Case study

Investigation of Immunopathological Processes Associated with the Development of Liver Fibrosis

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The aim of this study was to determine the dependence of the course of liver fibrosis on the functional state of the immune system, in particular, on the imbalance of pro-inflammatory and anti-inflammatory immune reactions that are formed in patients during the development of the disease. The study included 30 patients with chronic liver diseases (18 patients with chronic hepatitis C (CHC) and 12 patients with alcoholic liver disease (ALD), 15 healthy individuals were the comparison group. Liver elastography (FibroScan) was used to evaluate liver stiffness and determine fibrosis stages according to METAVIR classification. The following cytokine levels were measured in the serum samples of the group: IL-1β, TNF-α, IL-6, IFN-γ, IL-2, IL-4, IL-8, VEGF and TGF-β. According to the data presented in this work, in patients with CHC and ALD, there was a statistically significant increase in serum levels of pro-inflammatory cytokines, namely: IL-1β, TNF-α, IFN-γ, IL-2, IL-6 and IL-8. Interestingly, elevated TGF-β values were found in patients with CHC, but not in patients with ALD. Significantly lower concentrations of VEGF were observed in both study groups. There was also a significant decrease in serum IL-4 in patients with CHC, whereas in patients with ALD such a decrease was not statistically significant. Serum IL-1β content was approximately equally elevated in the early and late stages of fibrosis. A sharp rise in serum TNF-α levels occurred in the early stages of fibrosis. In the later stages, the rise in the level was replaced by a sharp fall. However, the serum levels of TNF-α in the later stages of liver fibrosis still significantly exceeded control values. The serum levels of IFN-γ in patients significantly exceeded control values without changes in different stages of fibrosis. Relatively high levels of serum IL-2 and IL-6 were noted only in the later stages of the disease. In both groups of patients, a clear dependence of serum levels of IL-8 in the stage of fibrosis was revealed. Analysis of the data allows us to conclude that immune mechanisms play a significant role in the pathogenesis of degenerative liver diseases. Therefore further studies of the mechanism and role of immune factors is required to explore possible diagnostic and therapeutic applications.

Keywords: immunopathology; liver fibrosis; hepatitis C; alcoholic liver disease

Introduction

Chronic diffuse liver diseases, the final expression of which, regardless of the etiological factor, is the process of fibrogenesis, represent an acute medical and social problem relating to the priorities of the national health systems of most industrialized countries of the world (Jeffers et al., 2007). To date, it has been established that fibrosis is the result of a time-repeated damage-recovery process of hepatocytes, which are the main target of most hepatotoxic factors, including hepatitis viruses, alcohol, bile acids, etc. (Corazza, Badmann, & Lauer, 2009).

The fact of the formation of immune response disorders in the development of various diseases has long been established (Ke, 2019; Vonghia, Van Herck, Weyler, & Francque, 2019). Numerous studies indicate that the balance of cellular immunity in the body is one of the most important conditions for maintaining an effective immune response to the various pathologic agents (Bidlingmaier, Zhu, & Liu, 2008; Patra, Ray, R. B., & Ray, R., 2019), where the key role is played by immune competent cells of peripheral blood, in particular lymphocytes. However, the interest of researchers in assessing the state of the cellular component of the immune system in patients with diffuse chronic liver diseases is dictated by the fact that it is the immune competent cells that regulate the inflammatory response to hepatocyte damage and modulate hepatic fibrogenesis. In light of the available data, there is reason to believe that the immune mechanisms, cellular and humoral, play a dominant role in the pathogenesis of degenerative liver diseases.
Methodology

The study included 30 patients (24 men and 6 women, mean age was 38 ± 6 years). Of these, 18 people with viral hepatitis C and 12 people with alcoholic liver disease. The control group consisted of 15 people (9 women, 6 men; mean age 36 ± 7.2 years), not suffering from chronic diseases. Fibro elastography was used to establish the stage of fibrosis (Klibansky et. al., 2012). We performed 3 consecutive series of 10 reliable measurements. Obtained results were evaluated and structured according to METAVIR liver fibrosis scoring system. Cytokines were determined in venous blood. The blood not stabilized by heparin was kept at 37 °C for 40-60 minutes and centrifuged at 1500 rpm for 10 minutes to separate the serum from the cell mass. The obtained serum samples were evaluated for the content of cytokines: IL-1β, TNF-α, IL-6, IFN-γ, IL-2, IL-4, IL-8, VEGF and TGF-β using ELISA kits. In the evaluation of the obtained data, the methods of statistical description and testing of statistical hypotheses, Student’s t-test, Wilcoxon test, Mann-Whitney test, Spearman method were used. Differences were considered significant at a significance level of p <0.05.

