Intradermal Autoleukocyte Immunization-Personified Method of Cell Therapy

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Abstract

The article is a review of our own investigation on intradermal immunization with inactivated autoleukocytes as a personified method of cell therapy. The method is used for treatment of autoimmune processes and stimulation of antiviral immunity. The obtained results prove that autoleukocyte immunization inhibits autoimmune processes, particularly the ones typical for chronic viral hepatitis: synthesis of antinuclear antibodies, antibodies to thyroglobulin and thyroperoxidase, cryoglobulins is reduced. Slowing of anti-thyroid immune process decreases threat of thyroiditis development. Cold tolerance improves; signs of systemic vasculitis, kidney insufficiency weaken or disappear; spermatogenesis indices become normal in men with idiopathic oligo- and azoospermia due to inhibition of synthesis of cryoglobulins. Thus, cryoglobulins of the 2nd and 3rd types were detected in a third of patients with idiopathic oligo- and azoospermia. Under the influence of immunization amount of spermatozoa increased to 20 ml/ml in most patients (85.71%), their motility and percentage of normal shapes improved. Autoleukocyte immunization promoted a significant decrease in high activity level of pro-inflammatory TNF-alpha cytokine [13, 14]; 4) use of autoleukocyte immunization on AIDS course have not been studied completely, the obtained results indicate perspectives for further conduct of appropriate research.

Immune tolerance with autoleukocytes, received from damaged cells, is recommended for treatment of other autoimmune conditions. Thus, in multiple sclerosis, autoreactive lymphocytes are isolated from cerebrospinal fluid, and in rheumatoid arthritis – from the inflamed joint [6].

However, for immune therapy of other autoimmune diseases, for example, multiple sclerosis or rheumatoid arthritis, various methods of manufacturing of T-cell vaccines, intended for intensification of immunological tolerance to one’s own antigens, are more often used. These methods usually imply cultivation of isolated lymphocytes for increasing of their amount, often with addition of certain antigen to culture medium to provide antigen-resistant properties [7-9].

Receptors of T-lymphocytes or their fragments may also be used. They provoke response in the form of lymphocyte generations, which have suppressive or cytotoxic action. However, this therapy has certain limitations, it is little effective in diseases with wide spectrum of autoreactive receptors [6]. Besides, methods of manufacturing of such vaccines are complex and expensive, which considerably restricts their use.

The aim of our research was trial of intradermal immunization with inactivated autoleukocytes in several directions: 1) for treatment of autoimmune processes, particularly a part of extra-hepatic manifestations in patients with chronic viral hepatitis C [10]; 2) for inhibition of cryoglobulin synthesis in patients with idiopathic oligoazospermia [11, 12]; 3) for decrease of high efficacy of pro-inflammatory cytokine – TNF-alpha [13, 14]; 4) use of autoleukocytes, obtained from patients with viral infection, as a curative vaccine [15, 16].

Technique Of Autoleukocyte Immunization

Leukocytes are isolated by means of precipitating patient’s heparinized venous blood. For this purpose, 40-50 ml of venous blood is taken with a preliminarily warmed syringe (37°C) into a heparin-containing vial (Heparini-Richter) with 50 units of heparin per 10 ml of blood (the volume depends on the number of leukocytes in 1 ml of blood). Then the blood is poured into test

Keywords: Intradermal autoleukocyte immunization; Autimmune processes; Cryoglobulinemia; Anti-thyroid process; Idiopathic oligoazospermia; TNF-alpha; Recurrent chronic herpes infection

The first report on this issue was in 2000 [21].

Viral load. This article highlights a new approach to therapy of such processes, especially cryoglobulinemia, is not sufficiently elaborated. Usually, it implies, especially in systemic vasculitis, mixed cryoglobulinemia is detected in almost half of patients with chronic hepatitis C, and HCV RNA is present in majority of patients with cryoglobulinemic mesangiocapillary glomerulonephritis and systemic vasculitis with purpura and nephropathy [17-20].

