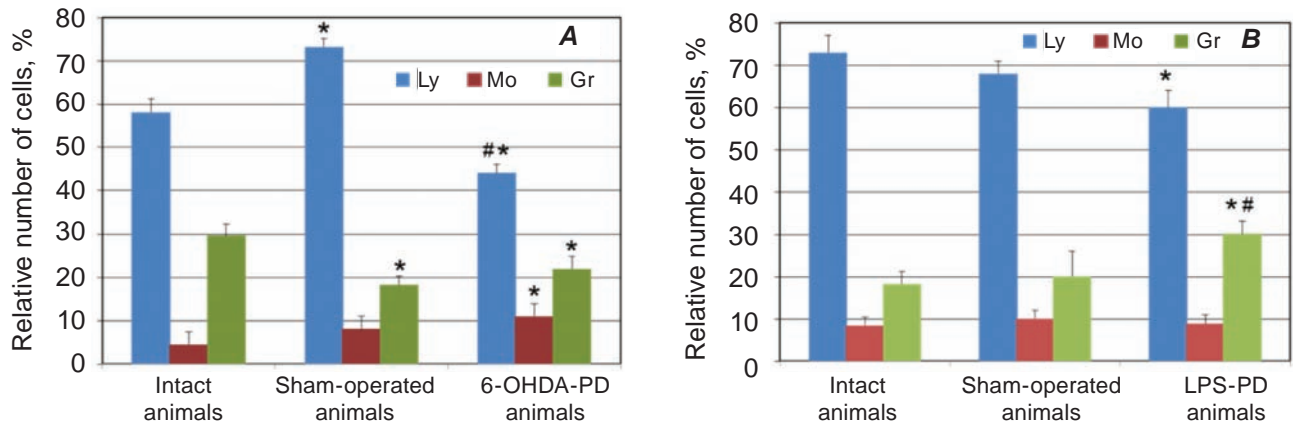
*Fig. 2. Differential leukocyte count in rats with different Parkinson's disease models. **A** – 6-OHDA-induced PD; **B** – LPS-induced PD. Data are presented as mean ± SD, n = 20. * and # indicate significant (P ≤ 0.05) differences as compared to the values in intact and sham-operated animals correspondingly (ANOVA with Tukey post-hoc test)*

LPS-PD did not differ from that in control intact animals, whereas the proportion of Gr was almost twice as high. Literature data concerning the relation of increased Mo fraction with SI are controversial. Namely, there is an assumption that Mo mobilization in the course of inflammation can be associated with their participation in tissue healing and initiation of adaptive immunity. The use of Mo assessment for the diagnostics of SI should be accompanied by the assessment of their phenotypic characteristics and functionality profile [32].

Increasingly attention is paid to the assessment of the differential leukocyte count (DLC) as an informative marker of SI. Even in the absence of generally recognized indicators of a systemic inflammatory process, such as an increased level of CRP in serum, a change in the DLC is a reliable marker of SI in the course of numerous inflammatory diseases,

including PD. According to the literature data, the DLC is applicable not only as a validated marker of the systemic inflammatory process in PD, but is also an important component in the complex of markers for the differential diagnostics of the disease subtypes [33]. Considering the aforementioned, we conducted a comparative assessment of neutrophil-to-lymphocyte (NLR), monocyte-to-lymphocyte (MLR), and platelet-to-lymphocyte (PLR) ratios in animals with 6-OHDA- and LPS-induced PD models (Table 2).