Results and Discussion

The content of pro- and anti-inflammatory cytokines in the blood of patients at different stages of liver fibrosis

In order to identify the most common patterns of changes in the levels of cytokines associated with the development of hepatic fibrosis (Lalor, Faint, Aarbdem, Hubscher, & Adams, 2007), serum samples were divided into 3 groups. Samples obtained from healthy donors were included in group 1, whereas samples from patients with CHC and ALD, respectively, were in group 2 and 3. This study revealed some common changes in cytokine levels in both CHC patients and ALD patients.

Interleukin 1 (IL-1) is synthesized by many cells of the body, primarily activated macrophages, keratinocytes stimulated by B-cells and fibroblasts. It was originally described as a factor that causes a rise in temperature, controls the activity of leukocytes, increases the number of bone marrow cells and leads to degeneration of the joints. There are two similar IL-1s: alpha and beta. Both proteins have a molecular weight of ~ 18 kDa. In the study, elevated serum IL-1 noted in both CHC patients and ALD patients. Elevated levels of IL-1 indicate the severity of inflammatory processes in the body. Since the increase was recorded in both groups of patients, it can be assumed that it is largely due to inflammation that is not directly associated with a viral infection.

Tumor necrosis factor α-alpha (TNF-α) is a glycoprotein with a molecular weight of 17,400 kDa. It is formed by macrophages, eosinophils and natural killers (14% of lymphocytes). The level of TNF increases with the entry of bacterial endotoxins into the body. This cytokine plays an important role in the pathogenesis of autoimmune diseases. From the data obtained, it follows that elevated levels of TNF-α occurred in both CHC patients and ALD patients. The data suggest that the production of TNF-α in patients with developing liver fibrosis may not be directly related to virus-specific immune responses.

Interferon – gamma (IFN–γ) is produced by activated T-lymphocytes (mostly Th1) and natural killer cells (NK) (Corazza et al., 2009). Was first identified as a natural antiviral agent (Dornmair, Meinl, & Hohlfeld, 2009). This property allowed us to classify this cytokine as interferon. Similar to type I IFN, IFN–γ exhibits pleiotropic biological properties, including the ability to induce the expression of antigens of the main histocompatibility complex type II (HLA-II) and Fc receptors, monocyte activation, stimulation of the functional activity of NK cells. IFN-γ is a regulator of immunoglobulin synthesis, including switching from one class to another (Ducoulombier et al., 2004). The biological activity of IFN-γ is realized through specific cellular receptors and the intracellular signaling protein kinase cascade, leading to the activation of relevant transcription factors and the transcription of a whole family of gene encoding factors of resistance to infectious agents. Data on serum IFN-γ in patients with hepatitis show that the development of hepatitis leads to an increase in serum IFN-γ. Interestingly in patients with ALD, the average level of this cytokine was higher than this of patients with CHC. This fact is difficult to explain, since viruses are effective inducers of Th1-mediated immune responses. Perhaps the long-term persistence of the virus in the body leads, ultimately, to a weakening of antiviral protection and, as a result, to a decrease in production of interferons.

Interleukin 2 (IL-2) is predominantly produced by CD4 + T cells in response to antigenic and mitogenic stimulation (Ochel, Tiegs, & Neumann, 2019). IL-2 is a major growth factor for T-cells. It is part of the cytokine family which also includes IL-4, 7, 9, 15, and 21. All of them act through the IL-2 alpha receptor (CD25), the IL-2 beta receptor (CD122) and the γc receptor (total gamma chain). IL-2 activates Ras / MAPK, JAK-STAT and PI 3-kinase and other signaling pathways that regulate the immune response. These serum levels of IL-2 in patients with hepatitis showed elevated serum IL-2 levels in patients with hepatitis C.

