SCIENTIFIC RESEARCH BEHIND MNRI®

Effect of Reflex Integration Techniques on Dynamic of Congenital and Adaptive Immunity in Herpes-Associated Patients with Multiforme Erythema

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Abstract

he purpose of this research was to study features of the functioning of congenital and adaptive links of immunity for patients with herpes-associated multiforme erythema (HAEM) and an assessment of efficiency in its treatment with the Masgutova Neurosensorimotor Reflex Integration (MNRI®) program. Patients with HAEM before treatment observed a decrease in the number of CD4+, CD8+, CD25+, CD95+ against an increase of CD16+/32+. When studying patients' blood serum,



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their cytokines profiles were initially observed as decreased in the IL-2, IFN-γ level and increased in IL-10. Patients on MLPB observed a high level of an expression of TLR2, TLR4 and TLR3, TLR9. During treatment with MNRI®, a consecutive increase of lymphocytes of CD4+, CD8+, CD25+ against decrease in CD16+ was observed. MNRI® therapy promoted the IL-2 and IFN-γ level increase in blood serum to normal values, raised induced production of IFN-γ, and lowered induced production of IL-4, IL-5. As a result of the use of MNRI® therapy, the effect of strengthening of an expression of TLR2,3,9 was noted. Inclusion of MNRI® therapy of patients with HAEM had an immunocorrective effect, promoting the turning on of mechanisms of congenital immunity. This research was carried out at the Laboratory for Studying the Mechanisms of Immunity Regulation, I. I. Mechnikov Scientific Research Institute of Vaccines and Serums at the Russian Academy of Medical Sciences in Moscow, Russia.

Key words: neuro-sensory-motor reflex patterns integration (MNRI®), herpes-associated erythema multiforme (HAEM), TLRs, cytokines, lymphocytes.

Introduction

Herpes-associated erythema multiforme is the most frequent form and occurs in 50-93% of patients with exudative erythema multiforme (EEM) (Mashkilleyson, Alihanov, 1983; Alihanov, 1986; Samgin, et all, 1990; Wetter, Davis, 2010; Drago, Romagnoli, Loi, Rebora, 1992). Erythema multiforme relapses in at least 30% of patients.

A considerable number of causes for the infectious-allergic form of erythema have been detected, and clinical manifestations of infectious herpes are found in about half of patients. Relapses of EEM are often accompanied by reactivation of HSV1 and 2. However, in the period of erythema relapse, clinical signs of HSV infection may not be found (Lamoreux, Sternbach, 2006).

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Selecting a method of therapy to treat erythema exudative multiforme (EEM) is determined by a trigger factor as well. The trigger role of herpes simplex virus in the pathogenesis of exudative erythema multiforme gives objective reasons to proscribe acyclic nucleosides to such patients (Ivanov, et all, 2002; Laeger, 2012; Lemak, Duvic, Bean, 1986; Huff, 1988; Weston, Morelli, 1997).

Therefore, the success in using immunomodulatory drugs in EEM therapy show that finding new combinations of methods of therapy is feasible. In the literature of the field, one can find statements that the monotherapy of EEM by acyclic nucleosides is not effective enough (Samgin, Khaldin, Khaldina, 2004; Samgin, Khaldin, 2000).

In many cases, psycho-emotional stress has a role in precipitating or triggering a herpes simplex relapse which comes prior to HAEM development. Because of what has been said, it appears important to study the connection between the immune system and the nervous system, and to search for new combined methods for adequate therapy to treat herpes-associated erythema multiforme, while taking into account the peculiarities of innate and adaptive functioning of the immune system.

This article uses the MNRI[®] (Masgutova Neurosensorimotor Reflex Integration[®]) therapeutic program developed by Dr. S. Masgutova, which includes diagnostic and therapeutic procedures (Masgutova, Akhmatova, 2005).

The purpose of this study is to investigate the effect of the MNRI[®] therapeutic program on the innate and adaptive immune systems functions, and the peculiarity of cells cooperation in the immune response at the patients with HAEM.

