Features of the immunoreactivity T and B lymphocytes subpopulations and cytokine imbalance in patients with hepatosplenomegaly of different etiology

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Abstract

The aim was to study the mechanisms of immunological dysregulation of cytokine and immunoglobulin production, changes in the CD expression of T and B lymphocyte subpopulations in patients with hepatosplenomegaly of different etiology.

Materials and methods. We examined 73 patients with liver cirrhosis complicated by portal hypertension, hepatosplenomegaly, and bleeding from phlebectasia. We identified three groups of patients depending on the triggering factors of cirrhosis: the first (I) group – HBV/HCV; the second (II) group – CMV/VEB; the third (III) group – hereditary enzymopathies. The study material was lymphocytes and blood serum. We used the methods of ELISA, immunofluorescence and flow cytometry.

Results. An increase in the concentration of IgA and IgM was revealed against the background of normal number of CD22+B lymphocytes with HBV/HCV (I group), high level of IgM and their producers, B lymphocytes, with CMV/VEB (II group), in the third group with hereditary enzymopathies, the concentration of all immunoglobulins was normal with an increased content of B lymphocytes. Multidirectional changes in the content of cytokines were revealed: in group I the synthesis of anti-inflammatory cytokines IL-4, IL-10 and in group II – pro-inflammatory IL-1β, INF-γ, TNF-α dominated; in group III the concentration of IL-6 and vascular growth factor (VEGF) was maximally increased. The number of activated CD3+CD4+CD25+ T cells was reduced in groups I and II – by 2.3 and 2.0 times respectively, in group III – increased by 1.2 times. The number of regulatory T lymphocytes CD3+CD4+CD25+CD127- was reduced by half in I and II groups. Expression of co-stimulatory molecules CD3+CD4+CD25+ was low in all groups and the maximum decrease was in group III. In patients with HCV/HBV, an increase in the expression of the late activation marker of lymphocytes CD3+HLA-DR+ by an average of 63 % was noted.

Conclusions. The revealed immune disorders in hepatosplenomegaly of different etiology are characterized by multidirectional changes. Approaches to the treatment of these patients should be complex, taking into account the trigger factors that cause dysregulation of immune responses, which leads to further destruction, and focuses on remodeling target organs.
Hepatosplenomegaly is manifested by an increase in the liver and spleen size, occurs often in viral hepatitis, cholestasis, biliary cirrhosis and hereditarily determined storage diseases. Structural and functional disorders of the spleen are caused by pathological changes in storage diseases. Functional viability of the immune system links in patients with various etiological factors of hepatosplenomegaly remains insufficiently studied.

Aim

The aim of this work was to study the mechanisms of immunological dysregulation of cytokine and immunoglobulin production, changes in the CD expression of T and B lymphocyte subpopulations in patients with hepatosplenomegaly of different etiology.

Materials and methods

The study was carried out in the Diagnostic Laboratory with Enzyme Immunoassay and Immunofluorescence Analysis of State Institution "Zaitsev V. T. Institute of General and Urgent Surgery of National Academy of Medical Sciences of Ukraine" (registration certificate No. 01-0170/2018).

We examined 73 patients with liver cirrhosis complicated by portal hypertension, hepatosplenomegaly, and bleeding from phlebectasia. These patients were admitted urgently for treatment to the clinic of the SI "Zaitsev V. T. Institute of General and Urgent Surgery of NAMS of Ukraine". We identified three groups of patients depending on the triggering factors of cirrhosis. The first (I) group included 32 patients (20 men and 12 women) with chronic hepatitis B (HBV) or/and C (HCV) viruses (47 % of the examined) with a mean age of 52.0 ± 5.6. The presence of chronic viral hepatitis B was established (47 % of the examined) with a mean age of 52.0 ± 5.6.

The role of the functional viability of the immune system links in patients with various etiological factors of hepatosplenomegaly remains insufficiently studied.
on the basis of detecting HBsAg in the blood serum by ELISA. The presence of viral hepatitis C was established on the basis of detecting total anti-HCV antibodies in the blood serum by ELISA.

