Induction of Antiidiotypic Immune Response with Autologous T-cell vaccine in Patients with Multiple Sclerosis

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Patients with different forms of multiple sclerosis were treated with a vaccine consisting of myelin-reactive T cells. It was found that after this treatment, lymphocytes from patients acquired the capacity to generate antiidiotypic proliferative response directed towards myelin-reactive T cells. The serum concentration of IFN-γ decreased about 2-fold 1.5-2.0 years after the start of vaccine therapy, whereas the concentration of IL-4 increased 2-3 fold. Myelin-reactive proliferative activity of peripheral blood mononuclear cells also decreased. The results of the 2-year follow-up study revealed no side effect of T-cell vaccination in patients with cerebrospinal form of multiple sclerosis and demonstrated its possible clinical efficiency in the treatment of this disease at early stages.

Key Words: *T cells; vaccine; multiple sclerosis*

The pathogenesis of multiple sclerosis (MS) is based on an autoimmune process mediated by type 1 T-helper cells reactive to surface antigens of nerve system cells (myelin basic protein, proteolipid protein, associated myelin glycoprotein, oligodendrocyte myelin glycoprotein). Recognition of these antigens by T cells and production of proinflammatory cytokines (IFN-γ and IL-2) triggers and maintains the myelin-destructive inflammatory process in the central nervous system [7].

Standard therapy of MS is based on immunosuppressive agents. An alternative and most perspective approach to the therapy of MS is based on stimulation of antiidiotypic immune response directed against variable fragments (idiotypes) of antigen receptors of pathogenic lymphocytes [6,7].

An original two-staged method for obtaining autologous antigen-specific T-cell vaccines was developed in our laboratory. This method considerably simplified the procedure of obtaining immunogenic T-cell vaccine and can be widely used in the therapy of autoimmune disease [1].

Here we studied immunological and clinical aspects of application of T-cell vaccine in the therapy of MS patients.

MATERIALS AND METHODS

Clinical studies were performed in accordance with the protocol approved by Scientific Council and Ethical Committee of Institute of Clinical Immunology, Siberian Division of Russian Academy of Medical Sciences. Informed consent was obtained from all participants. Immunotherapy was performed in 28 patients (age from 18 to 54) with cerebrospinal form of MS, history of the disease was not less than 2 years. Remittent, progredient-remittent, primary progredient, and secondary progredient course of the disease was diagnosed in 3, 4, 4, and 17 patients, respectively. Clinical diagnosis

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was confirmed by magnetic resonance study. Neurological status of patients was evaluated by Kurtzke expanded disability status scale (EDSS). The patients received no immunosuppressive therapy for at least 6 months before the start and during the trial.

Myelin was isolated from porcine brain as described previously [3].

The developed technology of obtaining T-cell vaccine consists in two stages. The first stage includes culturing of blood mononuclear cells (MNC) of the patients in the presence of myelin (50 μg/ml) for 7 days, the second stage consists in production of myelin-reactive T cells by unspecific mitogen stimulation with phytohemagglutinin (PHA, 5 μg/ml) and human recombinant IL-2 (Ronkoleukin, 100 U/ml). After culturing, the cells were inactivated, cryopreserved routinely in plasma with 10% dimethylsulfoxide (Sigma), and stored in liquid nitrogen. The total number of cells obtained from one patient was $1.8\text{-}2.7\times10^8$.

The inducing course of immunotherapy consisted in 4 subcutaneous T-cell vaccinations (once a week). Supportive treatment included vaccination with 1-2-month intervals. The vaccinal dose was $2\text{-}4\times10^7$ cells.

For evaluation of the specificity of antiidiotypic proliferative response, 10^5 MNC from the peripheral blood of vaccinated patients were cultured for 72 h in the presence of irradiated myelin-activated (vaccinal) T cells (10^5). In control samples, T cells specific to porcine articular collagen generated by the method similar to that for myelin-reactive T cells were added to MNC instead of vaccinal cells. Porcine articular collagen was isolated as described previously [8].