Materials and Methods

In this research, two groups of patients were involved: Control Group-A – 15 healthy individuals (8 males and 7 females, age range of 18-54), and Research Group (B and C) - patients with HAEM - 21 patients.

Research group of patients with HAEM. Twenty-one patients were chosen for research (age range of 25-40 years old). The duration of the HAEM disease varied from two to ten years with a frequency of relapse of one to two times per year. The laboratory DNA diagnostics identified the herpesviridae virus in these 21 patients: 11 patients were identified with HSV type 1 in salivary glands secretion and in foci, four patients had HSV-2 in the discharge of the urogenital tract, three patients were identified with EBV and HHV-6, two patients with EBV and HSV-1, and one patient with EBV DNA. The identification of viruses correlated with the specific titer of antibodies IgM and IgG to HSV-1 and HSV-2, and IgM to capsid antigen EBV (VCA), IgG to capsid protein, IgG to the early antigen (EA) and nuclear antigen (EBNA) while analyzing blood serum by ELISA.

The research group consisted of two other sub-groups: B) combined MNRI® and immunomodulation treatment – 16 patients (7 males and 9 females), that received the MNRI® therapeutic program and also standard medical immunomodulation treatment, and C) immunomodulation treatment– 15 patients (8 males and 7 females) that went through only standard medical immunomodulation treatment, whose results served as the criteria for comparison of the data in these two sub-groups and analysis of the effect of the MNRI® program. The 8 patients that participated in Group-B were from the international Svetlana Masgutova Educational Institute (Warsaw, Poland). The other 8 patients of Group-B and patients receiving immunomodulation treatment from Group-C (15 people) and also individuals of the Control Group (15 healthy individuals) were patients of the laboratory of immunity regulation of I. I. Mechnikov Research Institute of Vaccines and Serums (Moscow).

Determining the level of cytokines in the serum/plasma and blood of patients was determined by a flow cytometer Cytomix FC- 500 (Beckman Coulter, USA) with the help of the test system FlowCytomix Thl/Th2 11 plex (e-Bioscience, USA) according to the manufacturer's instructions.

Lymphocyte subpopulation structure and TLRs were determined by using monoclonal antibodies according to the manufacturer's instructions (Beckman Coulter, USA). The content of cells was determined by flow cytometer Cytomix FC-500 (Beckman Coulter, USA).

Combined Therapy of the Patients with HAEM

Results of treatments of patients with HAEM in Group-B (16 patients) who received the MNRI® and immunomodulation treatment were compared with results of Group-C (15 patients) who received immunomodulation treatment only. The Control Group-A of clinically healthy individuals (15 individuals) served as the criteria of the effect of both therapeutic programs. Patients with HAEM in Group-B received MNRI® therapy sessions for 14

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days (6-8 hours daily). The patients were observed for one year and were evaluated before and after the MNRI[®] sessions. The groups were comparable in age, sex, and severity of the disease.

Standard immunomodulation treatment (Group-C; 15 patients) included etiotropic treatment in the form of antiviral chemotherapeutic remedy-acyclic nucleosides (acyclovir, Zovirax, viroleks, valacyclovir, and famciclovir). The etiotropic treatment was administered taking into account clinical and laboratory activation indicators of viral infections.

Results and Discussion

All observed patients were evaluated and measured for immunological function before treatments began. The immunological research revealed some changes in the lymphocyte subpopulation reference ranges in the group of patients (see Table 1), which were taken before the treatment. In the whole group the percentage of T-helper CD4 + was on the lower level of normal (38.6%). They also revealed a decrease in the relative number of CD8+ regulatory lymphocytes to 22.2%. In the content of the subpopulation of natural killer-cells, CD16 + changes were observed in the form of increasing the percentage and absolute number up to 19.6% and 544.2 × 106 cells/ml. Lymphocytes with CD95 + marker were initially reduced to 34.6%. In peripheral blood lymphocytes of patients, a very low activation level of molecules CD25 + (a receptor for IL-2) was noticed, showing 2.4%.