The second (II) group included 32 patients (17 men and 15 women) with herpes viruses: cytomegalovirus (CMV) and Epstein-Barr virus (EBV) (42 % of the examined) with a mean age of 48.1 ± 4.7. The presence of the herpes viruses was established based on the detection of anti-infectious antibodies to CMV and EBV in the blood serum.

The third (III) group included 9 patients (2 men and 7 women) with hereditary enzymopathies, which are a consequence of gene polymorphism of the lysosomal enzymes – glucocerebrosidase (GBA) and chitotriosidase (CHIT1) (11 % of the examined) with a mean age of 37.0 ± 4.1.

The mean values of the results of 38 conventionally healthy individuals (24 men and 14 women) with a mean age of 45.2 ± 2.3 are presented as reference values. They did not have any clinical and laboratory signs of liver and spleen lesions, as well as somatic diseases. The material for the study was lymphocytes and blood serum.

Patients with HIV infection were exclusion criteria from the studies.

The enzyme-linked immunosorbent assay (ELISA) was used for the detection of class-specific serum antibodies (IgA, IgM, IgG). We used a kit of reagents “Vector-Best”, Novosibirsk. A two-stage sandwich was used with the appropriate monoclonal antibodies (mAbs) IgA, IgM, IgG. At the first stage, the analyzed samples were incubated with immobilized mAbs. At the second stage, the bound antibodies were treated with a conjugate with peroxidase of mAbs to the light chains of human immunoglobulin. The formed immune complexes were detected by enzymatic reaction with TMB (tetramethylbenzidine). The degree of staining was assessed by measuring the optical density at λ = 450 nm (StatFax 3200, USA), which is proportional to the concentration of immunoglobulin in the sample (in g/L).

Determination of the content of cytokines (IL-1β, IL-2, IL-4, IL-6, IL-10, TNF-α, INF-γ and vascular endothelial growth factor (VEGF)) in blood serum was carried out by the enzyme-linked immunosorbent assay (ELISA) with the appropriate mAbs (kits “Vector-Best”, Novosibirsk). At the first stage, the cytokine from the sample was bound with mAbs immobilized on the inner surface of the wells. Then, after washing, the bound antibodies interact during the second incubation with biotinylated antibodies to human cytokines (conjugate 1). Then, at the third stage, the bound conjugate 1 interacts during incubation with conjugate 2. Unbound conjugate 2 was removed by washing. The staining of the solution in the wells occurred during incubation with TMB. The optical density at λ = 450 nm (StatFax 3200, USA) was proportional to the concentration of immunoglobulin in the sample (in pg/L).

Expression of clusters of differentiation CD22+ was assessed by indirect immunofluorescence using monoclonal antibodies (“Sorbert”, RF) labeled with FITC-stain. In this method specific mAbs labeled with FITC not directly, but using a secondary serum, bind to the cell surface antigen. The cells stained by corresponding antibodies were visualized by fluorescent microscopy (Olympus BX53, Japan).

The analysis was performed 2 hours after blood sampling using test tube with the K3 EDTA according to the standard protocol. In each sample at least 5000 cells were analyzed. Various subpopulations of T lymphocytes were determined: CD3+HLA-DR+ (late activation lymphocytes marker), CD3+CD4+ (T helpers), CD3+CD4+CD25+ (activated T helpers), CD3+CD4+CD25+CD127neg (regulatory T lymphocytes), CD3+CD4+CD28+ (co-stimulatory molecules) using appropriate mAbs and dyes: CD3-PC5, CD4-PE, CD28-FTTC, CD25-FTTC, CD127-Pc7, anti-HLA-DR-PE (Beckman Coulter, USA). For correct exclusion of cells that did not meet the parameters from the analysis zone, the necessary logical constraints were introduced into the particle distribution histogram for low-angle, side scintillation (SSC). The evaluation of expression level of surface receptors was performed at mean intensity of fluorescence. For the lysis of red blood cells in the samples we used OptiLyse C (Beckman Coulter, USA). The analysis of stained cells was carried out on a flow cytometry Cytomix FC500 (Beckmann Coulter, USA).

