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TLR-mediated activation of peripheral blood monocyte phagocytosis in patients with multiple sclerosis

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

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The aim of the study: to reveal peculiarities of the phagocytic activity of TLR4, TLR7/8-activated monocytes of peripheral blood, depending on the type of multiple sclerosis and clinical effectiveness of the treatment.

Material and methods. *E. coli* or ssRNA40/LyoVec lipopolysaccharide as TLR4 and TLR7/8 agonists were added to the monocyte-enriched cell suspension, respectively, and incubated for 24 hours at t 37 °C under an atmosphere of 5 % CO_2. In parallel series, ram erythrocytes (RE) sensitized with hemolytic serum and inactivated *C. albicans* cells were used as phagocytosis objects; the incubation time was 30 minutes. The phagocytic index was calculated as the percentage of phagocytic monocytes and the phagocytic number as the ratio of the total number of absorbed REs or *C. albicans* cells to the number of monocytes that entered into phagocytosis. The study presents the results of examination of 58 patients with recurring remitting (RRMS) and 36 patients with progressive (PMS) multiple sclerosis.

Results. The differences in the activation mechanisms of peripheral blood monocytes in patients with PC, which consist in different phagocytic activity in response to stimulation of TLR4 and TLR 7/8 depending on the disease conditions, were presented. Phagocytic activity lesions of monocytes were observed both in patients with RRMS and PMS, associated mainly with FcR-mediated mechanisms of phagocytosis.

IFN-β therapy in patients with RRMS led to the correction of such disorders in patients with high treatment efficacy (responders), and TLR7/8-mediated activation of monocytes was accompanied by an increase in the number of phagocytic cells. In patients with low efficacy of IFN-β therapy (nonresponders), the nature of changes in the phagocytic activity of stimulated monocytes indicated a decrease in the functional reserve with regard to FcR-mediated phagocytosis.

Conclusions. The obtained results indicate differences in phagocytic activity indices during stimulation of TLR4 and TLR7/8 and may indicate the presence of functional and phenotypic alterations of peripheral blood monocytes depending on the effectiveness of MS treatment.

Ключові слова: розсіяний склероз, IFN-β, фагоцитарна активність моноцитів, Toll-подібні рецептори.

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TLR-опосередкована активація фагоцитозу моноцитів периферичної крові у хворих на розсіяний склероз

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Мета роботи – визначити особливості фагоцитарної активності TLR4, TLR7/8-активованих моноцитів периферичної крові залежно від типу перебігу розсіяного склерозу та ефективності лікування.

Матеріали та методи. У збагачену моноцитами суспензію клітин додавали ліпополісахарид *E. coli* або ssRNA40/LyoVec як агоністи TLR4 та TLR7/8 відповідно та інкубували протягом 24 годин за t 37 ºС при атмосфері 5 % CO_2. Як об'єкт фагоцитозу використали в паралельних серіях сенсибілізовані гемолітичною сироваткою еритроцити барана (ЕБ) та інактивовані клітини *C. albicans*, час інкубації – 30 хвилин. Розраховували фагоцитарний індекс (ФІ) як відсоток моноцитів, що фагоцитують, та фагоцитарне число (ФЧ) як відношення загальної кількості поглинутих ЕБ або клітин *C. albicans* до числа моноцитів, що вступили в фагоцитоз. Наведені результати обстеження 58 пацієнтів із рецидивно-ремітувальним (РРРС) і 36 осіб із типом (ПРС) розсіяного склерозу, що прогресує.

Результати. Показано різну фагоцитарну активність моноцитів периферичної крові у відповідь на стимуляцію TLR4 та TLR7/8 у хворих на РС, що свідчить про особливості механізмів активації мононуклеарних клітин залежно від типу перебігу захворювання.

Пригнічення фагоцитарної активності моноцитів спостерігали в пацієнтів із РРРС, більше – у хворих на ПРС, що передусім пов'язано із FcR-опосередкованими механізмами фагоцитозу.