Animals with LPS-induced PD showed a high NLR value (0.47 ± 0.02) compared to the same indicator in the groups of intact and sham-operated animals, while the values of this index in animals with 6-OHDA-induced PD did not differ substantially from corresponding control groups (0.21 ± 0.01 vs 0.33 ± 0.03 and 0.25 ± 0.04 correspondingly). High

T a b l e 2. Leukocyte ratios in rats with different models of Parkinson's disease

Coefficient	6-OHDA			LPS		
	Intact	sham-operated	PD	Intact	sham-operated	PD
NLR	0.33 ± 0.03	0.25 ± 0.05	0.21 ± 0.01*	0.31 ± 0.02	0.25 ± 0.06	0.47 ± 0.02*,#
MLR	0.14 ± 0.02	0.13 ± 0.03	0.18 ± 0.01*,#	0.10 ± 0.08	0.18 ± 0.03	0.12 ± 0.03
PLR	25.96 ± 0.11	24.84 ± 0.23	21.38 ± 0.13*,#	26.19 ± 0.13	25.87 ± 0.15	73.81 ± 0.10*,#
SII	35.22 ± 0.10	23.62 ± 0.12*	23.52 ± 0.04*	36.24 ± 0.17	45.05 ± 0.83*	115.13 ± 0.17*,#

Note: * and # indicate significant (P ≤ 0.05) differences as compared to the values in intact and sham-operated animals correspondingly (ANOVA with Tukey post-hoc test), mean ± SD, n = 10. NLR – neutrophil-to-lymphocyte ratio, MLR – monocyte-to-lymphocyte ratio, PLR – platelet-to-lymphocyte ratio, SII – systemic immune inflammation index

NLR value in rats with LPS-PD model indicates SI. Research by Sanjari et al. showed a positive correlation between NLR values and the severity of PD. Therefore, one can consider the studied indicator not only as a marker of SI, but also as an additional marker of the disease severity which can be reproduced in the LPS- induced PD model and is absent in the 6-OHDA-induced PD model [34].

The MLR values were moderately increased in comparison with those in control in animals with both PD models. PLR values in animals with LPS-PD was increased threefold as compared to control animals, while in animals with 6-OHDA-PD this indicator was lower than that in the groups of intact and sham-operated rats indicating the resolution of the inflammation.

Erdoğan et al. proved conclusively the high diagnostic and prognostic significance of the systemic immune inflammation index (SII) by the example of SI in patients with bronchial asthma [22]. This index was developed by Hu et al. in 2014 to assess the course of systemic inflammatory process in patients with oncological pathology. According to the results of our experiments, the SII value in animals with LPS- induced PD was more than 3 times higher than that in intact animals and more than 2 times as compared to sham-operated animals. Whereas in rats with 6-OHDA- induced PD, this index did not differ significantly from the control and experimental groups. It suggests that the complex SII, which is based on the quantitative ratio of lymphocytes to neutrophils and platelets, is a marker mirroring immune inflammation in PD, and this marker points out SI in LPS-lesioned rats only.

In addition to quantitative leukocyte characteristics, we have assessed metabolic profile of circulating phagocytes: Mo and Gr. For this purpose, we examined reactive oxygen species (ROS) generation, phagocytic activity, and the expression of phenotypic markers involved in phagocyte metabolic polarization. One of the key features of phagocytes, including Mo and neutrophilic Gr, is their high metabolic plasticity, through which these cells can be activated to numerous different functional states. According to commonly accepted hypothesis, activated phagocytes can acquire two opposite functional states: classically M1(N1) and alternatively M2(N2) polarized, reflecting the opposing ends of the full activation spectrum. Classically polarized phagocytes are participants of local and systemic inflammatory processes: both reparative and destructive nature

[35]. It is commonly accepted, that increased ROS generation along with decreased phagocytic activity is a metabolic marker of pro-inflammatory functional phagocyte shift. Pro-inflammatory phagocyte polarization is associated with continuous ROS production and acquisition of antigen-presenting capacity, both of these functions followed by delayed maturation of phagosomes and progressive decrease of phagocytic activity [36]. Classical phagocyte polarization is also associated with increased surface expression of co-stimulatory molecules CD80/CD86 (participating in phagocyte antigen presenting capacity) along with lack/decrease of CD206 (scavenger receptor involved in efferocytosis and tissue reparation) expression [37]. In our experiments, level of Mo and Gr ROS production in rats with 6-OHDA-PD was significantly lower compared to that in intact and sham-operated animals (Fig. 3, *A*). In addition to the baseline ROS generation we also examined reactivity reserve of this function (the residual capacity of a cell to perform given metabolic activity under stress, in this case, after the treatment with PMA *in vitro*). The existence of reactivity reserve of the oxidative metabolism was registered in intact and sham-operated animals, as well as in rats with 6-OHDA-PD. ROS generation values in circulating Gr and Mo from animals with LPS-PD significantly exceeded those in the control groups (Fig. 3, *B*). Moreover, there was no reactivity reserve of Mo oxidative metabolism denoting the extreme degree of activation of the oxidative metabolism in these cells. Mo and Gr phagocytic activity was slightly up-regulated in rats with 6-OHDA-PD (Fig. 3, *C*), and significantly decreased in in rats with LPS-PD (Fig. 3, *D*) as compared to intact and sham-operated animals.