Treatment Of Autoimmune Processes In Patients With Chronic Viral Hepatitis

Chronic hepatitis C is known to be accompanied by different autoimmune processes. In pathogenesis of autoimmune reactions in chronic viral hepatitis an important role belongs to tropism of HCV to lymphocytes. It is also important that building of viral antigens into superficial cell membranes results in their changes and formation of combined antigens with properties of autoantigens. Besides, lymphotropic property of HCV is manifested by stimulation and activation of B-lymphocytes, resulting in polyclonal and monoclonal proliferation in bone marrow and liver. Synthesis of these cells of wide spectrum of antibodies, in particular, of monoclonal rheumatoid factor, is the basis for the development of cryoglobulinemia. Mixed cryoglobulinemia is detected in almost half of patients with chronic hepatitis C, and HCV RNA is present in majority of patients with cryoglobulinemic mesangiocapillary glomerulonephritis and systemic vasculitis with purpura and nephropathy [17-20].

Treatment of patients with chronic hepatitis C with autoimmune processes, especially cryoglobulinemia, is not sufficiently elaborated. Usually, it implies, especially in systemic vasculitis, use of glucocorticosteroid hormones and cytostatic agents, which are often unfavorable or contraindicated for patients with intensive viral load. This article highlights a new approach to therapy of such patients by means of intradermal autoleukocyte immunization [20].

Influence on autoimmune processes was estimated by various factors, particularly, according to amount of antinuclear antibodies (ANA) in patients with chronic hepatitis C. 1 genotype virus (degree of fibrosis 1-2). Totally 166 patients with ANA in titre 1-80 and higher were immunized. The age of patients was 18-65 years, among them 95 women (57.22%), 71 – men (42.78%). Patients were not subjected to antiviral therapy, also medications, which can influence autoimmune processes, were not administered. ANA were determined before and in 10-12 days after immunization, further - in 1-2 months. It was established that amount of antibodies in blood serum considerably decreased in the examined patients as a result of immunization (Table 1).

Influence on autoimmune processes was estimated by many factors [22] in particular, according to content of antinuclear antibodies (ANA). It was established that after autoleukocyte immunization in most patients content of ANA in blood serum decreased, only in one female patient autoleukocyte immunization proved ineffective (Table 1).

Similar results were obtained in relation to other indices of autoimmune process. Thus, according to literature data, antithyroid antibodies are detected in 15-40% of patients with chronic hepatitis C. Though increased level of antibodies to autoantigens of the thyroid is not itself the reason for diagnostics of the thyroid impairment, it is an evidence of autoimmune process, which can lead to the development of thyroiditis. Especially dangerous is increase in antibodies to the thyroid antigens in patients with chronic hepatitis C, who receive interferon-therapy, since it intensifies autoimmune processes [23,24].

Ways of decrease in activity of cell-mediated and humoral immunities in relation to the thyroid antigens in patients without signs of thyroiditis are actually not elaborated. Thus, only monitoring is used concerning patients, who have increased level of antibodies to thyroid peroxidase and thyroglobulin, but there are no signs of the thyroid function impairment. But received data prove that autoleukocyte immunization has a positive influence on this autoimmune process (Table 2). The research was conducted in

<table>
<thead>
<tr>
<th>Number of the patients after being treated (ANA titres)</th>
<th>0</th>
<th>1:20</th>
<th>1:40</th>
<th>1:80</th>
<th>1:160</th>
<th>1:1280</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of the patients before being treated</td>
<td>1:80</td>
<td>34</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>1:160 – 1:320</td>
<td>1</td>
<td>39</td>
<td>52</td>
<td>15</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&gt;1:640</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1: Change of ANA titres in the patients with chronic hepatitis C treated by intradermal injection of autoleukocytes