For additional control, MNC were cultured in the presence of T cells activated by non-specific PHA and IL-2.

For obtaining antiidiotypic T-cell line, MNC (2×10^6/ml) were cultured with irradiated myelin-reactive idiotype-carrying T cells (10^6/ml). After 7 days, irradiated autologous MNC and idiotype-carrying T cells were added to the culture and the mixture was cultured for 7 days in the presence of IL-2 (100 U/ml). Then the cultures were irradiated (2000 rad) and their suppressor activity was evaluated in the proliferative test. To this end, 10^5 cells were cultured with autologous myelin-activated (idiotype-carrying) T cells (10^5) and irradiated MNC (10^5) in the presence of myelin (50 μg/ml) for 120 h.

For evaluation of myelin-induced proliferative T-cell response, MNC (2×10^5) were cultured with myelin (50 μg/ml) for 120 h.

Cell proliferation was evaluated by [^{3}H]-thymidine incorporation.

The content of cytokines IFN-γ and IL-4 in serum samples and supernatants of 72-h MNC cultures was measured using commercial kits (Vector-Best).

The results were processed using Mann—Whitney *U* test.

RESULTS

Antiidiotypic proliferative response of peripheral blood MNC was evaluated in 7 patients: in 1 patient with remittent, 1 with progredient-remmitent, 2 with primary-progredient, and in 3 with secondary progredient course of the disease. Antiidiotypic proliferative response of MNC from MS patients receiving 4 vaccination surpassed the control values by 4.4-5.7 times ($p<0.01$, Fig. 1). Stimulation with collagen-specific T cells and T cells activated by nonspecific mitogens (PHA+IL-2) did not potentiate MNC response.

Antiidiotypic T-cell lines reactive to myelin-specific T cells were obtained from 5 vaccinated MS patients (1 patient with remittent, 1 with progredient-remittent, 1 with primary progredient, and 2 with secondary progredient course of the disease). Cells of these lines suppressed antigen-induced proliferation of autologous myelin-activated T cells by 48-53% ($p<0.05$; Fig. 2). It can be hypothesized that T-cell vaccine can effectively induce specific immune response to autoimmune T cells.

Proliferative response of MNC to myelin was determined in 19 vaccinated patients (4 with remittent, 4 with progredient-remittent, 3 with primary progredient, and 8 with secondary progredient course of the disease). The course of vaccine therapy

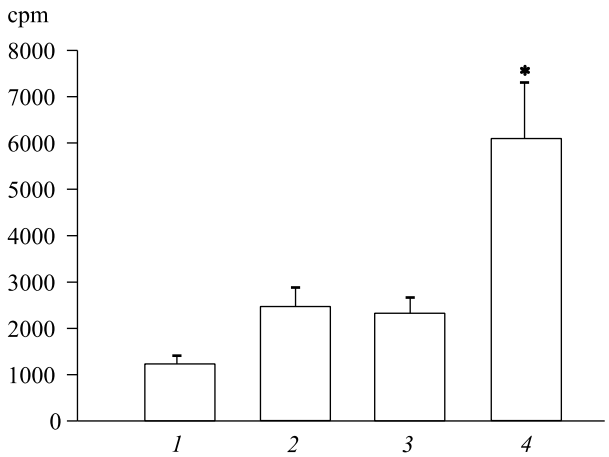

Fig. 1. Proliferative response of MNC from vaccinated patients (n=7) to autologous T cells specific to different antigens or nonspecifically activated. *1*) medium; *2*) nonspecifically activated cells; *3*) collagen-reactive T cells; *4*) myelin-reactive T cells. *$p<0.01$ compared to the control.