Ефективність лікування IFN-β у хворих на РРРС супроводжувалася нормалізацією фагоцитарних реакцій моноцитів, і TLR7/8-опосередкована активація моноцитів – підвищенням кількості клітин, що фагоцитують.

У пацієнтів із незадовільними результатами лікування IFN-β зниження фагоцитарної активності стимульованих моноцитів свідчить про зниження функціонального резерву щодо FcR-опосередкованого фагоцитозу.

Висновки. Результати вказують на різну активність мононуклеарних клітин при стимуляції TLR4, TLR7/8 і можуть свідчити про наявність функціональних і фенотипових альтерацій моноцитів периферичної крові залежно від ефективності лікування РС.

TLR-опосредованная активация фагоцитоза моноцитов периферической крови у больных рассеянным склерозом

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Ключевые слова: рассеянный склероз, IFN-β, фагоцитарная активность моноцитов, Toll-подобные рецепторы.

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Цель работы – определить особенности фагоцитарной активности TLR4, TLR7/8-активированных моноцитов периферической крови в зависимости от типа течения рассеянного склероза и эффективности лечения.

Материалы и методы. В обогащенную моноцитами суспензию клеток добавляли липополисахарид *E. coli* или ssRNA40/LyoVec в качестве агонистов TLR4 и TLR7/8 соответственно и инкубировали в течение 24 часов при t 37 ºС при атмосфере 5 % CO_2. В качестве объекта фагоцитоза использовали в параллельных сериях сенсибилизированные гемолитической сывороткой эритроциты барана (ЭБ) и инактивированные клетки *C. albicans*, время инкубации – 30 минут. Рассчитывали фагоцитарный индекс (ФИ) как процент фагоцитирующих моноцитов, фагоцитарное число (ФЧ) как отношение общего количества поглощенных ЭБ или клеток *C. albicans* к числу моноцитов, вступивших в фагоцитоз. Представлены результаты обследования 58 пациентов с рецидивирующим-ремиттирующим (РРРС) и 36 человек с прогрессирующим типом (ПРС) рассеянного склероза.

Результаты. Показана разная фагоцитарная активность моноцитов периферической крови в ответ на стимуляцию TLR4 и TLR7/8 у больных РС, что показывает особенности механизмов активации мононуклеарных клеток в зависимости от типа течения заболевания. Угнетение фагоцитарной активности моноцитов наблюдали у пациентов с РРРС, в большей степени – у больных ПРС, что в основном связано с FcR-опосредованными механизмами фагоцитоза.

Эффективность лечения IFN-β у больных РРРС сопровождалась нормализацией фагоцитарных реакций моноцитов, и TLR7/8-опосредованная активация моноцитов сопровождалась повышением количества фагоцитирующих клеток. У пациентов с неудовлетворительными результатами лечения IFN-β снижение фагоцитарной активности стимулированных моноцитов указывает на снижение их функционального резерва в отношении FcR-опосредованного фагоцитоза.

Выводы. Результаты указывают на разную активность мононуклеарных клеток при стимуляции TLR4 и TLR7/8 и могут свидетельствовать о наличии функциональных и фенотипических альтераций моноцитов периферической крови в зависимости от эффективности лечения РС.

Mononuclear phagocytes are known to be one of the initiators of inflammatory responses in various pathological conditions, including autoimmune demyelinating diseases. Therefore, the study of the role of these immune cells in the development of inflammatory processes in microglia may be a promising direction in investigating the pathogenic mechanisms involved in multiple sclerosis (MS) [1-4].

Acting as direct orthologues of glial cells of the central nervous system, peripheral blood monocytes are a valuable model system for studying the role of microglia in neurodegeneration [2]. Moreover, it is known that the functional properties of phagocytic cells may change in the course of inflammation, indicating that there is a direct link between these processes [1,4,5].

TLR-mediated activation (via Toll-like receptors) of peripheral blood monocytes is one of the physiological mechanisms of innate immunity resulting in the involvement of these cells in the process of immune response formation. Stimulation of TLRs of different types by agonists specific to them causes activation of the signaling pathway cascade, which in turn leads to the development of general and subpopulation-dependent functional reactions inherent in peripheral blood monocytes. It is known that TLR4 plays an important role in the development of inflammation and is part of one of the oldest signaling mechanisms of innate immunity [6–8], and an important role of TLR7/8 signaling in the development of neuroinflammatory processes has been shown [9,10].

