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INFLUENCE OF AUTOLEUKOCYTE VACCINATION ON ACTIVITY LEVEL OF TUMOR NECROSIS FACTOR ALPHA IN PATIENTS WITH CHRONIC HEPATITIS B

WPŁYW AUTOLOGICZNEJ SZCZEPIONKI LEUKOCYTARNEJ NA AKTYWNOŚĆ CZYNNIKA MARTWICY NOWOTWORU ALFA U PACJENTÓW Z PRZEWLEKŁYM ZAPALENIEM WĄTROBY TYPU B

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ABSTRACT

Introduction: data about influence of intradermal vaccination with native autoleukocytes on activity level of pro-inflammatory cytokine tumor necrosis factor alpha in patients with chronic hepatitis B have been presented in the article.

The aim: Based on positive results, obtained from autoleukocyte immunization in patients with psoriasis [14], the aim of our research was to use and study such therapy for reducing the synthesis of pro-inflammatory cytokine TNF- α in patients with chronic hepatitis B (chronic hepatitis B).

Materials and methods: Patients with chronic hepatitis B with high level of tumor necrosis factor alpha (≥ 30 pg/ml) were vaccinated with native autoleukocytes (23); simultaneously, the same procedure was performed to patients (11) with low level of this cytokine (5pg/ml). Leukocytes were isolated from heparinized peripheral venous blood of a patient with hepatitis B by centrifuging plasma, obtained after blood precipitation for 140-160 minutes at temperature 37^o C. The suspension was resuspended in 1-1.5 ml of a patient's blood serum and injected into the skin of the back in the dose 0.1 ml.

Results: in 30 days after immunization, reduction of tumor necrosis factor alpha was observed in all patients with its high level (100%), in 65.25% of individuals – to 5 pg/ml; in some patients, who had low or average level of pro-inflammatory cytokine, the level individually increased (41.67%).

Conclusions: The elaborated method of influence on activity of tumor necrosis factor alpha in patients with chronic hepatitis B is effective and worth implementing into clinical practice.

KEY WORDS: tumor necrosis factor alpha, vaccination with autoleukocytes, chronic hepatitis B

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INTRODUCTION

Current treatment strategy of a number of inflammatory processes implies inhibition of synthesis of pro-inflammatory cytokines, especially TNF-alpha with the medications, which are blockers of this cytokine or inhibit proliferation of Th1-lymphocytes, producing TNF [1-4].

Recently, drugs, action of which is aimed at inhibition and blockage of TNF- α biological activity (for example, infliximab, etanercept and adalimumab), are more often used for the treatment of immune-mediated diseases, such as rheumatoid arthritis, inflammatory diseases of the intestines and psoriasis [2, 3, 5]. Although clinical efficacy of these drugs is proved, however, inhibition of cytokine synthesis by means of antibodies to certain determinants of immunocompetent cells has a negative influence on immune system status. Long-term use of TNF- α inhibitors increases susceptibility to infectious diseases (or leads to exacerbation of the existing ones). Taking into account these side effects, it is obvious that

such methods of influence on TNF- α are unfavorable in infectious diseases [1, 6, 7].

In chronic viral hepatitis, excessive synthesis of TNF- α intensifies an inflammatory process with impairment of liver metabolism, playing a significant role in fibrosis pathogenesis and formation of liver cirrhosis [6, 7]. Thus, patients with chronic viral hepatitis with high level of TNF- α are administered medicines, which inhibit TNF- α content. However, it should be considered that biological inhibitors of TNF- α are foreign proteins and this may accelerate their excretion and lead to allergic reactions [8]. Concerning antibodies to certain determinants of immunocompetent cells (for example, chimeric monoclonal antibodies to CD20 antigen to B-lymphocytes), their long-term administration may lead to weakening of the immune response [4, 7]. Though viral hepatitis B may be referred to the diseases that negatively influence powerful immune processes, nevertheless, inhibition of immunity is undesirable, since it promotes intensification of virus

Table I. Influence of single autoleukocyte immunization on high TNF- α level (over 30 pg/ml) in blood serum of patients with chronic hepatitis B

TNF- α level before autoleukocyte immunization (pg/ml)	Number of patients	TNF- α level in 30 days after autoleukocyte immunization (pg/ml) ¹		
		0-5	10-15	20-30
		Number of patients		
30-50	15	11	4	
51-70	6	4	2	
71-90	1	0	0	1
100-180	1	0	1	
Total	23	15 (65.22%)	7 (30.43%)	1 (4.35%)

¹Influence of vaccination on TNF- α activity was manifested already in 10-12 days, however in some patients maximal indices were revealed later, thus, the results presented were obtained in 30 days.

reproduction and reactivation of the process, which is manifested by intensification of inflammatory process activity and acceleration of fibrosis development. After such therapy, there is a need for intensification (or renewal) of antiviral therapy [1, 6]. The risk for reactivation of chronic hepatitis B infection during therapy with TNF- α inhibitors is confirmed by data of many investigations, where authors emphasize that inhibitors of TNF- α may increase chronic hepatitis B replication and reactivate chronic hepatitis not only during, but also after cessation of treatment. It should be mentioned that a number of patients, who received TNF- α inhibitors before or simultaneously with the treatment, were treated with other immunosuppressants, sometimes for a long time, that significantly increases the risk for chronic hepatitis B reactivation and can be manifested by the appearance of fulminant hepatitis and even need in liver transplantation [8-11].