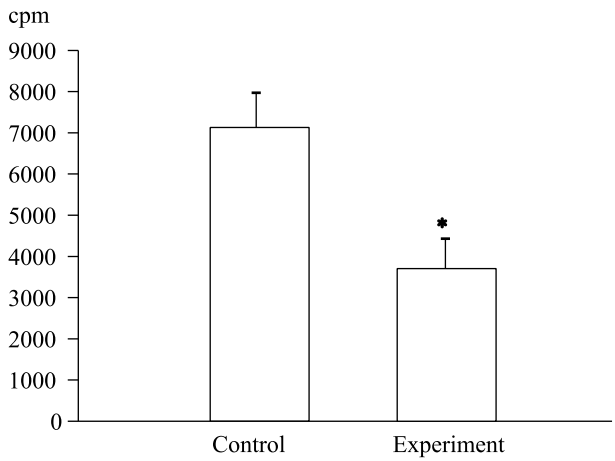

Fig. 2. Suppressor activity of antiidiotypic T cells. Myelin-activated T lymphocytes and irradiated MNC were cultured with myelin antigens in the absence (control) or presence of antiidiotypic T lymphocytes (experiment). Results of 5 experiments are presented. *p<0.05 compared to the control.

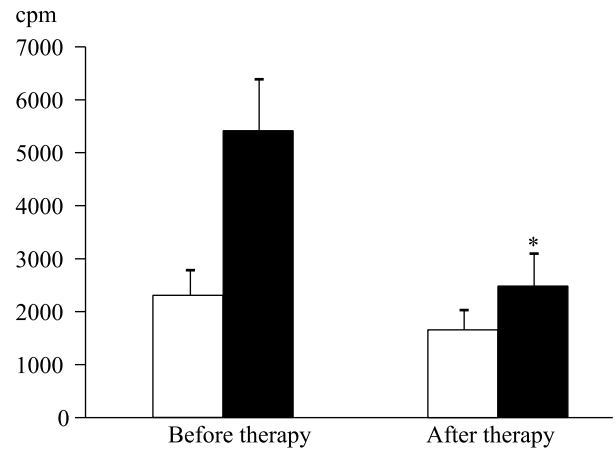

Fig. 3. Proliferative response of MNC from patients (n=19) to myelin before and after 2-year treatment. Open bars: medium; dark bars: myelin. *p<0.01 significant differences between the groups before and after therapy.

reduced proliferative response to myelin (p<0.01), which probably attested to a decrease in the content of myelin-reactive cells in the blood of patients after treatment (Fig. 3).

After 1.5-2-year immunotherapy, a decrease in serum level of IFN-γ and an increase in IL-4 concentration were noted in 11 patients (2 with remittent, 4 with progredient-remittent, 2 with primary progredient, and 3 with secondary progredient course of the disease, Fig. 4). These shifts corresponded to changes in the production of these cytokines by MNC from these patients in response to stimulation with myelin (p<0.01 for IFN-γ and p<0.05 for IL-4; Fig. 5). The spontaneous or PHA-stimulated production of cytokines by MNC remained practically unchanged.

No significant changes in clinical and biochemical laboratory tests were noted in vaccinated patients during 2-year monitoring. The percent of $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$, $CD20^+$ lymphocytes in the blood also remained unchanged.

In 3 patients with remittent MS, no exacerbation of the disease was observed, while in 1 patient neurological parameters considerably improved (Table 1). In 2 of 4 patients with progredient-remittent course of MS, no exacerbations of the disease were noted, patients status evaluated by Kurtzke scale remained stable (Table 2). In 1 patient, clinical state improved and EDSS decreased. Deterioration of the state in the form of increasing general fatigue was noted in 1 patient in this subgroup. In 3 of 4 patients with primary progredient course of MS, the general state was stable throughout the observation period. In 1 patient of this subgroup, deterioration of clinical state and EDSS increase were noted. In 10 of 17 patients with secondary progredient course of the disease, we observed stabilization or improvement (in 1 patient) of neurological parameters, while in 7 patients deterioration of the neurological status associated with progression of the disease was noted. No unfavorable side effects of vaccine therapy were found within the observation period.