Circulating phagocyte phenotypic profile was also different in 6-OHDA-lesioned and LPS-lesioned animals (Fig. 3, *E* an *F* correspondingly). The level of CD206 expression by circulating phagocytes in animals with 6-OHDA-PD was doubled in comparison with intact animals, whereas CD80/86 expression was significantly decreased as compared to both intact and sham-operated animals. In contrast, CD80/86 expression in LPS-lesioned rats was slightly increased as compared to intact animals, and CD206 expression values didn't differ significantly from those in intact rats.

Surprisingly, in the animals of the sham-operated group, we observed an increased level of expression of both studied phenotypic markers. It
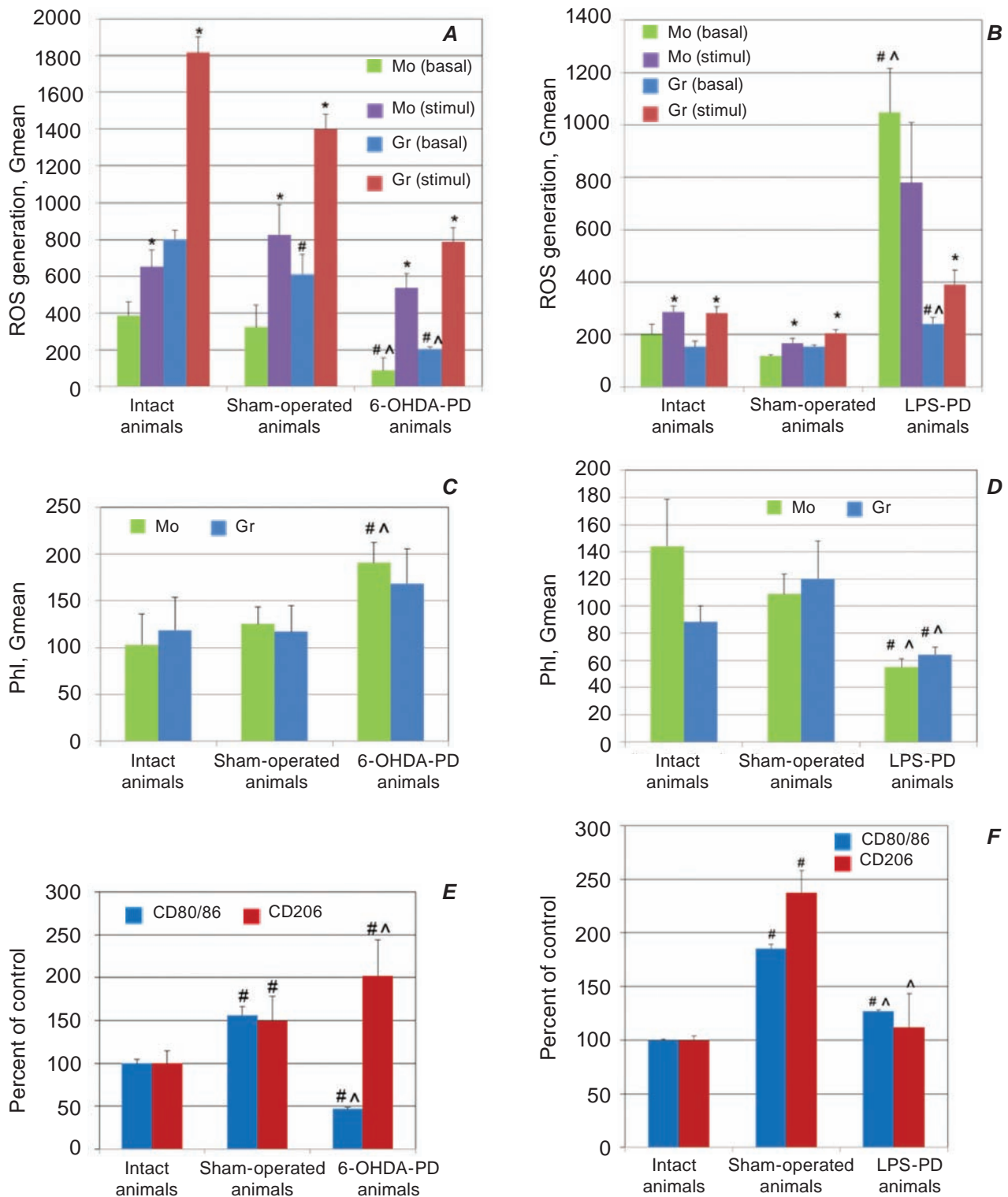
*Fig. 3. Circulating phagocyte functional and phenotypic characteristics in rats with different models of Parkinson's disease. **A**, **C**, **E** – 6-OHDA-PD; **B**, **D**, **F** – LPS-PD; **A** and **B** – phagocyte oxidative metabolism; **C** and **D** – phagocytic activity; **E** and **F** – phagocyte phenotypic marker expression. Data are presented as mean ± SD. # and ^ indicate significant (P ≤ 0.05) differences as compared to the values in intact and sham-operated animals correspondingly (ANOVA with Tukey post-hoc test), except for measurements in samples after the treatment with PMA in vitro, which were compared with untreated samples using a two tailed T-test, *P ≤ 0.05 versus corresponding untreated control*

