https://doi.org/10.17816/MAJ191S186-88

CELLULAR MECHANISMS OF NEUROIMMUNE INTERACTION IN MULTIPLE SCLEROSIS: IONOTROPIC GLUTAMATE RECEPTORS OF T-LYMPHOCYTES

U.Sh. Kuzmina[1], K.Z. Bakhtiyarova[2], Yu.V. Vakhitova[1]

[1] IBG UFRC RAS;
[2] Bashkir State Medical University, Ufa, Russia

КЛЕТОЧНЫЕ МЕХАНИЗМЫ НЕЙРОИММУННОГО ВЗАИМОДЕЙСТВИЯ ПРИ РАССЕЯННОМ СКЛЕРОЗЕ: ИОНОТРОПНЫЕ РЕЦЕПТОРЫ ГЛУТАМАТА Т-ЛИМФОЦИТОВ

У.Ш. Кузьмина[1], К.З. Бахтиярова[2], Ю.В. Вахитова[1]

[1] ФГБУН «Институт биохимии и генетики уфимского научного центра российской академии наук РАН», Уфа;
[2] ФГБОУ ВО «Башкирский государственный медицинский университет» Минздрава России, Уфа

juvv73@gmail.com

Using flow cytometry and real-time RT-PCR, we studied the effect of the blockade of glutamate NMDA receptors on the quantitative distribution of IFNγ- and IL-4-producing $CD4^+$ and $CD8^+$ T-lymphocytes and the expression of transcription factor of genes T-bet and GATA-3 in immune cells derived from healthy individuals and multiple sclerosis patients (MS). From the results of the study, it follows that blockade of NMDA receptors leads to a decrease in the proportion of studied effector subpopulations of T-lymphocytes, as well as differential modulation of gene expression of transcription factors (*TBX21, GATA3*). The severity of the negative effect of receptor blockade differs in different subpopulations of $CD4^+$ and $CD8^+$ T cells, which leads to a shift in the cytokine balance towards "proinflammatory" type 1 T-cells, to a greater extent in patients with MS. Thus, glutamate can regulate the Th1/Th2 and Tc1/Tc2 cytokine balance by modulating the activity of NMDA receptors of T lymphocytes in MS.

Keywords: glutamate; NMDA receptors; T-cells; multiple sclerosis.

С помощью методов проточной цитометрии и ОТ-ПЦР в реальном времени нами было изучено влияние блокады NMDA рецепторов глутамата на содержание IFNγ- и IL-4-продуцирующих $CD4^+$ и $CD8^+$ Т-лимфоцитов и экспрессию генов транскрипционных факторов T-bet и GATA-3 в иммунных клетках, полученных от здоровых лиц и больных рассеянным склерозом (РС). Из результатов исследования следует, что блокада NMDA рецепторов приводит к снижению доли изученных эффекторных субпопуляций Т-лимфоцитов, а также дифференциальной модуляции экспрессии генов транскрипционных факторов (*TBX21*, *GATA3*). Выраженность негативного эффекта блокады рецепторов отличается в разных субпопуляциях $CD4^+$ и $CD8^+$ Т-клеток, что приводит к смещению цитокинового баланса в сторону «провоспалительных» Т-клеток 1-го типа, в большей степени у больных РС. Таким образом, глутамат посредством NMDA рецепторов участвует в процессе поддержания цитокинового Th1/Th2 и Tc1/Tc2 баланса при РС.

Ключевые слова: глутамат; NMDA рецепторы; Т-клетки; рассеянный склероз.

Introduction. The heterogeneity of pathogenic processes in MS is due, among others, to a breakdown in the connections between the nervous and immune systems, in which neurotransmitters, such glutamate (Glu), play a significant role. Functionally active Glu receptors are found on human T-lymphocytes and their participation in the regulation of numerous processes in immune cells has been proven. However, the question of the role of glutamate and its receptors in the mechanisms, important for MS, are not understood. It is known that the terminal differentiation of $CD4^+$ and $CD8^+$ T-lymphocytes into effector cells is largely determined by the cytokine microenvironment, as well as the expression of transcription factors specific for a particular subpopulation. Based on this, the purpose of the study was to investigate the functional significance of the ionotropic NMDA receptors in the maintaining Th1/Th2 and Tc1/Tc2 cytokine balance in multiple sclerosis.

