Characterization of Immunogenic Properties of Polyclonal T Cell Vaccine Intended for the Treatment of Rheumatoid Arthritis

I. P. Ivanova, V. I. Seledtsov, D. V. Seledtsov, D. M. Samarin, G. V. Seledtsova, O. A. Herzsog, and V. A. Kozlov

Translated from *Kletochnye Tehnologii v Biologii i Medicine*, No. 4, pp. 221-225, October, 2007
Original article submitted May 28, 2007

Two-staged technology for obtaining polyclonal T cell vaccine intended for the treatment of rheumatoid arthritis is described. Stage 1 includes antigen-dependent cultural selection of patient's T cells and stage 2 consists in their reproduction in the needed amounts by nonspecific mitogenic stimulation. T cell vaccination induces an effective specific anti-idiotypic immune response against T cells reactive to joint antigens. Vaccine therapy significantly reduces plasma level of IFN-γ and increases IL-4 level. The results indicate immunological efficiency and safety of polyclonal T cell vaccine in patients with rheumatoid arthritis.

Key Words: *T cells; vaccine; rheumatoid arthritis*

The pathogenesis of rheumatoid arthritis (RA) is determined by autoimmune process mediated by type 1 T helpers. Type 2 collagen and other antigenic molecules expressed on synovial surfaces of the joint are involved in autoimmune induction during disease development. Specific recognition of these antigens by T cells triggers a chain of events leading to inflammation and destruction of the joints [10,11,15].

Traditional therapy for RA is based on long-term nonspecific immunosuppression, which in many cases fails to control the disease progress and is fraught with significant side effects [1].

A promising approach to the treatment of autoimmune diseases is immunization of patients with autoimmune T cells leading to generation of anti-idiotypic immune response. T cell vaccination leads to generation of two T cell types: one type recognizes idiotype associated with T cell receptor, the other (antiergotypic) suppresses T cell activation by a mechanism other than recognition of idiotypic determinants [4,16]. It was experimentally and clinically proven that this vaccination ensures long-lasting elimination of autoimmune T cells thus arresting the immunopathological process [5,6,12]. Since the production of autoantibodies is a T-dependent process, selective inactivation of autoimmune T cells arrests not only cellular, but also humoral autoimmune reactions.

By the present time, the safety and high clinical efficiency of T cell vaccination in the treatment of multiple sclerosis is proven [2-5,12]. There are reports that this technology was also used in the therapy of RA [7,8,14]; for instance, clinical efficiency of vaccination of RA patients with peptides corresponding to the variable site of autoantigen-recognizing T-cell receptor was demonstrated [8, 9,14].

Despite obvious good prospects of T cell vaccination in the therapy of multiple sclerosis and other autoimmune diseases, this method is not yet widely applied because of high costs, long period needed

Institute of Clinical Immunology, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk. ***Address for correspondence:*** irinaiki@rambler.ru. I. P. Ivanova

0007-4888/07/1444-0630

for obtaining T cell clones and difficulties associated with this procedure, and because is it often impossible to identify precisely the autoantigenic determinants involved in the pathological process.

The aim of the present study was to develop a method for obtaining immunogenic T cell vaccine without long-term cell cloning and to evaluate antigenic specificity and immunogenic activity of polyclonal T cell vaccine for the treatment of RA.

MATERIALS AND METHODS

Clinical and experimental studies were carried out in accordance with the protocol approved by the Academic Council and Ethic Committee of Institute of Clinical Immunology. Informed consent to participation in the study was obtained from all patients. Immunotherapy was carried out in 12 patients with rheumatic polyarthritis. All patients were females aged 21-70 years with the disease duration of 2-20 years, 1st-3rd degree process activity, X-ray arthritis stages II-IV, and 2nd degree functional insufficiency. The patients received basic methotrexate therapy ($\leq$10 mg/week).

The cartilaginous and synovial protein antigens (AG) were isolated from porcine joint tissue as described previously [13]. Protein content in the samples was measured by the method of Lowry.