Treatment of multiple sclerosis depends on the clinical form and nature of the disease. Treatment of exacerbations and progressive forms of MS involves administration of corticosteroids and cytostatics. Other pathogenic agents that can change the course of the disease and improve the survival of patients currently include interferons, synthetic glatiramer acetate polymer, and immunotherapy using monoclonal antibodies against specific integrin molecules [11].

It is known that monocytes are one of the most sensitive cells of the immune system capable of responding even to minor pathological processes in the body at the earliest stages of the immune response. Therefore, the analysis of the functional status of peripheral blood monocytes can be informative both for assessing the course of the disease and predicting the effectiveness of therapy [1,5,7–13]. All this indicates that mononuclear cells are unique and promising object that can be used as a biomarker to predict the course of multiple sclerosis.

Aim

The aim of the study was to reveal peculiarities of the phagocytic activity of TLR4, TLR7/8-activated monocytes of peripheral blood, depending on the type of multiple sclerosis and clinical effectiveness of the treatment.

Materials and methods

The study involved 94 patients with multiple sclerosis undergoing outpatient and inpatient treatment in the department of neuroinfection and multiple sclerosis of the State Institution “Institute of Neurology, Psychiatry and Narcology of the National Academy of Medical Sciences of Ukraine”. The study included 39 men and 55 women with a mean age of 34.8 ± 7.4 and 42.2 ± 7.7 years, respectively, as well as 27 practically healthy subjects (control group) of both sexes with a mean age of 35.3 ± 5.4 years. The study included patients with a verified diagnosis of multiple sclerosis (G35 code according to ICD-10 – Multiple Sclerosis). According to

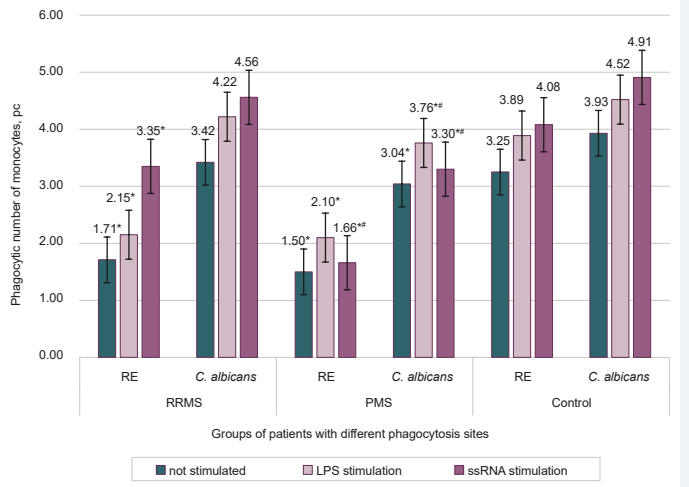

Fig. 1. Phagocytic index of peripheral blood monocytes relative to ram erythrocytes and *C. albicans* in patients with different types of MS.

***:** P < 0.05 when compared with control group; **#:** P < 0.05 when compared between patients with recurrent remitting MS and progressive MS.

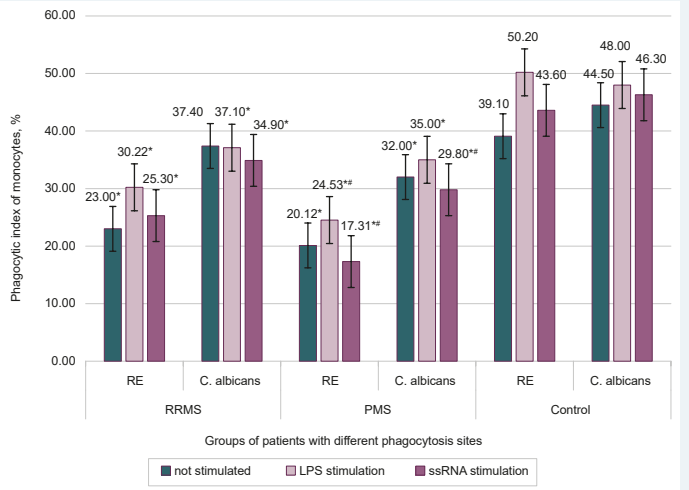

Fig. 2. Phagocytic number of peripheral blood monocytes relative to ram erythrocytes and *C. albicans* in patients with different types of MS.