<table>
<thead>
<tr>
<th></th>
<th>Patients without chronic hepatitis C, group 1 (n =28)</th>
<th>Patients with chronic hepatitis C and antiviral treatment, group 2 (n=22)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Decrease percentage in concentration of antibodies to thyroid peroxidase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-50 %</td>
<td>12</td>
<td>42.9</td>
<td>10</td>
</tr>
<tr>
<td>&gt;50%</td>
<td>9</td>
<td>32.1</td>
<td>4</td>
</tr>
<tr>
<td>100 %</td>
<td>7</td>
<td>25.0</td>
<td>0</td>
</tr>
<tr>
<td>Decrease percentage in concentration of antibodies to thyroglobulin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-50 %</td>
<td>14</td>
<td>50.0</td>
<td>10</td>
</tr>
<tr>
<td>&gt;50%</td>
<td>9</td>
<td>32.1</td>
<td>4</td>
</tr>
<tr>
<td>100 %</td>
<td>5</td>
<td>17.9</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2: Efficacy of single autoleukocyte immunization to reduce concentration of antibodies to thyroid antigens in patients without (group 1) and with (group 2) chronic hepatitis C. (*According Cochran-Armitage chi-square test for trend. Armitage, 1955) - squared test)
two groups: patients without chronic viral hepatitis C (group 1 – 28 patients: 23 women, 5 men; age 18-55 years) and patients with chronic viral hepatitis C (group 2 – 22 patients: 18 women, 4 men; age 18-55 years) were examined. Other therapy for inhibition of autoimmune processes was not conducted.

From data in table 2 it is seen that after single autoleukocyte immunization concentration of antibodies to thyroid peroxidase and thyroglobulin decreased in all patients, antibodies to thyroid peroxidase not detected in 25% of patients and antibodies to thyroglobulin not detected in 17.9% of patients from group 1.

Results of inhibition of autoimmune process in patients with chronic hepatitis C during antiviral therapy with interferon considerably differed from the results in patients of the 1st group - efficacy of autoimmune process inhibition was significantly lower [25,26].

Thus, the suggested method enables to decrease threat of autoimmune thyroiditis development, even in patients receiving interferon therapy, which often intensifies autoimmune processes.

The method also proved effective in the treatment of other autoimmune processes, in particular, cryoglobulinemia. Thus, the content of cryoglobulins of the 2nd and 3rd types decreased by 40% and more in majority of patients (280 from 310; 90.32%) after single immunization in 10-12 days. Efficacy of the procedure according to patients’ comments (improvement of general condition, cold tolerance, etc.) reached 90%. Stability of the achieved effect had individual character and ranged from several weeks to one year and longer. In some patients, especially in the period of antiviral therapy, autoleukocyte immunization was conducted repeatedly, depending on peculiarities of clinical course and duration of the achieved result.

Concerning influence on the course of systemic cryoglobulinemic vasculitis, efficacy of therapy depended on the severity of the disease. Thus, we did not manage to save a patient’s life with severe necrotic hemorrhagic vasculitis (over 15% of skin was damaged) and non-Hodgkin’s lymphoma. In patients with easier course of vasculitis, especially in case of past infection, efficacy of treatment approximated 90% [27,28]. The best effect was achieved in patients with moderate signs of vasculitis: purpura condition, arthralgia, primary manifestations of sensory neuropathy.

Therapy proved less effective in patients with severe damage of kidneys (cryoglobulinemic mesangiocapillary glomerulonephritis), though amount of cryoglobulins in the blood also decreased in these patients.

Attention should be paid to suggested method of cryoglobulinemia treatment, especially in patients with chronic hepatitis C, because treatment of cryoglobulinemic syndrome has not been sufficiently elaborated. Complication is associated with many factors; among them the following ones have an important meaning: 1) polymorphism of clinical manifestations, caused by simultaneous damage to various organs and systems; 2) presence of other, except cryoglobulinemia, autoimmune processes, which often occur in patients with chronic viral hepatitis; 3) viral damage to the liver, leading to disturbance of metabolism processes and restricting use of medications; 4) insufficient efficacy of etiotropic (antiviral) interferon therapy, which also contributes to worsening of autoimmune pathology. It should be mentioned that all these conditions have pathogenic connection, which is not always taken into consideration.