To assess the statistical significance of the differences in the groups, the nonparametric Mann–Whitney tests were used. Quantitative data in the tables are presented by the median, as well as the lower (25 %) and upper (75 %) quartiles for each group. The differences were considered significant at P < 0.05. The results were analyzed using the software package Statistica v.6.

Results

An evaluation of immunoglobulins in patients with liver cirrhosis complicated by hepatosplenomegaly, portal hypertension and recurrent bleeding showed significant increase IgA (more than 2 times) in group I (patients with hepatitis etiology of hepatosplenomegaly) and by 45 % in group II (patients with herpesvirus etiology of hepatosplenomegaly). An increased IgM content was also revealed – by 53 % in group I, and by 91 % in group II. The IgG content in all studied groups did not differ from the reference values, however, in groups I and II, this indicator was slightly higher than in group III (patients with hereditary enzymopathies) (Table 1).

The concentration of pro-inflammatory IL-1 was reduced by 6 times in group I and increased by 3 times in group II. The content of IL-2 was increased in all studied groups, but maximum increase by 7 times was found in patients with liver cirrhosis against the background of viral hepatitis (group I). The highest increase (20-fold) was IL-6 among all pro-inflammatory interleukins in all examined groups. The IFN-γ level was significantly higher in patients with herpesvirus infections (group II) and exceeded the reference values by 75 %, while in groups I and III IFN-γ did not significantly differ from the reference values. The tumor necrosis factor TNF-α was increased in groups I and II by 4 and 10 times, respectively, while in group III this cytokine was significantly lower than the reference values. The content of anti-inflammatory cytokines IL-4 and IL-10 was increased in all groups. Group I had the greatest increase in IL-4 (6 times) and IL-10 (7 times) relative to the reference values (Table 2).
Table 1. Serum immunoglobulins concentration in patients with hepatosplenomegaly of different etiology (Me, Q;

| Index, units | Reference values, n = 38 | Studied groups |
|--------------|--------------------------|----------------|
|              | Group I (HCV/HBV), n = 32 | Group II (CMV/VEB), n = 32 | Group III (storage diseases), n = 9 |
| IgA, g/L     | 1.92 (1.23; 3.54)        | 4.12 (2.51; 6.72)** | 2.78 (1.46; 4.4)** | 1.91 (1.76; 2.71) |
| IgM, g/L     | 1.20 (0.92; 1.56)        | 1.84 (1.14; 2.56)** | 2.30 (2.02; 3.85)** | 1.31 (1.09; 1.58) |
| IgG, g/L     | 11.55 (8.82; 14.65)      | 14.80 (11.78; 16.50) | 14.30 (11.60; 19.11) | 11.80 (8.70; 14.80) |

*: differences in comparison with the reference values are significant (P < 0.05); **: significance of difference between groups I and II (P < 0.05).

Table 2. Serum content of pro-inflammatory, anti-inflammatory and regulatory cytokines in patients with hepatosplenomegaly of different etiology (Me, Q;

| Cytokine | Reference values, n = 38 | Studied groups |
|----------|--------------------------|----------------|
|          | Group I (HCV/HBV), n = 32 | Group II (CMV/VEB), n = 32 | Group III (storage diseases), n = 9 |
| IL-1β, pg/L | 1.6 (0.0; 2.6)        | 0.2 (0.0; 0.4)*       | 4.3 (3.1; 5.6)** | 1.6 (0.2; 1.9) |
| IL-2, pg/L | 0.1 (0.0; 0.3)        | 0.8 (0.4; 0.9)*       | 0.3 (0.2; 0.5)* | 0.4 (0.2; 0.6)* |
| IL-6, pg/L | 2.0 (0.0; 4.0)        | 37.8 (19.3; 41.3)**   | 38.2 (17.5; 50.0)** | 43.3 (20.0; 62.3)** |
| IFN-γ, pg/L | 9.4 (0.0; 15.0)       | 14.1 (8.9; 16.6)**    | 37.6 (18.3; 45.4)** | 13.5 (8.2; 17.5) |
| TNF-α, pg/L | 0.5 (0.0; 2.0)       | 2.2 (1.3; 3.6)*       | 5.3 (2.9; 7.4)** * | 0.05 (0.0; 0.08)*** **** |
| IL-4, pg/L | 0.2 (0.0; 0.3)        | 1.2 (0.9; 1.9)**      | 0.4 (0.2; 0.6)** | 0.5 (0.1; 0.9) |
| IL-10, pg/L | 4.5 (0.2; 9.5)       | 27.8 (13.9; 38.6)**   | 19.2 (11.2; 24.6)** | 11.5 (5.5; 18.5)* |
| VEGF, pg/L | 170.0 (40.0; 280.0)   | 217.3 (90.2; 479.3)   | 270.0 (113.4; 532.0) | 328.0 (296.5; 927.0)*** **** |