***:** P < 0.05 when compared with control group; **#:** P < 0.05 when compared between patients with recurrent remitting MS and progressive MS.

the type of multiple sclerosis the patients were divided into two groups: 58 patients with relapsing-remitting MS (RRMS) and 36 patients with progressive MS (PMS). The biological material was blood samples from patients with multiple sclerosis and healthy subjects. All persons who participated in the study voluntarily gave their written consent to participate in the study.

In patients with RRMS, biological material was taken immediately prior to initiation of treatment and after completion of the annual course of treatment with recombinant human IFN-β "Betfer-1a" (Biopharma, Ukraine), administered according to the standard scheme. Patients who had at least one recurrence and/or increased EDSS disability score of 1 point or more during the year were classified as non-responders, and treatment efficacy was considered unsatisfactory. Patients who had a 1-point decrease in EDSS in the absence of MS relapses were classified as responders [14].

The Responder Group (MS (IFN+)) included 37 patients (64 %), the Non-Responder Group (MS (IFN-)) included 21 subjects.

Monocyte isolation was performed using a double percoll gradient (Sigma, USA) according to the method [15], adapted to small volumes of blood. The number of monocytes in suspension was determined by immunofluorescence method using anti-CD14-PE (EXBIO Praha, a.s., Czech Republic). The monocyte-enriched cell suspension was resuspended with complete RPMI medium with the addition of 1 μg/ml *E. coli* lipopolysaccharide (LPS) or ssRNA40/LyoVec (Invivogen, USA) as TLR4 and TLR7/8 agonists, respectively, or without the addition of inducers (intact cells), obtaining the final concentration of cells in the wells of 96-well plate $1x10^5$ cells/ml, and then incubated for 24 hours at t = 37 ºC under an atmosphere of 5 % CO_2.

Phagocytosis object was used in parallel series sensitized with hemolytic serum of ram erythrocytes (RE) and inactivated cells of 18-hour *C. albicans* culture, the incubation time was 30 minutes. At microscopic examination of the dyed preparations 200 monocytes were counted. The phagocytic index (PhI) was calculated as the percentage of phagocytic monocytes and the phagocytic number (PhN) as the ratio of the total number of absorbed REs or *C. albicans* cells to the number of monocytes entering phagocytosis (standard units) [15].

The obtained data were statistically processed using Statistica 11.0 (StatSoft, Inc.) and XLSTAT 19.6 (Addinsoft) software. The Mann–Whitney test was used to determine the significance of differences between the indices in the studied samples of RRMS and PMS, and the Wilcoxon test was used when comparing the indices before and after treatment. The data in the text, figures and tables are presented in the form of arithmetic mean and standard deviation: M ± SD.

Results

The study of phagocytic activity of peripheral blood monocytes in patients with different types of the course and different treatment efficacy was performed by modeling their activation by agonists, namely: *E. coli* lipopolysaccharides for TLR4, and ssRNA for TLR7/8.

The first stage of our study implied the assessment of the phagocytic activity of peripheral blood monocytes in patients with multiple sclerosis, depending on the form of the disease: recurrent remitting MS and progressive MS.

Regardless of the type of disease course in all patients with MS, abnormalities of phagocytic activity of non-stimulated monocytes were detected, indicating a simultaneous decrease in phagocytic number, as well as the number of cells capable of phagocytosis mediated, both to mannose receptors and to FcR receptors (*Fig. 1, 2*).

