REVIEW ARTICLE

CONSIDERATIONS FOR INTADERMAL APPLICATION OF IMMUNIZATION WITH NATIVE AUTOLEUKOCYTES IN MEDICINE

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ABSTRACT

The aim: Analyzing literature sources, to assess possibilities of using the method of intradermal immunization with native autoleukocytes to treat different diseases, to investigate the areas of usage, efficacy and expediency of the technique in clinical practice.

Materials and methods: Analysis of literature sources associated with intradermal immunization with native autoleukocytes.

Conclusions: The possibilities of using the method of intradermal immunization with native autoleukocytes in the treatment of various diseases are considered in the literature review. Intradermal immunization with autoleukocytes is one of the methods of personalized medicine. The application of the method results in normalization of the immune system condition as well as suppression of autoimmune and inflammatory processes. It also reduces the synthesis of pro-inflammatory cytokines and strengthens cellular antiviral immunity in a number of viral infections. It is proved, in particular, that the method reduces the synthesis of cryoglobulins, the formation of antithyroid antibodies, normalizes the level of tumor necrosis factor alpha, as well as reduces extrahepatic manifestations of chronic hepatitis and increases the effectiveness of antiviral therapy in patients with viral hepatitis B. Considering that immunization with native autoleukocytes has no contraindications, it can be used in many diseases.

KEY WORDS: antithyroid antibodies, antiviral therapy, cryoglobulinemia, antinuclear antibodies, immunization with autoleukocytes, tumour necrosis factor α

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INTRODUCTION

For complex treatment of a wide range of diseases, medicine involves the methods, which belong to cellular technologies [1-5]. The latter usually encompass a set of techniques that imply isolation of certain cells from the body, their cultivation for multiplication or imposing of antigen-presenting properties, frequently stimulation for the synthesis of metabolic products, in particular interleukins, with subsequent use of these cells or their synthesized substances [1]. Currently, application of stem cells is considered the main and the most perspective direction of the cell therapy [6-8].

However, we suggest considering intradermal immunization with native autoleukocytes (IINAL), isolated from peripheral venous blood, as one of the effective directions in the cell therapy [2, 3]. IINAL is the method of a personified cell therapy, which is a perspective method of treating various diseases, particularly autoimmune ones. Presence of different transmission factors in lymphocytes makes this procedure (immunization) similar to a vaccine by its mechanism of action [1]. Besides, replication of viruses occurs in leukocytes in a number of viral infections, thus, they can be used as individual virus-containing material, which definitely distinguishes this method from standard therapeutic vaccines due to various mutations of a virus [4].

THE AIM

Analyzing literature sources pertaining to intradermal immunization with native autoleukocytes, to investigate the areas of usage, efficacy and expediency of the technique in clinical practice.

MATERIALS AND METHODS

Analysis of literature sources associated with intradermal immunization with native autoleukocytes.

REVIEW AND DISCUSSION

Peculiarities of the method of intradermal immunization with autoleukocytes: 1) isolation of leukocytes from heparinized venous blood by its precipitation

for a few hours at t +37°C; 2) intradermal injection of leukocytes into the back region (between scapulae) into 8-10 points. This technique is described in detail in medical literature [2-5].

At present, the efficacy of such procedure has been proven for the treatment of many diseases by stimulating (or normalizing) the immune system, in particular, elimination of allergic conditions, decrease in intensity of inflammatory processes, as well as an antiviral vaccine. Some variations of curative therapy by means of intradermal immunization with native autoleukocytes will be discussed in the article.

I. APPLICATION OF INTRADERMAL IMMUNIZATION WITH NATIVE AUTOLEUKOCYTES IN THE TREATMENT OF AUTOIMMUNE PROCESSES:

1. Impact of immunization with autoleukocytes on cryoglobulinemia syndrome.

Cryoglobulinemia syndrome is an immune-dependent condition caused by the presence of pathological proteins – cryoglobulins in the blood serum. They belong to immunoglobulins and can form insoluble complexes (cryoprecipitate) in temperature reduction below 37^0C [5].

Cryoglobulinemia syndrome is known to be characterized by a variety of clinical manifestations, which depend on the degree and localization of affected vessels. Clinical symptoms of cryoglobulinemia (inflammation and necrosis of the vascular wall) can be manifested by damage to the following organs (systems): skin and mucosa, bones and joints, muscles, blood, immune system, ear, nose and throat, lungs, heart and vessels, digestive organs, kidneys, nervous system, eyes etc. [5].