The cytokine profile in the blood serum of patients at the beginning of the study showed a reduction of the proinflammatory cytokine IL-2 (9.1 \pm 0.2%), IFN- γ (18.4 \pm 1.1%) with increased anti-inflammatory cytokine IL-10 (51.8 \pm 14.6%) in comparison with the healthy subjects (see Table 2).

These cytokines were stabilized after the MNRI[®] method was performed, except IL-10. Its level remained elevated, probably as a controlling mechanism of hyperactivation of the immune system.

The failure of IFN-γ in the system of the observed patients showed a lower level in the serum with the reduction of induced production. With normal levels of serum IL-5 and IL-12, the patients with induction showed increased production of IL-5 and IL-12 (up to 267.8±55.2 and 6559.3±1798.2 pg/ml, accordingly) that may indicate that there are stored immunocompetent cells to induce switching B lymphocytes to IgA synthesis and activation of antigen-presenting cells.

The next stage of our research was the study of the Toll-like receptors (TLRs) of peripheral blood mononuclear leukocytes (PBMC) in patients with HAEM (see Table 3), our research revealed the following features: in the cells of the patients we initially observed higher levels of TLR3 (exceeding 2 times higher) and TLR9 (exceeding 2.3 times higher) expression, than in a healthy person.

It was established that TLR3 recognizes double-stranded RNA and molecular structure of viruses, but does not conduct a signal from single-stranded RNA or double-stranded DNA. TLR9 is involved in the recognition of DNA-containing viruses. TLR9 by interconnecting with nucleic acid sequences of bacteria or DNA viruses, induces the production of proinflammatory cytokines and interferon type I, which play an important role in the immunological defense. It can be assumed that prolonged viral replication in the observed patients may increase the number of cells with TLR3 and TLR9 expression (Akhmatova, Kiselevsky, 2008; Takeuchi, Kaufmann, Grote, et al., 2000).

Results of Treatments

Anamnestic data analysis showed that all of the patients previously received therapy with temporary clinical effect or without any effect at all. Twenty-one patients were prescribed with antiviral chemotherapeutic agents: acyclic nucleosides (acyclovir, Zovirax, viroleks, valacyclovir, and famciclovir). Eleven patients showed insignificant temporary improvement of blanching foci, reduction of frequency, and clinical severity of HAEM relapses. Patients also received vitamin A and B-group vitamins. Despite ongoing earlier etiopathogenic therapy the condition of HAEM was torpid. Our study revealed that the role of the trigger factor in all patients was the reactivation of the viral infection. Additionally, all patients showed impairment of the innate immune system, which was the basis for including the MNRI[®] method into the combined therapy. The goal of including this combined therapy was to correct immunological disorders and subsequent removal of the trigger factor.

As a result of applying the MNRI[®] therapy, the duration and severity of the disease significantly decreased (14 patients), which coincided with the dynamics of laboratory indicators of the herpes virus infection activation. Two patients also demonstrated similar result, but not on a significant level. In the group of traditional

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medical treatment, only 6 patients demonstrated similar results with lower level of significance. During the MNRI[®] test we observed, for example, a consistent significant increase of CD4+ lymphocyte

population up to 42.1%, CD8+ up to 27%, and CD25+ up to 3.7% with the CD16+ reduced to a normal range (18.1%) in 14 patients out of 16, and in Group-C non-significant increase of the same lymphocyte population in 5 patients out of 15.

The evaluation of the dynamics of the humoral immunity showed a decrease of IgA levels to normal levels after MNRI[®] therapy in the same 14 patients. The level of circulating immune complexes, which was significantly higher than normal prior the treatment, tended to decrease.