Stimulation of the Fc receptor in the group with recurrent type of disease using LPS resulted in an increase in PhI and PhN by 1.3 times (P < 0.05), and ssRNA40/LyoVec stimulation was associated with a two-fold increase in PM (P < 0.05). While in the group with progressive multiple sclerosis LPS and ssRNA40/LyoVec stimulation did not lead to significant changes in phagocytic activity

against intact monocytes in the study of phagocytosis against sensitized ram erythrocytes (*Fig. 1, 2*).

Assessment of indices of phagocytosis mediated by the mannose receptor revealed a decrease in phagocytic activity of intact monocytes in patients with progressive MS. At the same time, no significant differences in phagocytic activity were observed in the group with recurring remitting type of MS compared with the control group. Stimulation of cells by TLR4 and TLR7/8 agonists was not accompanied by significant changes in phagocytic index relative to non-stimulated cells, however, the PhI in both groups was significantly lower than in the control group ($P < 0.05$). The PhN index in the group of patients with RRMS with cell stimulation did not differ from the control, and in the group of patients with PMS was significantly lower in comparison with the RRMS group and control, $P < 0.05$ (*Fig. 1, 2*).

There is likely a number of mechanisms underlying the pathogenesis of multiple sclerosis that have common patterns that determine the effectiveness of multiple sclerosis therapy, especially when using IFN-β and other agents.

In the next step, we conducted a study of phagocytic activity of peripheral blood monocytes in patients with RRMS who received IFN-β therapy. Depending on the established efficacy of treatment, the patients were divided into two groups: MS (IFN+) responders and MS (IFN-) non-responders (*Table 1, 2*).

Evaluation of phagocytosis indices in patients with RRMS prior to treatment revealed significant inhibition mediated through the mannose receptor and especially through the FcR fragment of monocyte phagocytic responses. In addition, there was a decrease in the PhI and PhN indices of non-stimulated monocytes both in the responders and in the non-responders (*Table 1, 2*).

In the "responders" group, PhI was reduced relative to control by 1.75 times, PhN by 1.77 times, a corresponding decrease in phagocytic reactions was also observed in the "non-responders" group by 1.69 times and 2.07 times ($P < 0.05$). The PhI and PhN indicators of TLR4- and TLR7/8-activated monocytes in the responder and non-responder groups were also significantly lower than in the healthy subjects. When stimulated with ssRNA40/LyoVec and with LPS, PhI indicators remained below control ($P < 0.05$) (*Table 1, 2*).

After treatment in the MS (IFN+) group, there was an increase in the intact monocyte PhI and PhN against the control group ($P < 0.05$). In FcR-mediated phagocytosis in TLR4-stimulated monocytes, there was a 1.42-fold increase in PhI after treatment and a 1.33-fold increase in PhN. At the same time, TLR7/8 stimulation resulted in a more pronounced increase in PhI, almost 2-fold, and a 1.18-fold increase in PhN (*Table 1, 2*).

As for MS (IFN-) patients who showed poor IFN-β treatment results, PhI and PhN indices of non-stimulated monocytes remained lower relative to controls ($P > 0.05$). After TLR4 and TLR7/8 stimulation, the PhI values in this group remained significantly lower than the phagocytosis indices in the control group, and were also significantly lower than in the corresponding MS (IFN+) group. When stimulated with ssRNA40/LyoVec, the PhN remained lower than in the control group ($P < 0.05$). On the other hand, the PhN stimulation index did not differ from that of the MS (IFN+) group and control group (*Table 1, 2*).