Interleukin-4 (IL-4) is a regulator of the growth and
The decrease in this indicator in patients with CHC was insignificant. These data can be explained by the predominance of Th1 mediated reactions over Th2 mediated reactions in patients with CHC. Such a predominance may be due to the immune processes induced by the hepatitis C virus.

### Interleukin-6 (IL-6)

IL-6 is a 26 kD protein. Refers to multifunctional cytokines. Stimulates the proliferation of T-lymphocytes and endothelial cells. IL-6 is a growth and differentiation factor for B-lymphocytes, hepatocytes and neurons. When exposed to T-lymphocytes, IL-6 stimulates their production of IL-2. One of the most important effects of IL-6 is the stimulating effect on thrombocytopoiesis. A relatively high serum level of IL-6 was detected in both CHC patients and in ALD patients. The data suggests the fact that endogenous immunotropic factors play a predominant role in the development of the inflammatory process leading to liver fibrosis rather than viral infection.

### Interleukin-8 (IL-8)

IL-8 is a 72 amino acid residues. IL-8 producing cells are macrophages, lymphocytes, epithelial cells, fibroblasts, epidermal cells. IL-8 belongs to the group of chemokines, the main property of which is to provide chemotaxis to the zone of inflammation of various cells: neutrophils, monocytes, eosinophils, T-cells. IL-8 has pronounced pro-inflammatory properties, causing the expression of intercellular adhesion molecules and increasing the adherence of neutrophils to endothelial cells and subendothelial matrix proteins. A high level of IL-8 was observed in both study groups. Along with other data, these results point to the similarity of the immune processes occurring during CHC and ALD.

### Endothelial Growth Factor (VEGF)

VEGF is a glycoprotein which binds to endothelial cells and stimulates their proliferation. In addition to the angiogenic action, VEGF has the ability to enhance vascular permeability. A statistically significant decrease in serum VEGF in patients with hepatitis has no explanation yet. Perhaps this decrease is associated with increased consumption of this factor in the area of inflammation. The decrease in blood VEGF can contribute to the development of hypoxia of the liver tissue, and, thus, contribute to its fibrosis.

**Transforming growth factor beta (TGF-β)** is a member of a family of related molecules that have multiple effects on a large number of cell types by participating in the regulation of their growth and differentiation. TGF-β has immunosuppressive properties and, which is extremely important in connection with the present study, is a powerful stimulator of the growth of collagen fibers. A high level of TGF-β relative to control values was detected only in patients with CHC. Perhaps this is due to the fact that the processes of fibrosis in patients with CHC progress with greater speed in comparison with similar processes occurring in patients with ALD.

Thus, the study made it possible to register a number of similar unidirectional changes in patients with chronic hepatitis C and in patients with alcohol-related liver disease. In both groups, there was a statistically significant increase (relative to the control) in serum levels of pro-inflammatory cytokines, namely: IL-1, TNF-α, IFN-γ, IL-2, IL-6 and IL-8 (p <0.05). Elevated TGF-β values were detected in patients with chronic hepatitis C related liver disease (p <0.05), but not in patients with ALD. Significantly lower concentrations of VEGF were observed in both study groups (p <0.05). There was also a significant decrease in serum IL-4 (p <0.05) in patients with CHC, whereas in patients with ALD such a decrease was not statistically significant.

Further, it was of interest to evaluate the dependence of the content of serum cytokines which demonstrated common changes in patients with CHC and ALD on the stage of liver fibrosis. For this, all patients were divided into 3 groups: with early (n = 6), intermediate (n = 9) and late (n = 15) stage of fibrosis. From the obtained data it follows that the content of IL-1 in serum was approximately equally elevated in the early and late stages of fibrosis.

A sharp rise in serum TNF-α levels occurred in the early stages of fibrosis. In the later stages, the rise in the level was replaced by a sharp fall. However, the serum levels of TNF-α in the later stages of liver fibrosis still significantly exceeded control values. Serum levels of IFN-γ did not change depending on the stage of fibrosis, significantly exceeding control values (p <0.05). Relatively high levels of serum IL-2 and IL-6 were noted only in the later stages of the disease.

