

# Original treatment methods of frequently recurrent chronic herpetic infection caused by herpes virus type 1 and type 2

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## Abstract

*In the article we presented the results of clinical probation of new method of treatment of repeatedly recrudescing herpes simplex virus infection caused by types 1 and 2. Method of intradermal immunization with autoleukocytes was used in treatment of 32 patients (21 with herpes labialis and 11 with herpes genitalis) with frequent relapses – no less than 2-3 relapses a month during last 6 months (previous treatment with antiviral agents and immunostimulating/immunomodulating preparations failed). Leukocytes for immunization were isolated by sedimentation of heparinized blood at the temperature 37°C during 90-140 min. Blood plasma was centrifugated, then precipitated leukocytes were washed in cooled 0.9% NaCl solution. Leukocytes (in concentration 10-15 × 10<sup>6</sup> cells/ml) were injected with syringe intradermally in 10-12 skin sites of back between scapulas.*

*Stable remission occurred in 80.95% of patients with herpes labialis (in 17 of 21 patients) and 72.73% of patients with herpes genitalis (in 8 of 11 patients). Duration of surveillance was 3 years.*

*Effectiveness of treatment was a result of combined effects of leukocytes injected intradermally on the lymphocyte mediated immune response. Virus-containing autoleukocytes increase antiviral surveillance causing in leukocytes generation immune response (suppression or cytotoxicity) directed on themselves. This method is recommended for treatment of patients with repeatedly recrudescing herpes simplex virus infection with antiviral treatment failure.*

**Key words:** recurrent chronic herpetic infection caused by herpes simplex virus types 1 and 2, treatment of recurrent chronic herpetic infection, herpes-containing autoreactive cells, vaccination.

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## Introduction

Results of numerous researches confirm that 90% of adults are infected with herpes viruses, most often it is herpetic infection caused by herpes virus 1 and 2 types. According to the World Health Organization mortality rate, due to herpes virus infections is 15.8%; it is second in frequency after influenza (35.8%) [1-3]. It is known that neural ganglia cell persistency is characteristic feature of herpes viruses [4]. However, relation of the virus with white blood cells has been set in the early 80<sup>th</sup> of the last century; blood cells were used as a culture to detect the virus and for further study of its reproduction features [5].

Persistency of virus in leukocytes is accompanied by decrease of their functional activity and promotes the devel-

opment of secondary immunodeficiency. Occurring on a background of immunodeficiency, herpes virus infection affects immunocytes and supports immunodeficiency condition. This fact allows to apply herpetic infection to immune system diseases [6, 7].

Nowadays, many antiviral drugs, principally nucleoside analogues (acyclovir, idoxuridine, ganciclovir, ribavirin, valacyclovir etc.), are used for treatment of herpetic infection. Vaccination is the alternative method to treat herpetic infection. Investigations of the herpetic vaccines elaboration had been started at the beginning of fifties of the 20<sup>th</sup> century in the USSR. Based on experiences of the skin tests, the vaccine was used as a course of intradermal injections of the virus inactivated by formalin. There are

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numerous inactivated antiherpetic vaccines which contain inactivated virions (patents of Russian Federation: No. 2014084, 1994; No. 2085582, 1997; No. 2085583, 1997; patents of the USA: No. 4816250, 1989; No. 5215745, 1993; No. 5602023, 1997). Vaccines containing a part of viral DNA and some other viral components have been developed (patent of the USA No. 6387376, 2002; No. 6471965, 2002; NL No. 9900034, 2003).

In Ukraine, mostly an inactivated liquid herpetic vaccine, produced by "BIOPHARMA", is used for the treatment of the herpetic infection. The vaccine is an antigen of herpes virus type 1 and type 2, obtained in the chicken embryo culture, inactivated by formalin and contains 40 mcg of gentamycine. It is used in remission, after 2 weeks of complete disappearance of the symptoms. However, effectiveness of the vaccine therapy remains insufficient, and, therefore, today a comprehensive therapy is recommended: a vaccine is administered concurrently with antiviral drugs, immunomodulators, interferons (or inducers of endogenous interferon). The main reason for lack of effectiveness of the vaccine therapy is that the herpes virus infection with frequent relapses occurs in patients with severe immunosuppression, predefined failure of different parts of the immune system, mainly cellular, and thus, a reduced reactivity to antigens of the virus. The use of herpes virus vaccine is substantially restricted by frequent vaccine-induced relapses [8].

The aim of our study was to create more effective method of treatment. We proceeded from the fact that this might be achieved by strengthening the immune system response, mainly cellular immune response, triggering antiviral immune surveillance.

The considered method of treatment of frequently recurrent herpetic infection caused by herpes simplex virus type 1 and type 2 includes intradermal injection of pathogen. We used white blood cells, obtained from the patient's peripheral venous blood as the virus-containing material. We considered that the hematogenous spread plays an important part in prognosis for herpetic infection predetermined by significant virus predisposing to erythrocytes, and interaction with leukocytes which contain a large pathogen concentration [9].

## Material and methods

### Patients

The new method of treatment was used in 32 adult patients, age 18-70, including 17 women (53.13%) and 15 men (46.87%). All of them had frequent relapses of herpetic infection: more than 2-3 times per month during last six months. 21 patients had labial herpes (65.63%), 11 patients had genital herpes (34.37%). Among those with labial herpes 11 were men (52.38%), 10 women (47.62%), among patients with genital herpes – 4 vs. 7; 36.36% vs. 63.64% respectively,  $p = 0.1985$ .