Traditional therapy of MS is based on long-term nonspecific immunosuppressive therapy, which in many cases does not allow to control progression of the disease and has appreciable side effects. This necessitates the search for new approaches to the therapy of MS and other autoimmune diseases aimed at selective inactivation of pathogenic lymphocytes. One of these approaches is vaccination with

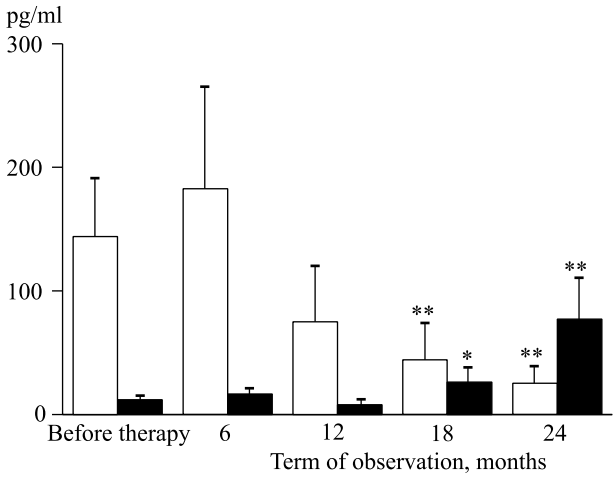

Fig. 4. Concentration of IFN-γ (light bars) and IL-4 (dark bars) in the serum of patients (n=11) in the dynamics of treatment. Here and on Fig. 5: *p<0.05, **p<0.01 compared to the level before therapy.

TABLE 1. Clinical Characteristics of Patients with Remittent Course of MS

Patient, age	History, years	Severity of the disease	Incidence of attacks		EDSS	
			before treatment	after 2-year treatment	before treatment	after 2-year treatment
G. D., 40 y.o.	10	I	2	No	2.5	2.5
S. E., 22 y.o.	9	II	2	No	2	2
M. S., 39 y.o.	4	II	2	No	6	4.5

autoimmune lymphocytes. It is known that there is no innate immunological tolerance to antigenic receptors on lymphocytes formed in the postnatal period; lymphocyte-lymphocyte idiotype-antiidiotypic interactions play an important role in the regulation of the function of the immune system.

Some clinical studies demonstrated induction of antiidiotypic T cell response directed towards circulating pathogenic autoimmune T cells in response to immunization with autologous myelin-reactive T cells [2,4,6,9,11]. This immunization leads to generation of anticlonotypic cytolytic $CD8^+$ T cells specifically recognizing idiotypic structures of T cell receptors involved in autoimmune process [2,5,10]. Moreover, T cell vaccination induces generation of anticlonotypic and antiergotypic $CD4^+$ T cells producing antiinflammatory cytokines IL-4, IL-10 [5,10] in activated state, thus preventing the development of the destructive process in tissues.

Obvious advantage of T-cell vaccination over other treatment methods consists in its selective direction against lymphocytes responsible for the development of the autoimmune process. It should be noted that vaccine technology explores the mechanism of immune memory and therefore long-lasting clinical effect can be attained.

The data on clinical application of T-cell vaccination are scanty and no definite conclusions on its practical value can be made. In previous studies, cloned T cells were used for immunization. These lymphocytes express identical idiotype-carrying antigenic receptors on their surface with a density sufficient for induction of highly specific antiidiotypic response [4-6,11]. However, generation of T-cell clones is an expensive, long-term, and in some cases ineffective procedure. Moreover, inactivation of one or several autoimmune clones can produce no effect on the development of the disease determined by polyclonal immune response directed against many antigenic determinants, and the value of a single clone in the autoimmune process is sometimes insufficient. These features limit the possibility of effective use of cloned T-cells in the treatment of MS and other autoimmune diseases.