should be noted that CD80 is over-expressed not only in case of an increase in the antigen-presenting activity of phagocytes, but also in case of differentiation of immature cells into myeloid-derived suppressor cells (MDSC) involved in the resolution of inflammation [38]. We are inclined to consider such phenotypic profile of circulating phagocytes in sham-operated animals as a sign of their anti-inflammatory polarization associated with post-surgery tissue reparation. Within these experiments, we were unable to differentiate MDSC, and additional studies are warranted to confirm this hypothesis.

Summarizing our data on phenotypic and metabolic characteristics of circulating phagocytes from animals with different PD models one can say the follows. Decreased oxidative metabolism and CD80/86 expression along with increased phagocytic activity and CD206 expression in phagocytic cells from 6-OHDA-lesioned rats indicate their anti-inflammatory functional shift, which can be stipulated by the spontaneous resolution of acute inflammation, initiated by the toxin introduction at the beginning of the experiment. The same assumption one can find in literature data. Highly activated oxidative metabolism and increased CD80/86 expression along with decreased endocytic activity in phagocytic cells from LPS-lesioned rats indicate their pro-inflammatory functional shift, as a sign of ongoing SI.

Conclusions. Taken together, our data indicate that PD model, based on stereotaxic LPS introduction, mimics SI inherent for clinical course of the disease as was confirmed by increased DLC values (NLR, PLR, SII), as well as pro-inflammatory shift of the metabolism of circulating phagocytes. Hematological and immunological indices in 6-OHDA-induced PD model indicate resolution of SI, which is typical for 'acute' nature of toxin-induced stereotaxic models of PD. Therefore, stereotaxic LPS-PD is useful model for the study of systemic inflammation involved in the PD pathophysiology.