Table 1. Indicators of phagocytic activity of peripheral blood monocytes against ram erythrocytes in patients with RRMS, depending on the effectiveness of treatment, (M ± SD)

Series	Indices	Groups of patients				Control
		Before treatment		After treatment		
		Responders	Non-responders	Responders	Non-responders	
not stimulated	PhI, %	22.4 ± 2.1*,#	23.1 ± 3.6*	37.0 ± 1.4#,§	26.7 ± 4.5*,§	39.1 ± 2.4
	PhN, pc	1.84 ± 0.12*,#	1.57 ± 0.22*	3.77 ± 0.14#,§	2.13 ± 0.21*,§	3.25 ± 0.20
LPS stimulation	PhI, %	29.5 ± 1.8*,#	25.3 ± 2.4*,**	46.4 ± 4.1#,§	34.5 ± 2.6*,**,§	50.2 ± 4.5
	PhN, pc	2.75 ± 0.21*,#	2.03 ± 0.31*	3.58 ± 0.26#	2.98 ± 0.34	3.89 ± 0.30
ssRNA stimulation	PhI, %	24.1 ±2.2*,#,§	18.7 ± 2.9*,**,§	49.1 ± 3.5#,§	23.9 ± 2.2*,**,§	43.6 ± 2.8
	PhN, pc	3.21 ± 0.12*,#,§	1.74 ± 0.31*,§	4.26 ± 0.15§	2.16 ± 0.35*,§	4.08 ± 0.20

*: $P < 0.05$ when compared with control group; #: $P < 0.05$ when compared with MS (IFN+) results before and after treatment; **: $P < 0.05$ when compared with MS (IFN-) results before and after treatment; §: $P < 0.05$ when compared between MS (IFN+) and MS (IFN-) patients.

Table 2. Indicators of phagocytic activity of peripheral blood monocytes against *C. albicans* in patients with RRMS, depending on the effectiveness of treatment, (M ± SD)

Series	Indices	Groups of patients				Control
		Before treatment		After treatment		
		Responders	Non-responders	Responders	Non-responders	
not stimulated	PhI, %	36.1 ± 1.5*	31.8 ± 2.2*	34.4 ± 3.1*	31.5 ± 4.1*	44.5 ± 2.3
	PhN, pc	3,5 ±0.2#	3.12 ± 0.20*	4.35 ± 0.30#,§	3.27 ± 0.20*§	3.93 ± 0.20
LPS stimulation	PhI, %	36.9 ±2.3*	35.3 ± 4.2*	39.1 ±1.6*	37.2 ± 3.1*	48.0 ± 2.1
	PhN, pc	4,17 ±0.10#	3.94 ± 0.20	5.06 ± 0.16#,§	4.15 ± 0.30§	4.52 ± 0.30
ssRNA stimulation	PhI, %	35.8 ±1.9*,#,§	30.2 ± 3.1*,**,§	40.5 ± 1.5#,§	48.8 ± 4.5**,§	46.3 ± 3.2
	PhN, pc	4.8 ± 0.2§	3.35 ± 0.30*,§	5.24 ± 0.20§	3.21 ± 0.20*,§	4.91 ± 0.30

*: $P < 0.05$ when compared with control group; #: $P < 0.05$ when compared with MS(IFN+) results before and after treatment; **: $P < 0.05$ when compared with MS(IFN-) results before and after treatment; §: $P < 0.05$ when compared between MS (IFN+) and MS (IFN-) patients.

With respect to the indices of mannose receptor-mediated phagocytosis, there was a decrease in PhI of unstimulated and stimulated monocytes in patients with RRMS in the MS (IFN+) group, prior to treatment, in contrast to PhN indices that had no significant differences from controls. Non-responders had a decrease in the levels of PhI and PhN of non-stimulated and stimulated monocytes.

After treatment in both groups, PhI values of non-stimulated and stimulated with LPS monocytes remained reduced. After stimulation with TLR7/8 agonists, the phagocytic index of monocytes did not differ from that of the control group. In the MS (IFN-) group, the PhN values of non-stimulated and ssRNA40/LyoVec stimulated monocytes remained low and did not differ from those before treatment (*Table 1, 2*).

The phagocytic features of mononuclear cells detected in non-responders on the one hand may indicate the presence of excessive, pathologically associated activation of non-stimulated cells, and on the other hand, indicate their functional depletion.

