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Research Article

Innate and Adaptive Immunity during Long-term Treatment of Multiple Sclerosis with Interferon Beta 1a

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Abstract

Long-term treatment of the multiple sclerosis (MS) with the interferon (IFN) β-1a can impair both innate and adaptive immunity of the patients. Analysis of the IFN gene expression in lymphocytes of blood of 18 patients with MS before treatment and during 1 year was performed by reverse transcription with real time PCR (RT^2-PCR) with fluorescent hydrolysis probes as well as ELISA to detect IFNα, β, γ, λ and IFNβ binding antibodies (BAbs); titers of neutralizing antibodies (NAbs) were estimated on the base of cytopatic effect of the vesicular stomatitis virus on permissive Vero-SF cells. Before a treatment the enhanced levels of IFN I and III types (in average, IFNβ RNA $2.2*10^3$ genome-equivalents (g-e) in 1 ml of blood and IFN β protein 37.3 pg/ml were detected in 90.9 ± 9.1% patients compared to healthy donors ($2.7*10^2$ g-e/ml and 11.7 pg/ml, respectively) ($P<0.01$). Treatment of the MS with the "Genfaxon44 (IFNβ-1a)" resulted in declining of IFNβ RNA up to $4.8*10^2$ g-e/ml in 6 months and after 1 year - until the normal values $3.6*10^2$ g-e/ml. The treatment was accompanied by the significant decrease of IFN α gene expression. Neither IFNγ nor IFNλ significant decrease were found. BAbs appeared in 6-12 months of the treatment in 2 from 17 patients with MS in the absence of anti-IFNβ antibodies in the control group of donors. NAbs were detected in both the sera with the IFNβ-BAbs. Thus, during the treatment of the MS with "Genfaxon44 (IFNβ-1a)" the significant decrease of IFNα and IFNβ gene expression along with unessential induction of the anti-IFNβ-binding antibodies with registration of neutralizing antibodies in 11.8±8.1% patients provide the stabilization of neurological status of the patients.

Keywords: multiple sclerosis; interferon (IFN) α, β, γ, λ, binding antibodies; neutralizing antibodies

Introduction

Innate and adaptive immunity are involved in pathogenesis of the autoimmune multiple sclerosis (MS) in 2.5 million patients in the world [1]. In the absence of etiotropic therapy interferon (IFN) β remains the most widely prescribed and widespread used treatment for MS. IFNβ is known to reduce inflammation and decreases relapses, alters the function of T cells and myeloid cells, maintains a population of BAFF-dependent regulatory B cells that suppresses cell-mediated inflammation of the central nervous system [2]. IFNβ also induces apoptosis of dendritic cells [1,3]. However, the exogenous IFN-β may serve as antigen inducing binding antibodies (BAbs) and neutralizing antibodies (NAbs) in patients with MS in 6-18 months of their treatment. NAbs against IFN-β are associated with a loss of IFN-β bioactivity and decreased clinical efficacy of the drug [4-8]. Our aim was analysis of IFN α, β, γ and λ gene expression, IFNβ binding and neutralizing antibodies in the process of the MS treatment with IFNβ-1a 44 μg.

Methods

Eighteen patients were randomly selected from the neurological department of the Moscow Regional Research Clinical Institute, Russia during 2012-2013. The patients (middle age 35.72 ± 9.46 years, 16 women and 2 men) had clinically definite MS according to the renewed diagnostic criterions of W. J. MacDonald in the modification of 2010: relapsing-remitting MS - 14 patients and secondary progressive MS - 4 patients; the duration of illness was 7.22 ± 4.10 years. Magnetic resonance imaging (MRI) for the patients with moderate disability revealed multiple foci of brain lesions with a variety of clinical manifestations such as numbness and weakness of the limbs, disturbances of gait and vision, dizziness and mild dysarthria. Results of the objective neurological

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examination were estimated according to the received scale of the clinical estimation of the functional condition of the conductive systems proposed by J. Kurtzke and the expanded disability status scale (EDSS). Before the research their average EDSS was 2.7 ± 1.2 points.

Patients with MS were treated with IFNβ-1a - "Genfaxon" ("Tutor S.A.C.I.F.I.A." Laboratory, Argentina) 44 μg subcutaneously, 3 times in a week. All the patients signed written informed consent and donated samples of blood. Their demographic and clinical data were recorded. The study protocol was approved by the local ethic committee. Control group consisted from 18 healthy donors (mean ± SD of age 41.78 ± 14.28 years).

Analysis of the IFN gene expression in lymphocytes of blood of 18 patients with MS before treatment and during 1 year was performed by reverse transcription with real time PCR (RT^2-PCR) with fluorescent hydrolysis probes [9]. ELISA to detect IFNα and IFNγ ("Vector-Best", Russia), IFNβ ("BioSourse", Japan and "pbl interferon source", USA), IFNλ ("Bender Med System", Austria); and IFNβ binding antibodies (BAbs) using "BŮHLMANN Laboratories AG", Switzerland with titers in Buhlmann Titer Units (BTU); titers of neutralizing antibodies were estimated on the base of cytopatic effect of the vesicular stomatitis virus on permissive Vero-SF cells in Neutralizing Units in 1 ml (NU/ml) [10].