According to our findings, intradermal immunization with autoleukocytes can be efficiently used for the treatment of cryoglobulinemia. It has been established that even after one procedure, the level of cryoglobulins decreases by 40% and more in 82% of patients. In some cases, immunization should be performed several times for better treatment efficacy [6].

A wide range of pathological processes, caused by cryoglobulinemia, indicates that it is expedient to use IINAL in numerous diseases provoked by inflammation and necrosis of the vascular wall.

Thus, for instance, vascular pathology is known to be a common cause of spermatogenesis impairment. Thus, patients with impaired synthesis of spermatozoa were examined for cryoglobulins, and we studied possible association between cryoglobulinemia and idiopathic oligo- and asthenozoospermia. It was established in these investigations that over the third of men with idiopathic infertility had elevated level of cold-precipitating proteins in the blood – cryoglobulins of the second and the third types. Inhibition of cryoglobulin synthesis by the IINAL method had a positive impact on spermatogenesis in the majority of patients with idiopathic oligoasthenoteratozoospermia, and resulted in normalization of spermogram indices or improvement of conditions for conduction of assisted reproductive technologies for couples [7-10].

This technique is also used in chronic viral hepatitis, since numerous extrahepatic signs of chronic viral hepatitis (especially chronic hepatitis C) are due to cryoglobulinemia [11]. Thus, intradermal immunization with native autoleukocytes inhibits synthesis of cold-precipitating proteins and has a positive impact on extrahepatic signs of chronic viral hepatitis, particularly glomerulonephritis, arthralgia, skin vasculitis and other manifestations of an elevated level of cold-precipitating proteins [3, 11, 12].

Although efficient antiviral therapy in chronic hepatitis C promotes normalization of the immune functions, cessation (or reduction) of immunopathological processes, signs of cryoglobulinemia syndrome can remain in many patients after elimination of the virus. Thus, for example, fatigue was observed in some patients with chronic hepatitis C with general weakness (201 patient) after achievement of a stable virological response (in 107 individuals; 53.23%). Cryoglobulinemia was observed in most patients (93%) with fatigue after successful completion of antiviral therapy [13]. Thus, expediency of cryoglobulin synthesis cessation by means of immunization with autoleukocytes often appears even after recovery from chronic hepatitis C.

However, in chronic hepatitis B, unlike in chronic hepatitis C, vascular damage is usually caused by harmful action of circulating immune complexes, which contain virus antigens and antibodies. Nevertheless, a certain fraction of vasculitis can be referred to cryoglobulinemic ones. In our previous investigations on peculiarities of different forms of chronic hepatitis B in Western region of Ukraine, it was established that in most patients with HBeAg-negative HBV DNA-positive, systemic vasculitis developed along with cryoglobulinemia (in seven out of eight patients with vasculitis signs), whereas in the group of patients with HbeAg-positive hepatitis, it was observed only in one patient with vasculitis out of 10 [14]. Clinically, cryoglobulenemic syndrome in patients with HBV is most frequently manifested by vasculitis, acrocyanosis, polyarthralgia, restricted movements, slight signs of sensory polyneuropathy, kidney damage, increased sensitivity to cold. Thus, in case of such extrahepatic signs, intradermal immunization with autoleukocytes is also indicated [2, 3, 5].

It has been established that treatment of cryoglobulinemia by IINAL technique is also highly efficient for patients with chronic toxoplasmosis with impairment of hypothalamic region. Injection of own leukocytes to such patients resulted in a significant reduction of the level of cold-precipitating proteins in the blood, considerable improvement of patients' general condition, and in most cases it was characterized by a stable remission [15].

Thus, the reported data prove the expediency of inhibition of cryoglobulin synthesis by intradermal immunization, since the method is efficient and does not have contraindications.