Discussion

Many studies have found that the association between the functional features of monocytes and their phenotype is not sufficiently conservative and is able to vary substantially both under physiological and pathological conditions [4,16,17]. Waschbisch A. et al. and Pinheiro C. et al. have shown that "classical", "intermediate" and "non-classical" monocytes have functional differences not only related to their properties, but also the specific ability to activate them in response to stimulation of MyD88-dependent TLRs of different types, in particular TLR4 and TLR7/8 [3,18]. Accordingly, we evaluated the composition of mononuclear cell subpopulations by influencing different types of TLR receptors. We have shown that activation of the Fc receptor of mononuclear cells by ssRNA40/LyoVec was stronger compared to the stimulating effect of LPS, possibly related to the involvement of a subpopulation of "non-classical" monocytes in phagocytosis.

Clarification of the role of peripheral blood monocytes in the pathogenesis of the disease, determination of appropriate immunophenotypes, according to some researchers may be of practical importance, in particular to be one of the criteria for predicting the effectiveness of therapy for stratification of patients with multiple sclerosis [4,7,9,18,19]. Zheng S. et al. have found out that the dysregulation of TLR7/8 signals on mononuclear cells may be considered as one of the key factors in the development of neuroinflammation in multiple sclerosis [7]. Hurtado-Guerrero I. et al. have demonstrated that TLR7 signaling mechanisms are disturbed in peripheral blood monocytes in patients with recurrent remitting disease [19]. The level of TLR7 expression on peripheral blood monocytes in MS patients was significantly reduced relative to mononuclear cells of healthy donors, and at the same time IFN-β therapy led to an increase in TLR7 expression to healthy donor levels. These researchers have also shown that TLR7 transcription in dendritic cells of monocytic origin depends on endogenous IFN-β production. A pattern has been revealed to indicate that impaired TLR7 expression in patients with RRMS is cell specific for the monocyte fraction of mononuclear cells, which also affects the production and modeling of IFN-β by these cells. However, the level of TLR7 expression on B-lymphocytes in these patients does not differ from that of healthy donors [9,18,19].

We have found a decrease in phagocytic activity of TLR4- and TLR7/8-activated monocytes in patients with recurrent remitting and progressive type of MS relative to healthy subjects ($P < 0.05$). The study has also shown a reduced reserve capacity of phagocytic mononuclear cells to LPS- and ssRNA-stimulated phagocytosis in patients with PMS compared with the corresponding RRMS group, $P < 0.05$ (*Table 1, 2*).

Therapy with recombinant interferon beta-1a in patients with RRMS resulted in the correction of phagocytic activity disorders in the responders, with TLR7/8-mediated monocyte activation accompanied by an increase in the number of phagocytic cells. In non-responders, the nature of changes in phagocytic activity of TLR4 and TLR7/8-stimulated monocytes indicated a decrease in functional reserve for FcR-mediated phagocytosis.

Thus, we believe that indicators of FcR-mediated phagocytosis of intact and TLR-activated monocytes can be used to predict the potential of non-responders in the treatment with IFN-β.

Conclusions

1. Modeling of TLR-dependent activation of monocytes and assessment of the functional state of intact and activated mononuclear cells allowed to obtain new data on the existence of functional-phenotypic heterogeneity of cells of the monocyte fraction of peripheral blood mononuclear cells, depending on the clinical form of its treatment.

2. As a result of a study of TLR4- and TLR7/8-mediated activation of cells of the monocyte fraction of peripheral blood mononuclear cells in patients with recurrent remitting and progressive type of MS, a decrease in phagocytic activity was observed, which was more significant in patients with PMS.

3. TLR-mediated stimulation of respondent monocytes in patients with RRMS after treatment was accompanied by an increase in the number of phagocytic cells, in contrast to non-responders. The nature of the changes in the phagocytic activity of stimulated monocytes indicated a decrease in their functional reserve in FcR-mediated phagocytosis.

Prospects for further research. The relevance of further studies of phagocytic responses of mononuclear cells with TLR-mediated activation in MS patients is undeniable. However, new progressive methods are needed to allow real-time detection of changes in the population of monocytes, their functional properties, and the ability to determine the level of Fc receptor expression on these cells. This will clarify the peculiarities of the functional status of monocytes of different subpopulations and their pathogenic role in the development and course of multiple sclerosis.

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