The method developed in our laboratory allows generation of a large number of myelin-reactive T cells over a short time. It is important that the composition of the vaccine is determined by initial individual reactivity of T cells and is presented by cells that were given selective growth preferences related to the presence of myelin antigen in the culture.

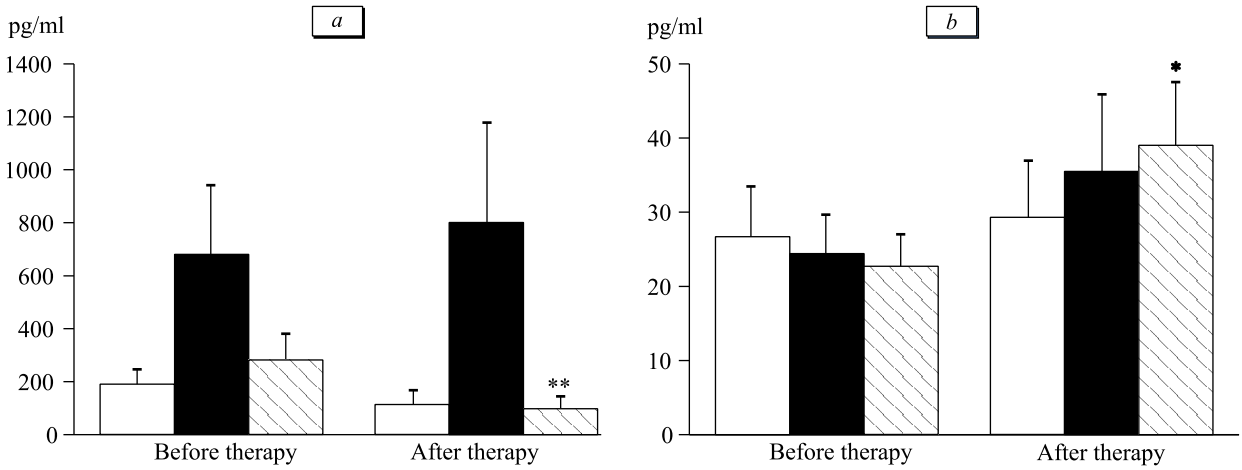

Fig. 5. Content of IFN-γ (*a*) and IL-4 (*b*) in supernatants of MNC from MS patients (n=11) before and after 2-year therapy. White bars: medium; dark bars: PHA; shaded bars: myelin.

TABLE 2. Clinical Characteristics of Patients with Progrediant Course of MS

Patient, age	History, years	Course of the disease	Severity of the disease	EDSS	
				before treatment	after 2-year treatment
K. O., 35 y.o.	13	PR	III	4.5	4.5
L. Yu., 21 y.o.	2	PR	III	4.5	3.5
N. N., 35 y.o.	11	PR	III	6	6.5
S. N., 30 y.o.	7	PR	II	3.5	3.5
M. N., 48 y.o.	4	PP	II	3	3
N. O., 42 y.o.	6	PP	III	4.5	5
G. V., 57 y.o.	11	PP	III	5	5
S. V., 45 y.o.	12	PP	III	6	6
K. Yu., 40 y.o.	15	SP	IV	7	7
A. M., 39 y.o.	13	SP	IV	6	6
B. N., 53 y.o.	29	SP	III	3.5	3.5
Z. V. 52 y.o.	21	SP	IV	5	6
V. A., 50 y.o.	14	SP	IV	5	5
V. N., 38 y.o.	12	SP	IV	6.5	7
G. K., 31 y.o.	9	SP	II	4.5	4.5
G. E., 30 y.o.	3	SP	III	4	3.5
D. A., 27 y.o.	8	SP	III	7	7
L. T., 45 y.o.	8	SP	III	5.5	5.5
M. T., 49 y.o.	9	SP	IV	6	8
P. I., 34 y.o.	4	SP	III	6	6.5
P. A., 46 y.o.	5	SP	III	4	4.5
P.E., 54 y.o.	9	SP	III	5	5
S.E., 23 y.o.	3	SP	III	6	7
S. O., 36 y.o.	13	SP	IV	6	7
T. I., 37 y.o.	9	SP	III	6.5	6.5

Note. PR: progredient-remittent; PP: primary progredient; SP: secondary progredient.