БІОМАРКЕРИ СИСТЕМНОГО ЗАПАЛЕННЯ ЩУРІВ ІЗ 6-OHDA- ТА ЛПС-ІНДУКОВАНОЮ ХВОРОБОЮ ПАРКІНСОНА

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На моделях хвороби Паркінсона (ХП), індукованих 6-гідроксидофаміном (6-OHDA) та ліпополісахаридом (ЛПС) досліджували гематологічні та імунологічні маркери системного запалення. Експерименти проводили на щурах лінії Вістар. Розвиток ХП констатували за результатами поведінкового тестування та імуногістохімічного виявлення втрати дофамінергічних нейронів. Досліджували гематологічні показники (повний аналіз крові та диференційну кількість лейкоцитів (ДКЛ)). Імунологічні показники включали фенотипові (CD206 і CD80/86) та метаболічні (киснезалежний метаболізм і фагоцитарна активність) характеристики циркулювальних моноцитів і гранулоцитів, а також рівні С-реактивного протеїну в плазмі крові. Показано, що у щурів із захворюванням Паркінсона, індукованого ЛПС, спостерігається нейтрофілія: у 1,9 раза збільшувалося співвідношення нейтрофілів до лімфоцитів, у 3 рази – співвідношення тромбоцитів до лімфоцитів та в 3 рази збільшувався індекс системного імунного запалення порівняно. Функціональний профіль циркулювальних фагоцитів у ЛП-індукованих щурів мав прозапальні і метаболічні зрушення. Так, у 5 разів були збільшені показники кисне залежного метаболізму та посилена експресія CD80/86 у поєднанні зі зниженням фагоцитарної активності та експресії CD206. У 6-OHDA-індукованих щурів були знижені показники ДКЛ порівняно з інтактними та хибно-оперованими тваринами. Функціональний профіль циркулювальних фагоцитів у цій моделі характеризувався протизапальним зсувом. Одержані результати продемонстрували, що ХП, стереотаксичним введенням ЛПС, є адекватною моделлю для вивчення системного запалення у щурів.

К л ю ч о в і с л о в а: хвороба Паркінсона, 6-OHDA, ліпополісахарид, системне запален-ня, лейкоцитарна формула, периферичні фа-гоцити, реактивні форми кисню, фагоцитарна активність, фенотипові маркери фагоцитів.

References

1. MacMahon Copas AN, McComish SF, Fletcher JM, Caldwell MA. The Pathogenesis of Parkinson's Disease: A Complex Interplay Between Astrocytes, Microglia, and T Lymphocytes? *Front Neurol.* 2021; 12: 666737.

2. Béjot Y, Yaffe K. Ageing Population: A Neurological Challenge. *Neuroepidemiology.* 2019; 52(1-2): 76-77.

3. Mishra A, Bandopadhyay R, Singh PK, Mishra PS, Sharma N, Khurana N. Neuroinflammation in neurological disorders: pharmacotherapeutic targets from bench to bedside. *Metab Brain Dis.* 2021; 36(7): 1591-1626.

4. Süß P, Lana AJ, Schlachetzki JCM. Chronic peripheral inflammation: a possible contributor to neurodegenerative diseases. *Neural Regen Res.* 2021; 16(9): 1711-1714.

5. Sadek HL, Almohari SF, Renno WM. The Inflammatory Cytokines in the Pathogenesis of Parkinson's Disease. *J Alzheimers Dis Parkinsonism.* 2014; 4: 148.

6. Wu Y, Hu Y, Wang B, Li S, Ma C, Liu X, Moynagh PN, Zhou J, Yang S. Dopamine Uses the DRD5-ARRB2-PP2A Signaling Axis to Block the TRAF6-Mediated NF-κB Pathway and Suppress Systemic Inflammation. *Mol Cell.* 2020; 78(1): 42-56.e6.

7. Beers DR, Zhao W, Neal DW, Thonhoff JR, Thome AD, Faridar A, Wen S, Wang J, Appel SH. Elevated acute phase proteins reflect peripheral inflammation and disease severity in patients with amyotrophic lateral sclerosis. *Sci Rep.* 2020; 10(1): 15295.