Studying the serum levels of cytokines after the therapy revealed that MNRI[®] therapy enhanced the lowered baseline level of IL-2 in serum and IFN-y to a normal level in the same patients of Group-B. Major producers of IL-2 are CD4 T-lymphocytes. IL-2 causes two principal physiological effects: to induce antigen-dependent proliferation of all types of T-cells and to promote the differentiation of certain functional lymphocyte subpopulations: Cytotoxic lymphocyte and regulatory T cells. The principal biological effects of a IL-2 signal was stimulation of T- cell and NK-cell proliferation. It is known that the immune defense against viruses is formed with the participation of many mechanisms of innate and adaptive immune systems and is implemented by using four main factors: interferon type I, natural killer cells, cytotoxic T cells, and neutralizing antibodies. Therefore, the tendency of increasing IL-2 during immunotherapy serves to enhance antiviral protection, and thereby helps to eliminate the trigger factor of the erythema development in the observed patients. IFN-γ (known as an immune interferon) is produced by immune T- lymphocytes subpopulations Th1, CD8+ CTLs, and NK- cells. IFN-y is the most potent activator of

 Table 1. Influence of the MNRI therapy, percentage lymphocyte subpopulations in peripheral blood of patients with HAEM

Indicator	Norm,	D.C. T.	Group 1	Group 2	
	Unit	Before Treatment	After MNRI	After Basic Therapy	
CD3+	60-76%	63,1±3,1	66,1±3,8*	53,7±4,9*	
	1100-1700	1748,3±331,7	1201,4±127,4	1026 112 6	
	x10 ⁶ cell/мcL			1030±112,0	
CD3 ⁺ /CD4 ⁺	38-46%	38,6±2,8*	42,1±2,3*	35,2±3,5*	
	700-1100 x10 ⁶	1015,6±172,6	1039±74,9	689,8±71,3	
	cell/мcL				
CD25+	8-12 %	2,4±0,6*	3,7±0,6*	2,9±0,9*	
	31-40%	22,2±2,1	27±2,6	23,1±5,8	
CD3+/CD8+	500-1000 x10 ⁶	608,7±170,7	524,5±84,4	612 4+91 5	
	cell/мcL			012,4±01,5	
CD16 ⁺ /CD32 ⁺	10-19%	19,6±2,3*	18,1±2,3*	11,9±1,6*	
	200-400 x10 ⁶	544,2±147,2	352,1±62,1	262 5+41 7	
	cell/мcL			202,3±41,7	
CD72+	11-16%	11,1±2,1*	16,1±2,6*	13,6±2,2	
	200-400 x10 ⁶	325±75,5	$346,3\pm40$	319,8±31,2	
	cell/мcL				
HLADR+	5-20%	13,7±3,1*	18,7±1,7*	12,0±1,3*	
	100-500x10 ⁶	416,7±80,9	401,8±40,7	249,3±49,7	
	cell/мcL				
CD95+	41-63%	34,6±4,2*	30,3±2,9*	28,9±6,4*	
	100-500x10 ⁶	540±44,5	603,7±77,9	566,3±52,3	
	cell/мcL				
CD21+	11-16%	12,2±2,8*	16,8±2*	11,2±1,2	
	100-500x10 ⁶	378,4±88,6 327,1±34,6 34		347,1±62,8	
	cell/мcL				

Note. * p < 0.05 - significance of differences between the indices before and after the treatment (Mann-Whitney U-test).

TABLE 2. The effect of MNRI on cytokine levels in the serum of patients with HAEM

Cytokine	Healthy	Cytokine level in the patients with HAEM, pg/ml				
	individuals	Before	MNDI	Dagia Thanany		
		Therapy	WINKI	Basic Therapy		
IL-1b	10,1±4,7	9,8±0,4	12±1,5	$10,1\pm 3,4$		
IL-2	18,3±6,5	9,1±0,2*↓	14,9±3,2*	11,6±1,7*		
IL-4	9,2±0,1	10,3±1,2	12,4±1,6	9,7±1,7		
IL-5	11,4±0,1	9,4±0,3*	11,3±2,1	12,5±0,9*		
IL-8	9,8±1,2	10,9±0,5	14,3±1,8	N/D		
IL-10	20,2±6,8	51,8±14,6*↑	71,6±23,3*↑	36,2±11,8		
IL-12	6,3±0,2	7,8±0,9	10,3±1,3	5,0±1,9*↓		
IFN-γ	23,6±5,9	18,4±1,1*↓	32,1±8,6*↑	21,4±4,2*		
TNF-β	8,8±0,2	10,2±1	11,7±0,8	N/D		
TNF-α	8,5±0,1*	9,5±0,2	10,2±0,6*↑	N/D		

Note. * p<0.05 - reliability of differences relative to the control group (healthy individuals);

#- p<0.05 - reliability of differences between the study groups (Mann-Whitney U-test).