2. Impact of immunization with autoleukocytes on antinuclear antibodies.

Detecting the level of antinuclear antibodies (ANA) is a laboratory test used for diagnosis of autoimmune diseases. ANA are present in the majority of patients with diffuse diseases of the connective tissue, can be detected in infectious diseases (especially chronic viral hepatitis) etc. After improvement of a patients' condition, ANA indices significantly decrease, even become normal. Thus, in the patients treated by means of immunization with autoleukocytes, ANA level was compared before and after the procedure as one of the methods for assessment of IINAL efficacy in patients with autoimmune processes, particularly in combination with HCV [3]. In a group of 20 patients, the initial ANA values were as follows: in 4 patients (20%) they were within the normal range (1:80), in 8 patients (40%) – 1:160, in 6 patients (30%) – 1:320 and 2 (5%) cases of antibody titers equaled 1:640 and 1:1280, respectively. Among patients with elevated ANA titers (16 people), the index decreased in almost all patients (15; 93.75%) in 28-72 hours after immunization with autoleukocytes. In 2 patients (12.5%), a 16-fold decrease was observed, in 4 - the titer fell 8-fold (25%), and in 9 (the largest number of observations; 56.25%) a 4-fold decrease in the level of antibodies was registered. Only in 1 patient (6.25%) with the highest ANA titer (1:1280), the level of antibodies remained at the previous level after immunization with autoleukocytes. It should be noted that as a result of a single intradermal immunization with native autoleukocytes, the ANA titer did not just decrease, but returned to the normal range in 14 out of 16 patients (87.5%) [2].

In other investigation of IINAL impact on ANA titres [11], the obtained results were similar, however, immunization with autoleukocytes proved ineffective in one patient, whose ANA titre increased from 1:80 to 1:1280 under the influence of antiviral therapy with pegylated interferon. After cessation of antiviral therapy, titre of antibodies spontaneously decreased to 1:640 and further remained at this level. However, even after single immunization, ANA were not detected already in 48 hours. Thus, this procedure also proved efficient in this observation.

3. Impact of immunization with autoleukocytes on recovery of tolerance to the thyroid antigens.

Data about IINAL impact on the synthesis of antibodies to the thyroid antigens, which was tested for the first time on patients with chronic hepatitis B and C, who often had thyroid peroxidase and thyroglobulin antibodies, were analyzed [16, 17].

Besides, 22 patients with laboratory signs of autoimmune thyroiditis were included in one of the studies on the renewal of tolerance to thyroid autoantigens. The levels of thyroid peroxidase antibodies (TPO) and thyroglobulin antibodies (TgAb) were assessed before and 10–12 days after a single autoleukocyte immunization. A decrease in the concentration of antibodies was detected in all immunized patients, and the group with a significant decrease in indices (by 50-100%) was the largest in number. This group in the study case of a decrease in the level of thyroid peroxidase antibodies included 8 patients (36.36%), and for thyroglobulin antibodies – 10 (45.45%) individuals. Some examined patients demonstrated a normalization of the indices: 6 cases (27.27%) in determining TPO level, and 3 cases (13.64%) in studying TgAb indicators [17].

Subsequently, decrease in the level of antibodies was significant in the majority of immunized individuals, however, these indices were individual, duration of the obtained results also differed. Thus, the number of procedures and period of recurrent immunization is determined individually for each case. However, a positive result of immunization with autoleukocytes in relation to the thyroid antigens has been confirmed [17]. It is of utmost importance as the risk of autoimmune thyroiditis development is reduced.

II. APPLICATION OF INTRADERMAL IMMUNIZATION WITH NATIVE AUTOLEUKOCYTES FOR REDUCTION OF INFLAMMATORY PROCESS INTENSITY.

Currently, a strategy of inflammatory processes therapy involves inhibition of high level of pro-inflammatory cytokines, in particular tumor necrosis factor alpha (TNF-alpha) by means of medicines, which block the formation of this cytokine or inhibit proliferation of Th-1-lymphocytes, producing TNF.

The drugs acting on inhibition and blockage of biological activity of TNF-α are used for the treatment of immune-mediated diseases, such as rheumatoid arthritis,

inflammatory diseases of the intestines and psoriasis. Clinical efficacy of such drugs is confirmed, however, inhibition of cytokine synthesis by means of antibodies to certain determinants of immunocompetent cells has a negative impact on immunity condition. Prolonged use of TNF-α inhibitors increases susceptibility to infectious diseases (or exacerbation of the existing ones). Considering these adverse manifestations, it is obvious that such methods are unfavorable. Therefore, IINAL was tested for inhibition of excessive TNF-α synthesis [18]. It has been established that this method has a positive impact on reduction of inflammatory process, particularly in patients with psoriasis [19].