Results

Before a treatment the enhanced levels of IFN I and III types were determined in blood samples of patients with MS in comparison with the control group of donors (Tables 1 and 2). One should note the IFNβ increased amounts (in average, $2.2*10^3$ genome-equivalents (g-e) in 1 ml of blood) and IFN β protein (37.3 pg/ml) in 90.9 ± 9.1% patients compared to healthy donors ($2.7*10^2$ g-e/ml and 11.7 pg/ml, respectively) (P<0.01). Treatment of MS with "Genfaxon44 (IFNβ-1a)" resulted in declining of IFNβ RNA up to $4.8*10^2$ g-e/ml in 6 months and after 1 year - until the normal values $3.6*10^2$ g-e/ml. Perhaps, the IFNβ gene expression level restoration was caused by the reverse regulation with the exogenous IFNβ-1a. Long-term treatment with IFNβ-1a for more than 12 months also resulted in significant decrease of IFN α gene expression in parallel with the stabilization of neurological status. Neither IFNγ nor IFNλ significant decrease were found.

In control group of donors BAbs (5-42 BTU) were revealed but NAbs were not detected. Before treatment of the MS patients BAbs titers varied in a range from 11 to 171 BTU; in 6 months of IFNβ-1a administration - 45-264 BTU; in 12 months – 26-232 BTU. BAbs were detected in 2 from 17 (11.8 ± 8.1%) patients with MS; in one from them NAbs (160-320 NU/ml) appeared in 6 months of the treatment, whereas in other patient - 80-100 NU/ml in 12 months.

Treatment of MS with other IFN β-1b/1a medications such as "Betaferon" (IFN-β-1b; Schering, Berlin, Germany), "Rebif" (Serono,Genevè, Switzerland) , "CinnoVex" (CinnaGen Company, Iran) induced BAbs in 22-38 % patients among them 80.6% contained NAbs [6]. According to our observations the long-term treatment of a patient with "Rebif" for 8 years caused both BAbs (1960 BTU) and NAbs (2000 NU/ml). BAbs rate after the administration of "Genfaxon" was significantly lower - in 11.8 ± 8.1% patients (P<0.01) and in all of them NAbs were revealed in 6-12 months of the treatment.

Long-term monitoring of the treatment of 18 patients with MS with "Genfaxon" for more than 12 months showed the stabilization of their neurological status estimated on the base of the EDSS

Table 1: Comparison of IFNα, IFNβ, IFNγ, IFNλ types gene expression in lymphocytes of blood from MS patients and healthy donors.

	IFNα	IFNβ	IFNγ	IFNλ
Percentage (%) of samples with IFN mRNA				
Control	23.5 ± 10.6	58.8 ± 12.3	11.8 ± 8.1	35.3 ± 11.9
MS before treatment	81.8 ± 12.2**	90.9 ± 9.1*	18.2 ± 12.2	81.8 ± 12.2*
MS after treatment				
3 months	80.0 ± 20.0	60.0 ± 24.5	20.0 ± 20.0	100
6 months	60.0 ± 24.5**	80.0 ± 20.0	20.0 ± 20.0	60.0 ± 24.5
12 months	54.5 ± 15.7	72.7 ± 14.1	45.5 ± 15.7	90.9 ± 9.1
>1 year	20.0 ± 13.3**	30.0 ± 15.3*	90.0 ± 10.0***	90.0 ± 10.0
Quantitative estimations of genome-equvalents in 1 ml of blood (among RT^2-PCR positive samples)				
Control	$3*10^1$	$2.7*10^2$	$7.2*10^5$	$6.2*10^2$
MS before treatment	$2.0*10^{3***}$	$2.2*10^{3**}$	$2.0*10^6$	$4.4*10^{3***}$
MS after treatment				
3 months	$3.0*10^2$	$6.3*10^2$	4***	$3.6*10^3$
6 months	$1.0*10^3$	$4.8*10^2$	$9.8*10^5$	$2.2*10^3$
12 months	$8.3*10^2$	$7.2*10^{1**}$	4***	$2.7*10^3$
>1 year	$1.9*10^{4**}$	$3.6*10^2$	$3.9*10^6$	$9.4*10^3$

*, **, ***, statistical significance: p <0.05, p <0.01, p<0.001, accordingly.

Table 2: Comparison of α, β, γ, λ type in sera of blood from MS patients and healthy donors.

Groups	IFN (pg/ml)			
	α	β	γ	λ
Control group of healthy donors	1.2	11.7	0.1	0
MS patients before treatment	0.5	37.3	3.12	0
MS patients after treatment with "Genfaxon" for 12 months	0.48	24.4	0.29	0

without evident complications. Our observations coincided with the previously described IFNβ-mediated remission [1,11,12]. Possible molecular mechanisms of IFNβ include the neuron growth factor induction; inhibition of the proinflammatory IFNγ and TNFα [13,14] induction of regulatory IL10 and interferon-stimulated genes and apoptosis of dendritic cells [3,11,13,15-19].

Conclusion

The treatment of MS with «Genfaxon 44 (IFNβ-1a)» resulted in significant decrease of IFNα and IFNβ gene expression, unessential growth of the anti-IFNβ-binding antibodies with registration of neutralizing antibodies in 11.8 ± 8.1% patients and stabilization of neurological conditions.

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