TABLE 3. The effect of MNRI therapy on the expression level of TLRs in PBMC in patients with HAEM								
	Number	Number of cells with expression,%						
	Of patients	TLR2	TLR4	TLR3	TLR9	TLR7	TLR8	
Before treatment	10	17,1±4,5*	23,4±5,8	34,2±6,7*	28,1±8,7*	49,9±8,1	52,4±4,9	
MNRI Method	11	26,8±6,1*	26±5,2*	41,4±3,1*	42,3±3,6*	52,4±6,2*	68,2±3,7	
Basic therapy	10	7,6±2,1*	12,3±1,5 *	21,5±6,1*	29,3±6,7	n/e	n/e	
Healthy individuas	15	4,8±0,6	4,2±0,3	15,4±3,4	11,2±1,3	n/e	n/e	
Note. The accuracy of the difference between the rates before and after the treatment: * $p < 0.05$, ** $p < 0.01$, $n/i \{n/e\}$, and - not examined.								

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macrophages. It also activates NK- cells, and induces the MHC I and MHC II protein cells expression, thereby facilitating antigen presentation (including virus) to T- lymphocytes, which leads to the formation of the antiviral immune response. IFN-γ which is produced by CD8+ cytotoxic T-lymphocytes contributes to the antiviral action of these cells.

The MNRI[®] therapy increased IFN-γ production to the normal level (452.7 pg/ml) and reduced initial elevated (high) levels of induced IL-4 and IL-5 production.

The observed patients initially had nine times higher levels of induced IL-5 production. IL-5 is produced by a subpopulation of immune CD4+ T- lymphocytes and mast cells, and promotes the differentiation and activation of eosinophils. IL-5 is described as a differentiating factor of B-lymphocytes. The increased IL-5 levels in the observed patients (showing a high rate of determined herpes virus infection), on one hand, may have a positive effect. This is accomplished by increasing levels of IgA which helps to increase mucosal defense. On the other hand, a significant increase in IL-5 levels can contribute to the formation of an allergic reaction. Considering that HAEM is a manifestation of a hypersensitivity reaction, reduction of the induced production of IL-5 during the course of therapy has a beneficial efficacy of the MNRI® therapy.

The study of the expression levels of TLRs the PBMC of the patients, as a result of MNRI® therapy, noted the enhanced expression of TLR2, 3.9 (to 26.8%, 41.4%, and 42.3 % accordingly). The very noticeable increase of the TLRs expression was TLR9 by a multiple of 1.5. MNRI® therapy did not affect the initially elevated (5 times) level of TLR4 expression. Therefore, the application of MNRI® therapy on the patients with HAEM contributed to the inclusion of the innate immunity.

After 12 months of monitoring the patients with HAEM, our research revealed a reduction of 2.7 times in the HAEM relapse rate and, in general, a reduction of clinical symptoms during relapses in Group-B (MNRI[®] combined with traditional medical treatment). The traditional medical treatment reduced the relapse rate by only 1.5 times. As a result of MNRI[®] therapy, we saw positive changes in the clinical and laboratory indicators of HSV infection activation, which is reflected in the reduction of herpes simplex relapse and reduction of laboratory indicators of active viruses.

In summary, based on our data, the use of the MNRI[®] therapeutic program allows correction of damaged mechanisms of the immune system and activation of the mechanisms of innate immunity in patients with HAEM. MNRI[®] therapy also helps to reduce the severity and duration of herpes simplex relapses by acting to influence the trigger factor and exerting a positive influence on the HAEM pathogenesis.

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We scientists thank all the adults who helped us in this research. We wish them good health along with a strong active and healthy lifestyle to keep their immune system activated! – Authors