*: differences in comparison with the reference values are significant (P < 0.05); **: significance of difference between groups I and II (P < 0.05); ***: significance of difference between groups III and II (P < 0.05).

Table 3. Content of T lymphocyte subpopulations in patients with hepatosplenomegaly of different etiology (Me, Q;

| T lymphocyte subpopulations, % | Reference values, n = 38 | Studied groups |
|-------------------------------|--------------------------|----------------|
|                               | Group I (HCV/HBV), n = 32 | Group II (CMV/VEB), n = 32 | Group III (storage diseases), n = 9 |
| T helper, CD3/CD4+            | 41.0 (32.0; 48.0)        | 38.0 (27.3; 43.5)**   | 51.0 (35.7; 66.4)**   | 48.4 (33.1; 56.7) |
| Co-stimulatory molecules CD3/CD4/CD28+ | 56.0 (48.7; 62.0) | 30.1 (20.2; 43.8)* | 36.7 (28.4; 46.6)* | 27.6 (22.4; 38.2)* |
| Activated T lymphocytes, CD3/CD4/CD25* | 3.5 (3.0; 4.5) | 1.5 (0.4; 3.1)** | 2.6 (1.6; 2.9)** | 4.6 (3.0; 5.8) |
| Regulatory T lymphocytes, CD3/CD4/CD25/CD127** | 4.5 (3.2; 5.4) | 1.9 (1.5; 2.3)** | 2.3 (1.1; 3.0)** | 4.3 (3.5; 6.0) |
| Late activation T lymphocytes marker CD3/HLA-DR- | 8.0 (5.5; 14.2) | 13.0 (11.1; 20.7)** | 3.5 (3.0; 4.6)** | 4.4 (3.8; 6.1)* |

*: differences in comparison with the reference values are significant (P < 0.05); **: significance of difference between groups I and II (P < 0.05).

The content of B lymphocytes expressing CD22+ was reduced in patients with cirrhosis of the liver against the background of viral hepatitis (group I). And in groups II and III, the level of CD22+ B lymphocytes was increased relative to the reference values (Fig. 1).

To form an adequate immune response to an antigen with subsequent elimination from the body, it is important to activate the lymphocytes of T helper population CD3/CD4+. The population of T helpers was reduced in group I (patients with hepatitis B or/and C viruses) (Table 3).

The expression of co-stimulatory molecules CD3/CD4/CD28* was reduced in group I (HCV/HBV) by 46 %, in group II (CMV/VEB) – by 34 %. The most pronounced deficiency of co-stimulatory molecules (2-fold decrease) was revealed in patients with hepatosplenomegaly against the background of hereditary enzymopathies in group III (Fig. 2).

The number of activated T cells CD3/CD4/CD25 was reduced in patients with hepatosplenomegaly of groups I and II – by 2.3 and 2 times, respectively, and in group III an increase in the number of activated T helpers by 1.2 times was revealed (Table 3). The level of Treg (regulatory T cells) CD3/CD4/CD25/CD127 (producers of IL-10) was decreased in groups I and II relative to the reference values (Table 3).

Multidirectional changes in the expression on T cells of the class II HLA-DR receptor involved in signal transduction by activated T lymphocytes have revealed.
Fig. 2. Individual values of the relative amount of co-stimulatory molecules (CD3+/CD4+/CD28+/lymphocytes): in a healthy donor (A), in a patient with hepatosplenomegaly on the background of HBV/HCV (B), CMV/VEB (C), in a patient with hereditary enzymopathies (D).