8. Ferrari CC, Tarelli R. Parkinson's disease and systemic inflammation. *Parkinsons Dis.* 2011; 2011: 436813.

9. Pajares M, Rojo AI, Manda G, Boscá L, Cuadrado A. Inflammation in Parkinson's Disease: Mechanisms and Therapeutic Implications. *Cells.* 2020; 9(7): 1687.

10. Ortiz GG, González-Usigli H, Pacheco-Moisés FP, Mireles-Ramírez MA. Physiology and Pathology of Neuroimmunology: Role of Inflammation in Parkinson's Disease. In book: Physiology and Pathology of Immunology. Edition 1. In Tech Open. 2017; 173-197.

11. Potashkin JA, Blume SR, Runkle NK. Limitations of animal models of Parkinson's disease. *Parkinsons Dis.* 2010; 2011: 658083.

12. Stefani A, Cerroni R, Pierantozzi M, D'Angelo V, Grandi L, Spanetta M, Galati S. Deep brain stimulation in Parkinson's disease patients and routine 6-OHDA rodent models: Synergies and pitfalls. *Eur J Neurosci.* 2021; 53(7): 2322-2343.

13. Deng I, Corrigan F, Zhai G, Zhou XF, Bobrovskaya L. Lipopolysaccharide animal models of Parkinson's disease: Recent progress and relevance to clinical disease. *Brain Behav Immun Health*. 2020; 4: 100060.

14. Pott Godoy MC, Tarelli R, Ferrari CC, Sarchi MI, Pitossi FJ. Central and systemic IL-1 exacerbates neurodegeneration and motor symptoms in a model of Parkinson's disease. *Brain.* 2008; 131(Pt 7): 1880-1894.

15. Talanov SA, Oleshko NN, Tkachenko MN, Sagach VF. Pharmacoprotective influences on different links of the mechanism underlying 6-hydroxydopamine-induced degeneration of nigro-striatal dopaminergic neurons. *Neurophysiology.* 2006; 38(2): 128-133.

16. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 6th Edition, San Diego: Academic Press, 2007; 456 p.

17. Hoban DB, Connaughton E, Connaughton C, Hogan G, Thornton C, Mulcahy P, Moloney TC, Dowd E. Further characterisation of the LPS model of Parkinson's disease: a comparison of intra-nigral and intra-striatal lipopolysaccharide administration on motor function, microgliosis and nigrostriatal neurodegeneration in the rat. *Brain Behav Immun.* 2013; 27(1): 91-100.

18. Leite-Almeida H, Almeida-Torres L, Mesquita AR, Pertovaara A, Sousa N, Cerqueira JJ, Almeida A. The impact of age on emotional and cognitive behaviours triggered by experimental neuropathy in rats. *Pain.* 2009; 144(1-2): 57-65.

19. Iancu R, Mohapel P, Brundin P, Paul G. Behavioral characterization of a unilateral 6-OHDA-lesion model of Parkinson's disease in mice. *Behav Brain Res.* 2005; 162(1): 1-10.

20. Walsh S, Finn DP, Dowd E. Time-course of nigrostriatal neurodegeneration and neuro-inflammation in the 6-hydroxydopamine-induced axonal and terminal lesion models of

Parkinson's disease in the rat. *Neuroscience.* 2011; 175: 251-261.

21. Pauletti G, Dandekar S, Rong H, Ramos L, Peng H, Seshadri R, Slamon DJ. Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: a direct comparison of fluorescence in situ hybridization and immunohistochemistry. *J Clin Oncol.* 2000; 18(21): 3651-3664.

22. Erdogan T. Role of systemic immune-inflammation index in asthma and NSAID-exacerbated respiratory disease. *Clin Respir J.* 2021; 15(4): 400-405.

23. Rudyk MP, Pozur VV, Voieikova DO, Hurmach YV, Khranovska NM, Skachkova OV, Svyatetska VM, Fedorchuk OG, Skivka LM, Berehova TV, Ostapchenko LI. Sex-based differences in phagocyte metabolic profile in rats with monosodium glutamate-induced obesity. *Sci Rep.* 2018; 8(1): 5419.