There is no generally accepted scheme of cryoglobulinemia treatment in patients with viral hepatitis. Antiviral therapy is considered to be an effective method of cryoglobulinemia treatment, because hepatitis virus is a trigger factor in formation of pathological cold-shock proteins. However, in some patients cryoglobulinemia remains after achievement of a stable virological response as a result of antiviral therapy.

Decrease in amount of cryoglobulins has a positive impact on a patient’s condition with chronic hepatitis C. Thus, hair loss, caused by interferon therapy, signs of arthralgia, signs of depression decreased or disappeared in some patients.

According to our results, presence of cryoglobulins in blood serum may also be the cause of idiopathic oligo- and azoospermia [11,12] (unfortunately detection of cryoglobulins is not included into examination algorithm of these patients). According to our data, reduction of cryoglobulin synthesis improves indices of spermogram. Thus, among 55 examined men with idiopathic oligo- and azoospermia, cryoglobulins were detected in 19 (34.55%), usually of the 2nd and 3rd types (cryoglobulinemia of the 1st type was diagnosed only in one person).

In patients undergoing autoleukocyte immunization, improvement of spermatogenesis indices was observed. Thus, in 12 men (85.71%) from 14, showing decrease in concentration of cryoglobulins by 65%-100% as a result of treatment, amount of spermatoza increased to 20 ml/ml and higher [11,12].

A positive influence of intradermal immunization with inactivated autoleukocytes on the course of autoimmune hepatitis (AIH) was also established. This method was tested for the treatment of 12 patients, for whom basic standard scheme of immunosuppression proved ineffective or was contraindicated. As a result of autoleukocyte immunization, the condition of ten patients significantly improved, remission was observed, and in four of them it was durable. In two patients remission did not occur, but improvement of psycho-emotional condition was observed [29].

Positive results of autoleukocyte immunization of patients with systemic vasculitis and other autoimmune processes enable to suggest the influence of the procedure on the condition of pro-inflammatory cytokines. It became the basis for trial of autoleukocyte immunization as a method of decreasing high TNF level in blood serum. A group of patients with psoriasis and high level of cytokine was chosen for investigation of possible influence of immunization on TNF synthesis. The group included 24 patients aged 18-58 years [14]. Peculiarities of psoriasis clinical picture were not taken into consideration; only patients with high level of TNF-alpha were chosen.

Decrease in TNF-alpha content occurred in all patients (100%). In most of them (16 from 24; 66.7%) the level of pro-inflammatory cytokine after immunization became normal. However, in some patients, despite a considerable reduction, its content was still high. In such cases immunization was repeated. In the majority of patients with low level of TNF-alpha (in 6 from 9) autoleukocyte immunization did not influence its synthesis, nevertheless, in three patients a moderate increase in its content occurred [14]. The latter enables to suggest that influence of intradermal autoleukocyte immunization on TNF-alpha synthesis may depend on the condition of immunocompetent cells and correlation of regulatory cytokines (pro-inflammatory and anti-inflammatory) in a patient’s blood.

**Autoleukocyte Immunization as a Means of Treatment of Chronic, Frequently Recurrent Herpes Infection**

Reproduction of simplex herpes virus is known to occur in polymorphonuclear leukocytes and monocytes [30,31], thus, in our opinion, blood autoleukocytes may be used as a virus-containing material.
Immunization of patients with frequently recurrent herpes, which was not almost influenced by combined antiviral and immunomodulation therapy. Autoleukocyte immunization was commenced not earlier than three months after cessation of antiviral therapy. Antiviral and immunomodulation medications were not administered during immunization and monitoring periods. Immunization of patients with frequently recurrent herpes, which was not almost influenced by combined antiviral and immunomodulation therapy, was conducted on the 5-7th day after temporary cessation of the process. Clinical monitoring for three years showed that 22 patients (from 32; 68.75%) had durable remission (up to 3 years - monitoring period). One-two relapses were observed in three patients (9.37%) in 1-3 months after immunization, but in a mild form, relapses did not occur after repeated immunization. Thus, permanent remission was achieved in 78.12% (68.75% + 9.37%) of patients. In four patients (12.5%) incidence of relapses decreased, but permanent remission was not achieved.