A clear dependence of the serum concentration on the stage of fibrosis was demonstrated by IL-8. At an early stage, there was a 2-fold increase in this indicator compared with the control (p <0.05). At the
intermediate stage of fibrosis, it exceeded the control value by 4 times, whereas at the late stage, it was almost 6-fold increase. According to the literature, elevated serum IL-8 is associated with chronic and acute inflammatory conditions. It correlates with neutrophil infiltration of inflammatory foci.

Conclusion

According to the data presented in this work, in patients with CHC and ALD, there was a statistically significant increase in serum levels of pro-inflammatory cytokines, namely: IL-1, TNF-α, IFN-γ, IL-2, IL-6 and IL-8. Interestingly, elevated TGF-β values were found in patients with CHC, but not in patients with ALD. Significantly lower concentrations of VEGF were observed in both study groups. There was also a significant decrease in serum IL-4 in patients with CHC whereas in patients with ALD such a decrease was not statistically significant. Serum IL-1 content was approximately equally elevated in the early and late stages of fibrosis. A sharp rise in serum TNF-α levels occurred in the early stages of fibrosis. In the later stages, the rise in the level was replaced by a sharp fall. However, the serum levels of TNF-α in the later stages of liver fibrosis still significantly exceeded control values. The serum levels of IFN-γ in patients significantly exceeded control values without not depending on the stage of fibrosis. Relatively high levels of serum IL-2 and IL-6 were noted only in the later stages of the disease. In both groups of patients, a clear dependence of serum levels of IL-8 on the stage of fibrosis was revealed. It is possible that the definition of serum IL-8 may have diagnostic and prognostic significance for liver diseases of different aetiology.

References

Исследование иммунопатологических процессов, связанных с развитием фиброза печени

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Целью данного исследования было определение зависимости течения фиброза печени от функционального состояния иммунной системы, в частности, от дисбаланса провоспалительных и противовоспалительных иммунных реакций, которые формируются у пациентов при развитии болезни. В исследование были включены 30 пациентов с хроническими заболеваниями печени (18 пациентов с хроническим гепатитом C (CHC) и 12 пациентов с алкогольным заболеванием печени (ALD), 15 человек без данных заболеваний составили группу сравнения. Эластография печени (FibroScan) использовалась для оценки жесткости печени и определения стадии фиброза в соответствии с классификацией METAVIR. В образцах сыворотки этой группы были измерены следующие уровни цитокинов: IL-1β, TNF-α, IL-6, IFN-γ, IL-2, IL-4, IL-8, VEGF и TGF-β. Согласно данным, представленным в данной работе, у пациентов с CHC наблюдалось статистически значимое повышение уровней провоспалительных цитокинов в сыворотке крови, а именно: IL-1β, TNF-α, IFN-γ, IL-2, IL-6 и IL-8. Было замечено, что повышенные значения TGF-β были обнаружены у пациентов с CHC, но не у пациентов с ALD. Значительно более низкие концентрации VEGF наблюдались в обеих группах исследования. Содержание IL-1 в сыворотке было примерно одинаково повышено на ранних и поздних стадиях фиброза. Резкое повышение уровня TNF-α в сыворотке крови наблюдалось на ранних стадиях фиброза. На более поздних этапах состояние было менее выраженным. Однако, уровень TNF-α на поздних стадиях фиброза печени все же значительно превышал контрольные значения. Уровни IFN-γ в сыворотке крови у пациентов значительно превышали контрольные значения без изменений на разных стадиях. Относительно высокие уровни провоспалительных IL-2 и IL-6 были отмечены только на более поздних стадиях заболевания. В обеих группах пациентов была выявлена четкая зависимость уровней IL-4 от стадии фиброза. Анализ данных позволяет сделать вывод, что иммунные механизмы играют значительную роль в патогенезе дегенеративных заболеваний печени. Поэтому необходимы дальнейшие исследования механизмов и роли иммунных факторов для изучения возможных диагностических и терапевтических применений.

Ключевые слова: иммунопатология, фиброз печени, гепатит С, алкогольная болезнь печени

References


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