Patient's blood samples were examined for the presence of anti-HIV using Labsystems 3<sup>rd</sup> generation HIV EIA (Ani Labsystems Ltd. Oy; Vantaa, Finland) by the IFA test system (positive patients were not included in the study). CD4 level of all patients were greater  $0.5 \times 10^9/l$  in  $mm^3$  (investigation conducted in Synevo-Ukraine laboratory in Lviv by flow cytometry using flow cytometer "Eclipse XL", Beckman Coulter, France). Treatment was compatible with local ethics committee.

### Blood sampling

Patient's blood samples have been taken in amount 50-60 ml into the vial with *Heparinum natricum* (Heparini-Richter) at the rate 50 units of heparin for 10 ml of blood.

### Leukocytes isolation

Leukocytes were isolated by sedimentation of heparinized blood at the temperature 37°C during 90-140 minutes. Blood samples were poured into 5-6 tubes 10 ml each and placed on rack at the angle 45° to speed up leukocytes isolation.

The light part of settled blood was aspirated and moved into the other test tube. Cells were precipitated at 200 xg during 5 minutes. Centrifugation was performed at 37°C to avoid possible loss of cryoglobulins (according to our data, cryoglobulinemia occurs in 17% patients with recurrent herpetic infection). The supernatant was removed; the sediment was washed with 10 ml 0.9% NaCl solution cooled to +4-5°C (washing out was conducted twice). After washing, 1-1.5 ml of isotonic NaCl solution were added into the precipitated leukocytes and carefully mixed, autoleukocytes were used for patients' immunization (white blood cells count  $10-15 \times 10^6$  cells/ml).

The washed leukocytes cannot be stored. The procedure should be carried out not later than 15-20 minutes after preparation of cell suspension. Cells are carefully but thoroughly mixed in an isotonic solution and injected with the use of syringe intradermally into the skin of the back between scapulas (the control is the formation of "lemon peel"). The distance between the injection sites should be 3-4 cm. The total amount is 10-12 injections. In case of 2 or more relapses of herpes, even mild, after intradermal autoleukocytes injections, repeated immunization is required.

## Results and discussion

We considered that it was possible to include into the group with stable remission one patient – a 56-year-old man with relapse of labial herpes after 19 months of an isoimmunization. Hypothermia and severe stress preceded this relapse. In the next three years, the patient had no clinical manifestation of herpes virus infection.

Isoimmunization had a positive effect in patients who failed to achieve stable (3-years) remission. Thus, in 4 of

7 patients (57.14%) the number of relapses decreased, their course became milder, but in 5-6 months frequency of relapses increased and in 11-12 months returned to the initial level. In two patients from that group in the next 3 years only 2-3 relapses per year occurred. Only in one of 32 patient it wasn't possible to achieve even a temporary improvement.

The clinical form of herpetic infection in some degree influenced the effectiveness of treatment. Thus, the stable remission as a result of treatment occurred in 17 of 21 patients (80.95%) with labial herpes and in 8 of 11 patients with genital herpes (72.73%);  $p = 0.6096$ .

The advantage of the proposed method is the combined effect of virus-containing autoreactive leukocytes on different parts of immunological homeostasis because of its wide range of therapeutic effects. Thus, after autoleukocytes intradermal injections significant decrease of the autoimmune processes occurs (in our research laboratory it was manifested by decrease of serum anti-nuclear and anti-neutrophil antibodies). In Ukraine this method is used for treatment of autoimmune disorders in patients with chronic viral hepatitis [10], systemic vasculitis [11] and cryoglobulinemias [12]. In recent years, the autoreactive T-cell vaccines (or TCR, as well as their fragments) are used for treatment of autoimmune diseases, multiple sclerosis (cells are isolated from CSF), inflammatory joint diseases (leukocytes are isolated from the synovial fluid) [13].

The assumption for using this method in treatment of autoimmune disorders is the fact that autoimmune diseases can be transmitted passively with autoreactive T-lymphocytes. Therefore, the autoreactive lymphocytes which preserved their immunogenicity are used for treatment of autoimmune diseases by analogy with vaccines used for preventing infectious diseases. The mechanism of the effects of these vaccines action is attributed to the fact that the injection of autoreactive cells creates a condition of the lymphocyte mediated immune response activity due to the cytotoxic lymphocytes generation. The Jerne grid correction – an anti-idiotypic regulation of the immune response evidenced by increased serum anti-idiotypic antibodies after leukocyte immunization is also important. Another mechanism of this immunization includes combinations of: CD3+, CD4+, CD25+ and CD3+, CD8+, CD28+ T-lymphocytes activation, along with the Fc-receptors block, glycoprotein and lectin receptors on B-lymphocytes [13]. In our case, virus-containing autoleukocytes increase antiviral surveillance causing in leukocytes generation immune response (suppression or cytotoxicity) directed on themselves.

## Conclusions

The intradermal immunization of herpes-virus-containing autoleukocytes enhances the antiviral surveillance and leads to suppression of infection caused by her-

pes virus type 1 and type 2 providing the large impact on immune system.

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*The authors declare no conflict of interest.*

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