Our technology for preparation of T cell vaccine consists of 2 stages. Stage 1 includes antigen-specific cultural selection of cells and stage 2 is reproduction of the needed amount of cells by non-specific mitogenic stimulation. The optimal concentrations of reagents added into the culture were determined in preliminary experiments. During stage 1, patient's mononuclear cells (MNC) isolated from the peripheral blood were cultured in a concentration of 2×10^6 in 1 ml RPMI-1640 with 10% inactivated autologous plasma, 5 mM HEPES, 2 mM L-glutamine, and 5×10^{-5} M mercaptoethanol (all reagents from Sigma) in the presence of synovial (1 μg/ml) and cartilaginous (1 μg/ml) AG for 5-7 days in a humid atmosphere with CO_2. During stage 2, antigen-specific cells were reproduced by their stimulation with phytohemagglutinin (PHA, 5 μg/ml, Sigma) and recombinant IL-2 (100 U/ml, Roncoleukin, Biotech) for 5 days. After the end of culturing, the cells were irradiated in a dose of 2000 rad, cryopreserved by the standard method in the plasma with 10% dimethylsulfoxide (Sigma), and stored in liquid nitrogen vapor until use as vaccine. Total number of cells obtained from one patient was $1.8\text{-}2.7\times10^8$.

Inducing immunotherapeutic course included 4 weekly subcutaneous T cell vaccinations. Maintenance therapy included vaccinations with 1-month intervals. Vaccine dose was $2.0\text{-}4.0\times10^7$ cells.

Surface markers of vaccine cells were detected using monoclonal antibodies (MAb) LT3 (CD3), LT4 (CD4), LT8 (CD8), LKN16 (CD16) (Sorbent) conjugated with fluorescein isothiocyanate, and ICO-180 (CD20) MAb (MedBioSpectr), labeled with phycoerythrin. The count of positive cells was determined on a FACS Calibur immunocytometer (Becton Dickinson) using CELLQuest software (Becton Dickinson).

Immunological specificity of T cells intended for vaccination was evaluated by their antigen-induced proliferative response as follows. Vaccinal T cells (10^5) were cultured for 72 h with irradiated (2000 rad) autologous MNC in the presence of joint AG (cartilaginous and synovial, 1 μg/ml each) in wells of a 96-well round-bottom plate (Costar). Control cells were cultured with myelin AG (50 μg/ml) isolated from porcine brain by Debler's method.

In order to evaluate the degree and specificity of anti-idiotypic response, MNC (10^5) isolated from the peripheral blood of vaccinated patients with RA were cocultured for 72 h with irradiated vaccinal or myelin-reactive T cells (10^5); the latter cells were obtained similarly as the vaccinal cells. In one more control series MNC were cultured with T cells non-specifically activated with PHA (5 μg/ml) and IL-2 (100 U/ml).

In order to obtain anti-idiotypic T-cell strain, MNC (2×10^6/ml) from vaccinated patients were cultured with irradiated T cells (10^6/ml) reactive to joint AG. After 7 days, irradiated autologous MNC and idiotype-carrying T cells were added to the culture. Cell culturing was continued with IL-2 (100 U/ml) for 7 days. The cells were then irra-

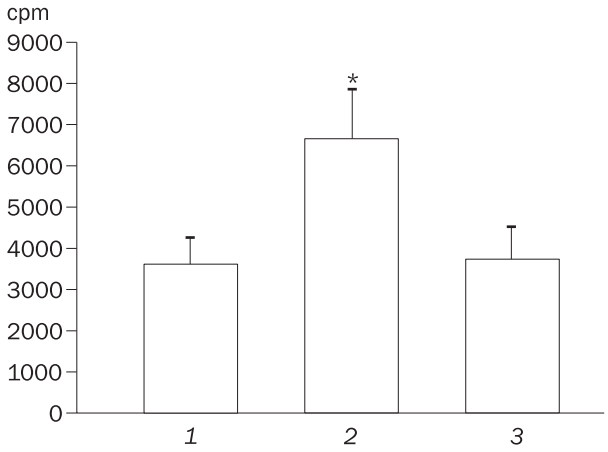

Fig. 1. Proliferative response of vaccinal T cells in the presence of tissue AG. *1*) medium; *2*) joint AG; *3*) myelin AG. Here and in Figs. 2-5: *$p<0.05$ compared to the control.

diated (2000 rad) and their suppressor activity was evaluated in the proliferation test. These cells (10^5) were cocultured for 72 h with autologous AG-reactive T cells (10^5) and irradiated MNC (10^5) with joint AG (1 μg/ml).