Fig. 3. Individual values of the relative amount of the late activation marker of lymphocytes (CD3+/HLA-DR+): in a healthy donor (A), in a patient with hepatosplenomegaly on the background of HBV/HCV (B), CMV/VEB (C), in a patient with hereditary enzymopathies (D).
In patients with hepatitis viruses HCV/HBV, an increase in the expression of the late activation marker of lymphocytes CD3+HLA-DR+ by an average of 63 % was noted (Fig. 3). In patients with the herpesvirus CMV/VEB (group II) and in patients with hereditary enzytopathies (group III) a decrease in the expression of CD3+HLA-DR+ by 56 % and 45 %, respectively, was revealed (Fig. 3).

**Discussion**

The involvement of the spleen in the pathogenesis of liver cirrhosis is due to the anatomical participation of both organs in the portal circulation, blood filtration and recurrent bleeding are hepatitis B viruses (HBV), hepatitis C viruses (HCV), herpes viruses – cytomegalovirus (CMV), Epstein-Barr virus (VEB), Helicobacter pylori infection, toxoplasmosis (the causal agent of Toxoplasma gondii), and various genetic defects of lysosomal enzymes (storage diseases) [7,8]. The presence of certain trigger factors leads to the induction of specific mechanisms of the pathological process, which differ in the features of biochemical, immunological, hemostatic reactions [9].

The study of indexes of the immunity humoral link in patients with hepatosplenomegaly indicates an imbalance in the content of different classes of immunoglobulins depending on etiological factors. In particular, in group I a significant increase in IgA concentration was revealed with a normal content of antibody-producing CD22+ B lymphocytes. This phenomenon can be explained by a violation of the antigens elimination of the intestinal microflora due to insufficiently of liver function caused by viral cytolysis of hepatocytes. Antigens of the intestinal microbiota enter the systemic circulation through the portosystemic anastomoses of the affected liver induce the production of antibodies mainly in the spleen. In addition, IgA which is found in large quantities in the intestinal mucosa can enter the bloodstream through portosystemic anastomoses [10]. It can also be assumed that the high concentration of IgAs a consequence of the activation of CD5+ B1 lymphocytes subpopulation, which in the abdominal cavity can respond to both T dependent and T independent antigens [11].

In contrast to group I, in group II the increase in the concentration of IgA was less significant. We detected a high content of IgM as a marker of an acute infectious process against a background of 35 % increased expression of the CD22+ marker on B lymphocytes. This indicates that in the group with autoimmune liver damage, the mechanism of IgM increase is associated with the reactivation of viral infection and chronic infection induced an increase in the synthesis of antiviral IgM. In addition, the increased content of antibody-producing CD22+ B lymphocytes in patients with herpesvirus etiology of autoimmune hepatitis (group II) may be the result of VEB reactivation which is known to enhance the proliferation of B lymphocytes [12]. Reinfection or reactivation of lysogenic forms of CMV and/or VEB in the spleen induces antibody production by B lymphocytes against the background of our revealed sharply reduced suppressed activity of CD6+ T lymphocytes in this group, which stimulated inflammatory reactions in these organs [11,13].

The inflammatory process is initiated and mediated by the participation of a wide range of cytokines, which are capable of exerting both protective and damaging effects. But at the final stage of chronic diseases the increased level of various functional classes of cytokines is mainly associated with their damaging effects, such as the maintenance of local and systemic inflammation, apoptotic death of hepatocytes, progression of fibrosis and the development of extrahepatic complications [14].

An increased serum content of pro-inflammatory cytokines in liver cirrhosis was revealed in groups I and II. A significant increase in the concentration of IL-6 in all groups indicates the development of an acute inflammatory reaction. In group I a low level of IL-1β against the background of a high concentration of IL-6 indicates the resolution of an acute inflammatory reaction, since one of the main functions of IL-6 is self-limitation of the inflammatory response by suppressing the production of TNF-α and IL-1β, and stimulation synthesis of an antagonist of the IL-1 receptor and the soluble TNF-α receptor [15]. Through stimulating B lymphocytes, IL-6 induces the synthesis of immunoglobulins and also participates in the differentiation of cytotoxic T lymphocytes [16].