Material and methods. The study involved 12 healthy individuals and 15 patients with relapsing-remitting MS (remission stage). An intracellular staining of marker cytokines was applied for evaluation of quantitative distribution the subsets of T-lymphocyte in cultures of peripheral blood mononuclear cells activated by phorbol-myristate-acetate (PMA, 25 ng/ml) and ionomycin (ion, 1 μg/ml) and treated with antagonist of NMDA receptor (+)-MK801 (10^{-4} M) with subsequent analysis by flow cytometry. $CD4^+$ T cells (Th) producing IFNγ were defined as Th1, and IL-4 as Th2. IFN-γ-producing $CD8^+$ T cells were identified as Tc1, and IL-4 — producing as Tc2. The

Table

Influence of blockade of NMDA receptors on T-cells on the studied parameters

Parameter	Healthy (n = 12)		MS (n = 15)	
	PMA/ion	PMA/ion+ (+)-MK801	PMA/ion	PMA/ion+ (+)-MK801
Cytokine-secreting T-lymphocytes (Me (Q_{25}; Q_{75}))				
$CD4^+IFN\gamma^+$ T-cells,	8.0	5.5#	14.0	11.6#
% of total $CD4^+$	(7.1; 8.1)	(5.1; 5.9)	(7.0; 15.9)	(5.2; 11.7)
$CD4^+IL\text{-}4^+$ T-cells,	0.5	0.4#	1.8	1.1#
% of total $CD4^+$	(0.4; 2.4)	(0.4; 2.1)	(1.1; 2.1)	(0.8; 1.3)
Th1/Th2 ratio	**7.9**	**8.2**	**9.0**	**12.8#**
	(3.6; 16.9)	**(2.7; 14.1)**	**(7.5; 13.1)**	**(9.3; 20.8)**
$CD8^+IFN\gamma^+$ T-cells,	25.0	17.6#	31.2	28.0#
% of total $CD8^+$	(24.2; 25.1)	(14.0; 21.1)	(30.8; 33.3)	(26.3; 30.1)
$CD8^+IL\text{-}4^+$ T-cells,	2.1	1.6#	2.8	2.0#
% of total $CD8^+$	(2.0; 3.2)	(1.3; 2.2)	(0.7; 6.8)	(0.5; 3.9)
Tc1/Tc2 ratio	**11.7**	**17,9**	**6.8**	**8.4#**
	(8.8; 15.9)	**(7.7; 21.3)**	**(3.7; 8.6)**	**(7.1; 10.8)**
Gene expression of transcription factors (Me (Q_{25}; Q_{75}))				
TBX21		1.2	1.5#	0.7
		(0.5; 1.4)	(0.6;2.3)	(0.4; 1.2)
GATA3		0.2	0.2#	0.4
		(0.2; 0.3)	(0.1;0.3)	(0.3; 0.6)
***TBX21/GATA3* ratio**	**3.2**	**7.0#**	**3.1**	**5.9#**
		(2.3; 3.4)	**(6.0; 8.9)**	**(2.7; 4.0)**

N o t e. # $p < 0.05$ relative to the corresponding group "PMA/ion".

mRNA level of genes coding the transcription factors (*TBX21* and *GATA3*) was evaluated using real-time RT-PCR.

Results and discussion. The blockade of NMDA receptors on PMA/ion-activated T-cells is accompanied by a decrease in the proportion of IFNγ- and IL-4-producing cells in both groups of donors (table). The intracellular level of IL-4 in $CD4^+$ T-lymphocytes of patients with MS to compared with healthy donors decreases by 2.7 times stronger. (+)-MK801 causes the inhibition of the production of IFNγ in $CD4^+$ cells to a lesser extent in patients with MS. Similar trends were observed in the population of $CD8^+$ T-lymphocytes, while the sensitivity of IFNγ production to the blockade of NMDA receptors in MS was 3.2 times lower than in healthy donors. Analysis of the ratios of Th1/Th2 and Tc1/Tc2 showed that blockade of NMDA receptors leads to a shift of the cytokine balance towards the cellular level of immunity (Th1 and Tc1 cells), more significantly in MS. According to obtained data the blockade of NMDA receptors in the conditions of pharmacological activation of T-cells induces an increase in the relative mRNA level of the *TBX21* while simultaneously suppressing the expression of the *GATA3* (table). The consequence of this change in gene expression is an increase in the ratio of *TBX21/GATA3* and shift in the balance towards the pattern of proinflammatory cytokines. Thus, the results of the study indicate the involvement of NMDA glutamate receptors in the mechanisms of maintaining a balance between the effector subpopulations of T lymphocytes. The obtained data on the effects of blockade on T-lymphocytes of patients with MS emphasize the importance of Glu and its receptors in the pathogenesis of the disease and the relevance of their further research.