The main question is whether idiotypic determinants presented in T-cell vaccine can induce a specific antiidiotypic response. The results obtained by us confirm this possibility. The concentration of idiotypic determinants in T-cell vaccine is sufficient for induction of the antiidiotypic immune response. MNC of vaccinated patients demonstrated an increase in proliferative activity in the presence of vaccinal myelin-reactive, but not collagen-reactive T cells. It is also important that T cells initially activated by nonspecific stimuli did not stimulate proliferation, which also confirmed specificity of the immune response.

Another principal question is whether lymphocytes generated as a result of T-cell vaccination can affect pathogenic immune reactions. According to our findings, vaccine therapy reduced proliferative activity of autoimmune T cells of patients in response to myelin antigens. However, previous studies showed that the duration of this effect is determined by the duration of vaccine therapy [1], therefore long-term supportive treatment is a rational and scientifically grounded measure.

It is known that type 1 T-helpers producing IFN-γ play a key role in the development of pathological myelin-destructive process [7], whereas type 2 T-helpers producing IL-4 can suppress activity of type 1 T-helpers, thus preventing the development of cytodestructive immune reactions. The reduced synthesis of IFN-γ and enhanced production of IL-4 in the organism of vaccinated patients can attest to functional changes in myelin-reactive cells and involvement of nonspecific immune mechanisms into inhibition of the immunopathological process.

One of the objectives of the present pilot clinical study was evaluation of side effects of T-cell

vaccination. Our findings definitely proved safety of this treatment. None complications were detected throughout the observation period. The results also indicate significant clinical efficiency of T-cell vaccination during treatment of MS. We believe that T-cell vaccination can be most effective at early stages of the disease, but it cannot be excluded that appreciable results of this treatment can be also obtained at later stages. However, final conclusions are premature. Large-scale clinical studies are required for evaluation of the role of T-call vaccination in the treatment of MS and other autoimmune diseases.

REFERENCES

1. I. P. Ivanova, V. I. Seledtsov, N. V. Banul, *et al*., *Med. Immunol*., **7**, No. 1, 27-32 (2005).
2. J. Correale, B. Lund, M. McMillan, *et al., J. Neuroimmunol*., **107**, No. 2, 130-139 (2000).
3. G. E. Deibler, R. E. Martenson, and M. W. Kies, *Prep. Biochem*., No 2, 139-165 (1972).
4. N. Hellings, J. Raus, and P. Stinissen, *Autoimm. Rev*., **3**, 267-275 (2004).
5. G. Hermans, R. Medaer, J. Raus, and P. Stinissen, *J. Neuroimmunol*., **102**, No. 1, 79-84 (2000).
6. P. Stinissen, J. Zhang, R. Medaer, *et al., J. Neurosci. Res*., **45**, 500-511 (1996).
7. P. Stinissen and J. Raus, *Acta Neurol. Belg.*, **99**, 65-69 (1999).
8. S. C. Strom, *Methods Enzymol.*, **82**, Pt. 2, 554-555 (1982).
9. A. A. Vandenbark, E. Morgan, R. Bartholomew, *et al*., *Neurochem. Res*., **26**, 713-730 (2001).
10. Y. C. Q. Zang, J. Hong, M. V. Tejada-Simon, *et al., Eur. J. Immunol.*, **30**, 908-913 (2000).
11. J. Zhang, P. Stinissen, R. Medaer, and J. R. Raus, *J. Mol. Med*., **74**, 653-662 (1996).