Antigen-induced proliferative response was evaluated by culturing peripheral blood MNC in a 96-well plate in a concentration of 2×10^5 in complete medium with cartilaginous (1 μg/ml) and synovial (1 μg/ml) AG or without them (control) for 5 days. Cell proliferation was evaluated by the standard method by [^{3}H]-thymidine incorporation.

Plasma cytokines (IFN-γ and IL-4) were measured by enzyme immunoassay using Vector-Best commercial kits.

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

RESULTS

At the initial stage of the study we developed optimal conditions for obtaining AG-reactive (vaccinal) T cells, subsequently used for immunotherapy. Using the above method we obtained polyclonal population of T cells reactive to joint AG (T cell specimens of 7 patients with RA were studied). Proliferation of vaccinal T cells in the presence of cartilaginous and synovial AG was 1.8-2 times higher than in the control ($p<0.01$; Fig. 1). Cytofluorometric marker analysis of these T cell populations indicated that the percent content of CD3$^+$, CD4$^+$, CD8$^+$, CD16$^+$, and CD20$^+$-cells in them was 90-93, 55-62, 25-35, 3-9, and 2-3%, respectively.

Initially low anti-idiotypic proliferative response of MNC from 9 patients to vaccinal T cells after 4 vaccinations 3-fold surpassed the control level ($p<0.01$; Fig. 2).

Strains of T cells reactive to antigen-specific vaccinal T cells were obtained from 5 vaccinated patients. Cells of these strains 2-fold suppressed T cell proliferation induced by joint AG (Fig. 3).

The proliferative response of MNC (evaluated in 12 patients) to cartilaginous and synovial AG decreased significantly as a result of immunotherapy in comparison with the pre-treatment level ($p<0.01$; Fig. 4). On the other hand, significant and stable reduction of plasma IFN-γ level and an increase in IL-4 level ($p<0.01$; Fig. 5) were observed 6 months after vaccine therapy.

Standard treatment of autoimmune diseases is based on the use of hormones, cytostatics, and other agents with nonspecific immunosuppressor activity. This treatment impairs the immunity in general and is associated with high risk of serious side effects. The obvious advantage of T cell vaccine therapy is its selective direction to inactivation of lymphocytes responsible for the development of autoimmune process [6,12]. This treatment technology activates the immune memory mechanism, which seems to produce a long-lasting clinical effect.

On the whole, these data suggest that polyclonal T cell vaccine induces immune response selectively directed against autoantigen-reactive T cells in RA patients. This means that the concentration of idiotypic determinants in the resultant T cell vaccine is sufficient for induction of an effective anti-idiotypic immune response. Our technology for preparation of T cell vaccine has obvious advantages in comparison with the methods previously used for generation of anti-idiotypic response [4,14]. This technology does not include long-term, difficult, and expensive procedure of T cell cloning. During the preparation of vaccinal T cell popu-

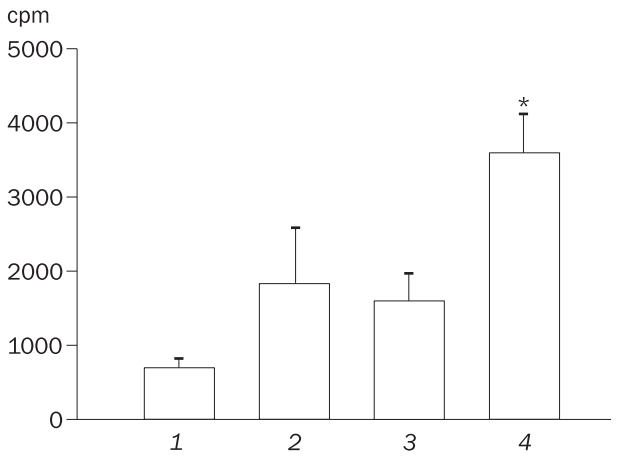

Fig. 2. Proliferative response of MNC from vaccinated patients to autologous T cells specific to different AG or nonspecifically activated. *1*) medium (control); *2*) nonspecifically activated T cells; *3*) myelin-reactive T cells; 4) vaccinal T cells.