Thus, in the treatment of inflammatory processes (for example, in patients with rheumatoid arthritis, psoriasis, etc.) with inhibitors of pro-inflammatory cytokine, it is expedient to prescribe lamivudin to the patients, who have at least anti-HBc, for prevention of possible exacerbation of hidden hepatitis B [12, 13].

In our prior investigations, it was established that the method of cell therapy as intradermal vaccination with native autoleukocytes significantly inhibits high level of TNF- α in patients with psoriasis, having a positive impact on the disease course [14].

THE AIM

Based on positive results, obtained from autoleukocyte immunization in patients with psoriasis [14], the aim of our research was to use and study such therapy for reducing the synthesis of pro-inflammatory cytokine TNF- α in patients with chronic hepatitis B (chronic hepatitis B).

MATERIALS AND METHODS

To study influence of intradermal autoleukocyte immunization on TNF- α synthesis in patients with chronic

hepatitis B, a group of patients with high level of this cytokine (≥ 30 pg/ml) was chosen. The investigation involved patients who did not take any medicines that influence the level of cytokines.

The group of patients with chronic hepatitis B included 23 patients aged 20-60 years (13-females, 10 – males). An obligatory condition was mono-infection (hepatitis C and AIDS were excluded in patients). Test-system “Corbett Research” (Australia) was used for quantitative determination of HBV DNA by PCR method. Hepatitis C was excluded determining HCV RNA by qualitative PCR method (real-time), using test-system “Corbett Research” (Australia); the patients were examined for HIV-infection by ELISA method with test system of the fourth generation Genscreen Ultra HIV Ag-Ab (Bio-Rad, France).

Simultaneously, patients with chronic hepatitis B (12), in whom TNF- α could not be detected or its level in blood serum did not exceed 5 pg/ml, were vaccinated.

TNF- α in blood serum was detected by the method, which is based on “sandwich” variation of ELISA test using mono- and polyclonal antibodies to TNF- α (manufacturer “Vector Best”, Russia).

The method of reducing activity of pro-inflammatory cytokine by means of autoleukocyte vaccination was performed in two stages: isolation of leukocytes from peripheral blood and their intradermal injection to a patient. The method of immunization and mechanism of its action was described earlier [14].

In 10-12, 30, 60 and 180 days after immunization, the content of TNF- α in blood serum was investigated. Further, cytokine level was monitored, if necessary, the immunization was repeated (individually, considering peculiarities of response to the procedure). The research was performed analogously to the previous one [14].

Patient consent: The health, privacy, and confidentiality of personal information and rights of patients, involved in medical research, were taken into consideration according to Helsinki declaration. The study was approved by the local Ethical Committee of Danylo Halytsky Lviv National Medical University.

RESULTS AND DISCUSSION

The influence of autoleukocyte immunization on intensive TNF- α synthesis in blood serum of patients with chronic hepatitis B is confirmed by the results of monitoring its content before and after immunization (table I).

From the data given in table 1, it is seen that reduction of TNF- α in blood serum was observed in all patients with chronic hepatitis B due to autoleukocyte immunization. At the same time, their general condition improved, which was manifested by the reduction of fatigue and positive influence on extrahepatic manifestations: skin vasculitis; the level of creatinine in blood serum returned to norm or decreased in patients with various forms of liver damage (6).

It is impossible to explain the improvement of clinical manifestation only by the influence on TNF- α , because autoleukocyte vaccination has a positive impact on different autoimmune processes that are present in many patients with chronic viral hepatitis. However, in our investigation the most important is the proof that this method of treatment inhibits TNF- α production. In addition, in all patients with high level of ALT, it decreased (or returned to norm), which in fact indicates weakening of an inflammatory process in hepatocytes.

As a result of immunization of patients with chronic hepatitis B, in whom TNF- α level in blood serum could not be detected or did not exceed 5 pg/ml (12), its level did not change in 7 individuals, however in 5 (41.67%) it increased: in four patients to 10 pg/ml, and in one – to 175 pg/ml). It is important that in the patient, whose level of cytokine significantly increased, general condition improved and concentration of HBV DNA decreased almost twice. In 2 weeks, reduction of TNF- α level to 35 pg/ml was recorded. This patient was repeatedly immunized, and in 10 days after immunization, the level of pro-inflammatory cytokine was already 6 pg/ml. In fact, intensification of cytokine production in patients with chronic hepatitis B and low TNF- α level indicates that autoleukocyte immunization may intensify the activity of CD8+ T-cells, which produce different cytokines, in particular TNF- α . It should positively influence the efficacy of chronic hepatitis B treatment, since CD8+ T-cells are thought to control HBV replication via non-cytolytic route and this effect is initiated through interferon-gamma and TNF- α [15].

Thus, the results of investigation indicate the expediency of implementing this method into complex therapy of chronic hepatitis B with high TNF- α activity and intensive inflammatory process in the liver. Moreover, a significantly wider range of a positive influence of this procedure results in reduction of the content of precipitating cold-shock proteins – cryoglobulins [16-19], and unlike antibodies-inhibitors of TNF- α , may promote improvement of antiviral immunity [20].

CONCLUSIONS

It has been established that immune cell therapy in the form of intradermal autoleukocyte vaccination has a regulatory effect on TNF- α synthesis in patients with chronic hepatis

is B. Thus, it will be expedient to use this method for the treatment of patients with high level of this cytokine. In addition, this method, according to previously obtained results, positively influences different extrahepatic manifestations in patients with chronic viral hepatitis.

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Authors' contributions:

According to the order of the Authorship.

Conflict of interest:

The Authors declare no conflict of interest.

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