Discussion

Efficacy of autoleukocyte immunization has not been studied completely. However, it can be explained by complex influence of autoreactive cells on mechanisms of immune response. T-lymphocytes are known to passively transmit autoimmune diseases. Presence of transmitting factor in lymphocytes makes such immunization similar to vaccination for prevention of infectious diseases. Thus, in case of intradermal injection, autoreactive cells may provoke condition of activity of cellular lymphocyte-mediated immune response by generation of cytotoxic leukocytes. The process of correction of Jerne's network – idiotype-anti-idiotype regulation of the immune response is also important, which is confirmed by increase in anti-idiotype antibodies in a patient's blood serum after leukocyte immunization. One more mechanism may be involved in this manipulation: activation of CD3+, CD8+, CD25+ lymphocytes, as well as CD3+, CD4+, CD28+ lymphocytes, simultaneously with blockage of Fc-receptors and glycoprotein, lectin receptors on B-lymphocytes [6]. It has been established that lymphocyte immunization induces not only T-, but also B-cell reactions, thus promoting synthesis of antibodies, which influence autoreactive T-cell clones inhibiting them. It is also considered that leukocyte immunization leads to generation of CD4+ T cells, producing anti-inflammatory cytokines IL-4 and IL-8, which has a positive influence on autoimmune process inhibition [32-34].

Determined ability of autoleukocyte immunization of inhibiting excessive synthesis of pro-inflammatory TNF-alpha cytokine also has an important clinical significance. Theoretically, it is associated with increasing Th2 level and, accordingly, normalization of Th1/Th2. However, it requires further special investigation.

It is known that the cells, isolated from body tissues, subjected even to the simplest, non-traumatic manipulations, become foreign to the body to some extent. Likely, it intensifies immunological response, possibly by means of activation of non-specific immunity factors (complement system, macrophages, etc.). Cross-reaction by means of partial identity of antigenic structures is also important.

Anyway, rapid decrease in concentration of antibodies may be explained only by their interaction with immunizing cells. It is also proved by in vitro investigations that ANA react with leukocytes; for detection of certain other autoantibodies, use of neutrophils fixed with ethanol as a substrate is possible. However, remote results of treatment cannot be explained by interaction with autodies. In some patients, who showed a rapid and effective response, not only signs of skin vasculitis disappeared, but also “old” skin pigmentation decreased. The latter is difficult to explain by the influence on the immune system. It is known that slight amount of stem cells is in peripheral blood. However, in case of their intradermal injection together with lymphocytes, influence of these cells on skin regeneration is likely, because skin renovation, in our opinion, cannot be explained by other factors. The level of leukocyte loading with antigens of a disease agent also has an important meaning. Immunization with leukocytes, which contain virus and its antigens, may intensify virological surveillance. It is confirmed by the efficacy of treatment of frequently recurrent chronic herpes. Influence of viral load on the course of other diseases requires further investigation.

Conclusions

Intradermal immunization with inactivated autoleukocytes decreases activity of various autoimmune processes, particularly to the thyroid proteins, inhibits synthesis of cryoglobulins, leads to improvements of spermogram indices in some patients with idiopathic impairment of spermatogenesis. Autoleukocyte immunization has a positive influence on the course of autoimmune hepatitis, promotes reduction of extracellular manifestations of chronic hepatitis C, even in patients during antiviral therapy.

Intradermal autoleukocyte immunization has a positive impact on the condition of frequently recurrent herpes of the 1st and 2nd types. Expediency of administration of such immunization as a curative vaccine in other viral infections requires further investigation.

According to our data, intradermal autoleukocyte immunization inhibits excess synthesis of pro-inflammatory TNF-alpha cytokine. Thus, it is expedient to investigate possibility of administration of autoleukocyte immunization for the treatment of such diseases as psoriasis and rheumatoid arthritis.

Conflict of Interest

All authors have no conflicts of interests to disclose.

References