24. Bezard E, Yue Z, Kirik D, Spillantini MG. Animal models of Parkinson's disease: limits and relevance to neuroprotection studies. *Mov Disord.* 2013; 28(1): 61-70.

25. Liu M, Bing G. Lipopolysaccharide animal models for Parkinson's disease. *Parkinsons Dis.* 2011; 2011: 327089.

26. Eidson LN, Kannarkat GT, Barnum CJ, Chang J, Chung J, Caspell-Garcia C, Taylor P, Mollenhauer B, Schlossmacher MG, Ereshefsky L, Yen M, Kopil C, Frasier M, Marek K, Hertzberg VS, Tansey MG. Candidate inflammatory biomarkers display unique relationships with alpha-synuclein and correlate with measures of disease severity in subjects with Parkinson's disease. *J Neuroinflammation.* 2017; 14(1): 164.

27. Minalyan A, Gabrielyan L, Pietra C, Taché Y, Wang L. Multiple Beneficial Effects of Ghrelin Agonist, HM01 on Homeostasis Alterations in 6-Hydroxydopamine Model of Parkinson's Disease in Male Rats. *Front Integr Neurosci.* 2019; 13: 13.

28. Luo J, Chen C, Li Q. White blood cell counting at point-of-care testing: A review. *Electrophoresis.* 2020; 41(16-17): 1450-1468.

29. Lin JX, Lin JP, Xie JW, Wang JB, Lu J, Chen QY, Cao LL, Lin M, Tu R, Zheng CH, Huang CM, Li P. Complete blood count-based inflammatory score (CBCS) is a novel prognostic marker for gastric cancer patients after curative resection. *BMC Cancer.* 2020; 20(1): 11.

30. Jensen MP, Jacobs BM, Dobson R, Bandres-Ciga S, Blauwendraat C, Schrag A, Noyce AJ, IPDGC. Lower Lymphocyte Count is Associated With Increased Risk of Parkinson's Disease. *Ann Neurol.* 2021; 89(4): 803-812.

31. Jiang S, Wang Y, Gao H, Luo Q, Wang D, Li Y, Yong Y, Yang X. Cell Ratio Differences in Peripheral Blood between Early- and Late-Onset Parkinson's Disease: A Case-Control Study. *Biomed Res Int.* 2019; 2019: 2072635.

32. Ingersoll MA, Platt AM, Potteaux S, Randolph GJ. Monocyte trafficking in acute and chronic inflammation. *Trends Immunol.* 2011; 32(10): 470-477.

33. Kara SP, Altunan B, Unal A. Investigation of the peripheral inflammation (neutrophil-lymphocyte ratio) in two neurodegenerative diseases of the central nervous system. *Neurol Sci.* 2021; 1-9.

34. Sanjari Moghaddam H, Ghazi Sherbaf F, Mojtahed Zadeh M, Ashraf-Ganjouei A, Aarabi MH. Association Between Peripheral Inflammation and DATSCAN Data of the Striatal Nuclei in Different Motor Subtypes of Parkinson Disease. *Front Neurol.* 2018; 9: 234.

35. Sica A, Mantovani A. Macrophage plasticity and polarization: *in vivo* veritas. *J Clin Invest.* 2012; 122(3): 787-795.

36. Tan HY, Wang N, Li S, Hong M, Wang X, Feng Y. The Reactive Oxygen Species in Macrophage Polarization: Reflecting Its Dual Role in Progression and Treatment of Human Diseases. *Oxid Med Cell Longev.* 2016; 2016: 2795090.

37. Orecchioni M, Ghosheh Y, Pramod AB, Ley K. Macrophage Polarization: Different Gene Signatures in M1(LPS+) vs. Classically and M2(LPS-) vs. Alternatively Activated Macrophages. *Front Immunol.* 2019; 10: 1084.

38. Dilek N, Vuillefroy de Silly R, Blancho G, Vanhove B. Myeloid-derived suppressor cells: mechanisms of action and recent advances in their role in transplant tolerance. *Front Immunol.* 2012; 3: 208.