Since the level of pro-inflammatory cytokine became normal almost in all patients and their condition improved after the procedure, the decision was made to test this method for reduction of TNF-α synthesis in patients with HBV [20]. Reduction of TNF-α in blood serum was also recorded in examined patients due to immunization. Their overall condition improved, which was manifested by reduction of general weakness and clinical signs of extrahepatic signs: skin vasculitis and kidney damage.

Emphasis should be made on the group of patients with HCV, who had very low level of TNF-α in blood serum or it was not detected at all. In some of them (approximately 30%), moderate increase in cytokine level occurred, which also promoted improvement of their condition.

III. APPLICATION OF INTRADERMAL IMMUNIZATION WITH NATIVE AUTOLEUKOCYTES AS A THERAPEUTIC ANTIVIRAL VACCINE.

It is known that viruse reproduction of some infectious diseases occurs in polymorphonuclear leukocytes and monocytes. Thus, blood immune cells can be used as a vaccine, which contains "own" patient's virus [1]. For example, hematogenous route of transmission, caused by interaction with leukocytes plays a significant role in pathogenesis of herpes infection (substantial accumulations of herpes simplex virus type 1 and 2 are found in leukocytes). Therefore, autoleukocytes can be used as an antiviral vaccine for this disease. IINAL proved an effective vaccination for the treatment of patients with severe form of type 1 and 2 chronic herpes infection [4].

It was also determined that immunization with patient's own native leukocytes temporarily inhibits intensity of hepatitis B virus replication [21]. Thus, the technique for intensification of antiviral therapy for chronic hepatitis B was also elaborated [22, 23]. The investigation included patients, in whom DNA HBV replication remained despite prolonged treatment with tenofovir (at least two years). This group included patients, who demonstrated reduction of viral load to a certain degree after achievement of "individual effect" of antiviral therapy, and it remained stable throughout observation period (8 months). Thus, immunization was performed three times with an interval of 30-40 days along with tenofovir treatment. Inhibition of virus reproduction resumed due to conducted immunization. Besides, patients with high viral load demonstrated better response to such therapy.

Thus, based on a trial of this method in patients with type 1 and 2 chronic recurrent herpes and chronic hepatitis B, it is obvious that it should be used as an antiviral therapy in other viral infections as well, provided the virus (virus antigens) is present in blood lymphocytes.

Expediency of applying IINAL method in the treatment of a wide range of diseases has been proven. For instance, clinical examples of IINAL immunization for patients with chronic hepatitis B, C and autoimmune hepatitis are presented in one medical article [24]. The examples demonstrate that in patients with hepatitis, the level of cryoglobulins significantly decreases, synthesis of antinuclear antibodies and thyroid antibodies is inhibited, overall condition improves after such immunization.

Thus, a wide range of intradermal immunization with native autoleukocytes enables to use them in various diseases. According to our previous data, the procedure is also efficient in rheumatoid arthritis, Bekhterev's disease, early stages of osteoarthrosis, Raynaud's disease, pollinosis, bronchial asthma etc.

The mechanism of positive impact of immunization with autoleukocytes on a wide range of immunopathological processes requires further investigations. However, it has been proven that IINAL amplifies an immune response by activating congenital immunity factors, cross reactivity by means of partial identity of antigen structures, antiviral surveillance by regenerating cytotoxic lymphocytes etc. The process of correction of Jerne immune network – idiotypic-anti-idiotypic regulation of the immune response, activation of CD3+, CD8+, CD25+-lymphocytes, as well as CD3+, CD8+, CD28+-lymphocytes is also important along with blockage of Fc-receptors and glycoprotein, pectin receptors on B-lymphocytes [1, 2].

CONCLUSIONS

Intradermal immunization with autoleukocytes is one of the methods of personified medicine. This method significantly improves the results of treatment of many diseases, has a positive impact on autoimmune processes, decreasing synthesis of pro-inflammatory cytokines,

amplifying cell-mediated antiviral immunity in numerous viral infections. Considering that immunization with native autoleukocytes does not have contraindications, it is expedient to use this method in many diseases, in particular those, where cryoglobulins, factors of autoimmune aggression, high level of pro-inflammatory cytokines, and the presence of a virus in leukocytes play an important role in pathogenesis.

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