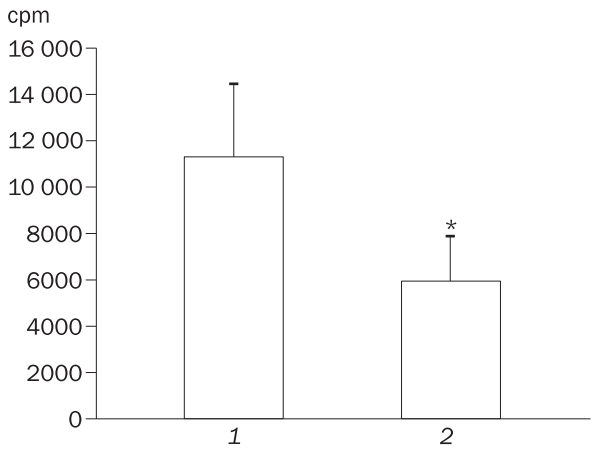

Fig. 3. Effects of anti-idiotypic T cells obtained from vaccinated patients on proliferation of AG-reactive T cells. *1*) culturing without AG (control); *2*) culturing with anti-idiotypic T cells.

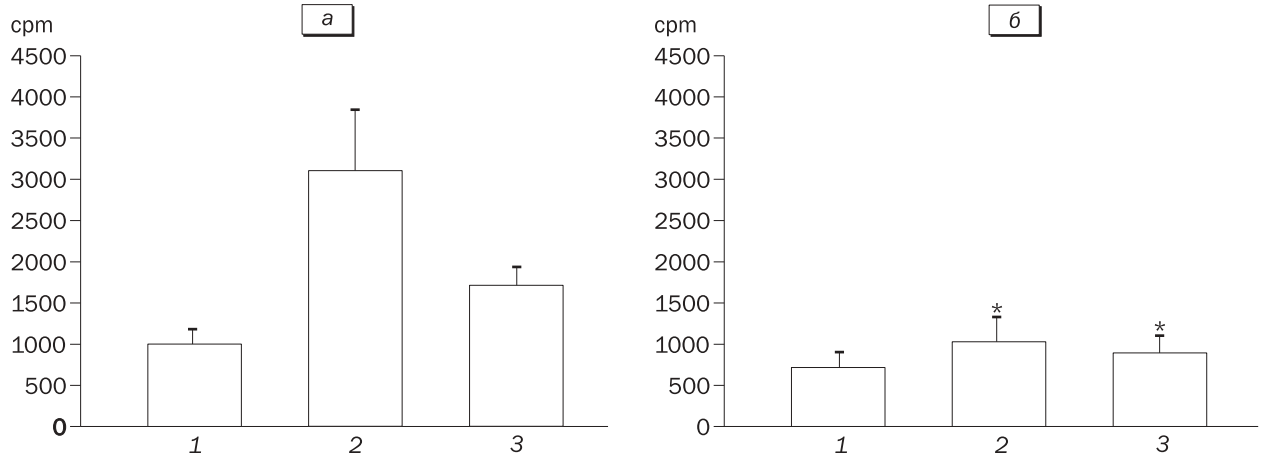

Fig. 4. Proliferative response of MNC from RA patients to cartilaginous and synovial joint AG before (*a*) and 1 year after treatment (*b*). *1*) MNC; *2*) MNC with cartilaginous AG; *3*) MNC with synovial AG.

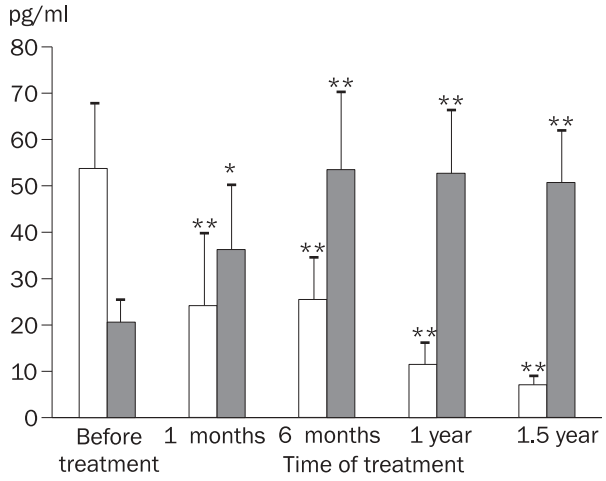

Fig. 5. Dynamics of IFN-γ (light bars) and IL-4 content (dark bars) in the plasma of patients during treatment. $^{**}p<0.01$ compared to the control.

lation, the cells most reactive to autoantigen have selective advantages at the first stage of culturing. Hence, the anti-idiotypic immune response induced in the patient should be aimed primarily against the most autoreactive cells which predominate in the vaccine and are most likely to be involved in the development of the autoimmune process.