An increase in the synthesis of IL-10 was observed in all groups, but most pronounced in groups I and II. This led to a decrease in anti-infectious protection, the development of immunosuppression, disruption of reparative processes due to a shift in the balance towards Th2 [16]. The high production of IL-10 is probably associated with a high level of immunoglobulins in these groups and as a consequence a high immune complexes concentration which acting on macrophages induces the secretion of many cytokines, including IL-10.

We have shown the concentration of TNF-α increases by 5 and 10 times in patients with cirrhosis of the liver of viral HBV/HCV etiology (group I) and in patients with cirrhosis of the liver against the background of herpes infection (group II), respectively. Since this cytokine is involved not only in defense reactions but also in the processes of destruction and repair of tissues. So, this increase enhances the inflammatory process. In long-term chronic inflammation TNF-α, that is one of the mediators of tissue damage and activation of fibrogenesis, which is also associated with the ability of TNF-α to induce fibroblast proliferation and collagen deposition. A high concentration of TNF-α entails chronic inflammation, destruction and contributes to the unfavorable course of the pathological process. An excessive increase in the production of TNF-α, IL-6, IL-10 against the background of the T-cell link deficiency and activation of the humoral link immunity enhance the progressive nature of inflammation and liver tissue destruction [17]. This is most pronounced in I and II groups.
It is known that hereditary defects of lysosomal enzymes (glucocerebrosidase, chitotriosidase, angiotensin converting enzyme, acid lipase, etc.) lead to pathological accumulation of metabolites in cells and hyperplasia of many organs, including the liver and spleen [18]. There is evidence Gaucher cells (lipid-filled macrophages) express the vascular endothelial growth factor (VEGF) in patients with storage diseases. This cytokine not only stimulates angiogenesis, but also promotes the attraction of monocytes, which may be the reason for the local accumulation of Gaucher cells in various organs [19]. In group III a significant increase in VEGF was revealed, which indicates the activation of neangiogenesis in the liver, endothelial dysfunction and can serve as an indirect marker of liver fibrosis. The development of intrahepatic angiogenesis can be considered as a compensatory mechanism aimed at decumpression of the portal system. At the same time, the newly formed vessels carrying blood bypassing the sinusoids are unable to provide oxygen and nutrients to the liver tissue, which leads to the portal hypertension progression in patients of group III.

The interaction of antigens with antigen-recognizing receptors is a signal for the activation of T lymphocytes (CD3+). This is manifested by the secretion of cytokines that enhance the processes of proliferation and differentiation of various subpopulations of T lymphocytes, B lymphocytes and macrophages [16]. We have shown the content of the total number of CD3+ T lymphocytes in all patients with hepatosplenomegaly was reduced [8]. A decrease in the number of CD3+ T lymphocytes in all patients with hepatosplenomegaly may be due to secondary immune deficiency. Its formation is probably due to the damaging effect of toxic metabolites in viral infection and a genetic defect of lysosomal enzymes against the background of impaired detoxification function of the liver. In group II long-term course of herpesvirus led to a pronounced decrease in the number of T lymphocytes in all patients with hepatosplenomegaly [20]. This leads to inhibition of the processes of proliferation and differentiation of cytotoxic T lymphocytes along with an increase in the HLA receptors class II expression [26]. We also found a similar tendency in patients with hepatosplenomegaly associated with viral hepatitis; however, in groups II and III the expression of CD3+CD4+CD28+ T suppressors was reduced.

According to various authors, T regulatory cells (CD3+CD4+CD25+CD127low) inhibit the functional activity of virus-specific cytotoxic CD8+ T lymphocytes in hepatitis [21,22]. Therefore, it can be assumed they play a central role in the viral infection and may be the target of immunotherapy. In patients of group I a decrease in regulatory T lymphocytes CD3+CD4+CD25+CD127low was revealed while in group III (patients with hepatosplenomenagaly of non-infectious etiology) the expression of Treg cells did not differ from the reference values. According to Mengshol (2010) galectin-9 produced by Kupffer liver cells contributes to the maintenance of the number of Treg cells CD4+CD25+FoxP3+CD127low. This leads to inhibition of the effects of CD4+ cells and contributes to apoptosis of cytotoxic T lymphocytes [23,24].