The immunological efficiency of T cell vaccination can be seen not only from a significant reduction of proliferative immunoreactivity of patients' MNC aimed against joint AG, but also from changed concentrations of IFN-γ (reduction) and IL-4 (elevation) detected in the plasma of vaccinated patients. This indicates restructuring of the immune system, presumably aimed at inhibition of the immunopathological process. It is assumed that IFN-γ produced by type 1 T-helpers plays the main part in the involvement of macrophages and other immunocompetent cells in pathological inflammation in RA, while IL-4 produced by type 2 T-helpers can reduce this IFN-γ-mediated effect and hence, exhibits an antiinflammatory effect.

At present we carry out pilot clinical studies on evaluation of side effects, immediate and remote results of treatment of RA patients by autologous T cell vaccine. The fact that this treatment is safe causes no doubt. Not a single complication was detected during the period of observation. It is not yet time to make conclusions on the efficiency of this therapeutic method. We supposed that T cell vaccination could be most effective in patients receiving no immunosuppressor therapy. But according to our preliminary data, this treatment can be effective not only at the early, but also at late stages of the disease in patients receiving basic therapy.

REFERENCES

1. E. L. Nasonov, *Revmatologiya*, **10**, No. 6, 294-301 (2002).
2. I. P. Ivanova, V. I. Seledtsov, N. V. Banul, *et al.*, *Med. Immunol.*, **7**, No. 1, 27-32 (2005).
3. J. Correale, B. Lund, M. McMillan, *et al.*, *J. Neuroimmunol.*, **107**, No. 2, 130-139 (2000).
4. N. Hellings, J. Raus, and P. Stinissen, *Autoimmun. Rev.*, **3**, No. 4, 267-275 (2004).
5. G. Hermans, R. Medaer, J. Raus, and P. Stinissen, *J. Neuroimmunol.*, **102**, No. 1, 79-84 (2000).
6. A. Honda, A. Ametani, T. Matsumoto, *et al.*, *Int. Immunol.*, **16**, No. 5, 737-745 (2004).
7. J. M. van Laar, A. M. Miltenburg, M. J. Verdonk, *et al.*, *J. Autoimmun.*, **6**, No. 2, 159-167 (1993).
8. L. W. Moreland, L. W. Heck, W. J. Koopman, *et al.*, *J. Rheumatol.*, **23**, No. 8, 1353-1362 (1996).
9. L. W. Moreland, E. E. Morgan, T. C. Adamson 3rd, *et al.*, *Arthritis Rheum.*, **41**, No. 11, 1919-1929 (1998).
10. J. B. Smith and M. K. Haynes, *Ann. Intern. Med.*, **136**, No. 12, 908-922 (2002).

11. I. Stamenkovic, M. Stegagno, K. A. Wright, *et al.*, *Proc. Natl. Acad. Sci. USA*, **85**, No. 4, 1179-1183 (1988).
12. P. Stinissen, J. Zhang, R. Medaer, *et al.*, *J. Neurosci. Res.*, **45**, No. 4, 500-511 (1996).
13. S. C. Strom and G. Michalopoulos, *Methods Enzymol.*, **82**, Pt. A, 544-555 (1982).
14. A. A. Vandenbark, E. Morgan, R. Bartholomew, *et al.*, *Neurochem. Res.*, **26**, No. 6, 713-730 (2001).
15. A. Vanderborght, P. Geusens, J. Raus, and P. Stinissen, *Semin. Arthr. Rheum.*, **31**, No. 3, 160-175 (2001).
16. Y. C. Zang, J. Hong, M. V. Tejada-Simon, *et al.*, *Eur. J. Immunol.*, **30**, No. 3, 908-913 (2000).