The development of a strong and prolonged T cell response with the predominant synthesis of cytokines characteristic of Th1 T lymphocytes (IFN-γ, IL-2) and also the activation of effector cytotoxic CD8+ lymphocytes can lead to the termination of the infectious process. But the previously identified suppression of the CD8+ subpopulation of T killer cells in group II indicates a long-term course of the pathogen with the development of a chronic process [8]. In the case when as a result of the opposition of the virus the body fails to implement the antiviral strategy due to the activation of Th1 T lymphocytes, the balance of Th1/Th2 T lymphocytes shifts towards the Th2 subpopulation with the predominance of pro-inflammatory cytokines [17,25]. Thus, in group I activation of Th1-dependent reactions is noted while in group II simultaneous activation of both Th1 and Th2-dependent reactions is noted.

The interaction of CD28 co-stimulatory molecules on T lymphocytes with ligand B7 (CD80/CD86) on antigen-presenting cells increases the production of cytokines by T cells, but further stabilizing the mRNA of these molecules and enhancing the transcription of their genes. Horst et al. (2020) has shown that insufficient formation of the B7/CD28 complex leads to a low level of T lymphocyte cytokines production the development of anergy and therefore blocks the immune response [16]. All examined patients showed a decrease in the expression of co-stimulatory molecules CD3+CD4+CD28+ reflecting the activation of the immune system, participating in cell-cell interaction and determining T helper-dependent antibody production.

According to R. Oba et al. the most severe forms of liver cirrhosis are characterized by insufficiency of the immunity cellular component manifested by a decrease in the level of cytokotic T lymphocytes along with an increase in the HLA receptors class II expression [26]. We also found a similar tendency in patients with hepatosplenomegaly associated with viral hepatitis; however, in groups II and III the expression of CD3+HLA-DR+ receptors was reduced.

Immune disorders in hepatosplenomegaly depending on the etiology can be characterized by both changes in immunoreactivity and immunoresistance. Therefore, approaches to the treatment of patients with hepatosplenomegaly must be in complex. It is necessary to take into account the triggering infectious factors that cause the formation of various immune responses which can be one of the elements of further destruction and remodeling of organs.

Conclusions

1. In the overwhelming number of patients with hepatosplenomegaly trigger infectious factors were detected: hepatitis form B or/and C viruses (in 47 %), herpes virus – cytomegalovirus or/and Epstein-Barr virus (in 42 %) and a small number (in 11 %) were diagnosed with enzymopathies – quantitative disorders of lysosomal enzymes – glucocerebrosidase and chitotriosidase.

2. Activation of the immunity humoral link in response to infection was revealed in group I – a significant increase in the IgA and IgM concentration, while the number of B lymphocytes was at the level of reference values. In group II antibody production for the herpes infection presence was more pronounced in IgM, while the number of B lymphocytes was 2 times increased.

3. In group II the concentration of IL-1β was many times higher than reference values and this induced the entire cascade of humoral and cellular sensitization. TNF-a concentration was increased tenfold in this group. In group III we revealed a maximum increase in IL-6 (22 times), which indicates the presence of a cytokine storm...
in patients of this group who were admitted to the clinic at the height of bleeding. Group III showed a significant increase in VEGF. This factor stimulates angiogenesis and is a chemoattractant of Gaucher cells and an additional factor of their accumulation in the spleen and liver.

4. The activity of regulatory cells CD3+CD4+CD25+CD-127hi (as producers of IL-10) manifested itself as a manifold increase in this anti-inflammatory cytokine in response to the presence of a cytokine storm.

5. An increase in CD3+CD4+ was revealed in groups II and III. The number of co-stimulatory molecules CD3+CD4+CD28+ was reduced in all groups, which indicates a violation of intercellular signaling. The deficiency of these molecules was revealed in patients with hereditary enzymopathies (group III).

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