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The level of lipopolysaccharide-binding protein in acute intestinal infections, the effect of IL-1β and IL-10 on its production

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Abstract

Aim. To determine the level of lipopolysaccharide-binding protein (LBP) in acute intestinal infection, depending on the etiology and severity of the disease, and the effect of interleukin (IL)-1β and IL-10 levels on the expression of LBP.
Methods. Serum samples of 62 patients were assayed by solid phase enzyme-linked immunosorbent assay using a set of reagents ELISA (USA) and Vector-best (Novosibirsk) for levels of LBP, IL-1β, IL-10. The first group included 33 patients with bacterial intestinal infection, the second group consisted of 29 patients with viral diarrhea, and the control group comprised 20 conditionally healthy patients. Analyses were performed using Microsoft Excel 2010 and Statistica version 6.0 software. Statistical differences were determined by using the Mann–Whitney U Test, the p-value ≤0.05 were regarded as statistically significant. Spearman's correlation coefficient was used to examine relationships. Shapiro–Wilk W test was used to check for normal distribution of the features.
Results. We detected the presence of LBP in all the studied groups, with the content of LBP was significantly increased in the group of bacterial intestinal infections compared with other groups. With regard to disease severity, LBP level was the highest for mild acute intestinal infections caused by bacteria, and for viral diarrhea, fluctuations in LBP did not exceed the norm. In intestinal infections caused by bacteria, the levels of LBP were directly related to the levels of IL-1β and IL-10.
Conclusion. Detection of LBP concentration can be used for the initial differential diagnosis of intestinal infections caused by bacteria, which would significantly narrow the diagnostic search and determine the tactics of etiotropic therapy; also, considering the relationship between the concentration of LBP and disease severity, this indicator can be used as a predictive sign of the course of the disease.
Keywords: intestinal infectious diseases, lipopolysaccharide-binding protein (LBP), cytokines.

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Background. Acute intestinal infections (AII) rank second among infectious pathologies for all population groups. The study of the immunopathogenesis mechanisms is of interest to infectious disease specialists. The etiological factor of AII is mainly represented by viruses and the range of gram-negative bacteria, where endotoxin, a lipopolysaccharide, which is involved in both local and general inflammatory reactions, becomes a damaging factor [1,2].

In the case of massive lipopolysaccharide-mediated endotoxemia, the systemic response of the body may become uncontrollable, and is accompanied by marginal leukostasis, granulocytopenia, depletion of myelopoiesis, release of lysosomal enzymes by neutrophils, increased vascular permeability ("capillary leakage"), and redistribution of fluid from the vascular bed to the adjacent tissues [3]. During the development of inflammatory reactions in response to the entry of endotoxin in the body, the synthesis of lipopolysaccharide-binding protein (LBP) is stimulated. LBP is synthesized in the liver, intestinal epithelium, and the lungs under the influence of proinflammatory cytokines with subsequent formation of a low-toxic lipopolysaccharide + LBP complex [4]. LBP levels are regulated by inflammatory mediators.

This work aimed to study the level of LBP in patients with AII, the dependence of its concentration on the disease severity, and the effect of interleukins (IL-1β and IL-10) on LBP synthesis.

Material and methods. We performed a retrospective analysis of case histories and a study of

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Table 1. The level of lipopolysaccharide-binding protein (LBP) and interleukins (IL-1β and IL-10) in acute intestinal infections (AII)

Parameter	Group 1, bacterial AII (n = 33)	Group 2, viral diarrhea (n = 29)	Group 3, control (n = 20)
LBP, mg/ml	5533 [4856.5–6374.5]. p* ≤0.05. p** ≤0.05	5372 [4342–6139]. p* ≥0.05. p** ≤0.05	4177 [3880–4868]
IL-1β, pg/ml	0.534 [0–1.866]. p* ≥0.05. p** ≥0.05	0 [0–1.6]. p* ≥0.05. p** ≥0.05	1.046 [0.409–1.613]
IL-10, pg/ml	5.518 [0.345–7.931]. p* ≤0.01. p** ≥0.05	5.518 [0–11.25]. p* ≤0.01. p** ≥0.05	0 [0–0]

Note: *p** — the level of significance of differences when compared with the control group; *p*** — the level of significance of differences when comparing viral diarrhea with bacterial AII (nonparametric Mann–Whitney method U-test; median, interquartile range between the 25th and 75th percentiles).

the blood serum of 62 patients with AII who were treated at a regional hospital. Group 1 consisted of 33 patients with AII of bacterial etiology. The mean age of the patients was 27.2 ± 8.7 years. Group 2 (comparison group) included 29 patients with viral diarrhea, and the mean age of the patients was 23.2 ± 8.4 years.

The main criteria for the inclusion of patients in the studies were the following conditions:

– age of 15 to 55 years;

– laboratory-confirmed intestinal infection in the acute period; and

– the absence of concomitant diseases in the stage of decompensation and/or in the period of exacerbation, as well as the absence of any other acute pathology.

In all patients, the etiological factor of AII was confirmed by bacteriological and serological studies and polymerase chain reaction.

The control group, formed by the random sampling technique, consisted of 20 somatically healthy people of the corresponding age (mean age 23.3 ± 1.4 years).

The concentrations of LBP and the cytokines IL-1β and IL-10 in blood serum, taken with the written consent of the patients, were determined by the method of enzyme-linked immunosorbent assay using a set of ELISA (USA) and Vector-Best (Russia, Novosibirsk) reagents.

Statistical processing of the data obtained was performed using the electronic programs Microsoft Excel 2010 and Statistica 6.0. To compare the data obtained, we used the methods of nonparametric analysis using the Mann–Whitney U-test. In the event that the calculated value of the U-test was equal to the critical value or less, the differences were considered statistically significant. The distribution of characteristics was assessed using the Shapiro–Wilk W test.

The study presented was approved by the regional ethical committee of the Chita State Medical Academy of the Ministry of Health of the Russian Federation, protocol No. 57 of November 10, 2013.

Results and discussion. In the course of the work, in all studied groups, the presence of LBP was established in the blood serum. The highest protein level was registered in the group of patients with AII of bacterial etiology. It amounted to 5,533 (4,856.5–6,374.5) mg/mL, which exceeded significantly the indicators of the control group, where the concentration of LBP was within 4,177 [3,880–4,868] mg/mL ($p \leq 0.05$).

The high level of LBP is due to the fact that the bacteria that cause intestinal infections are gram-negative and, being destroyed by nonspecific protective factors, release a significant amount of endotoxin. Because lipopolysaccharide is a powerful structural component of gram-negative bacteria [1], for its recognition, primarily by TLR4 receptors, sufficient synthesis of LBP is required. TLR4 receptors are the most important participants in the signaling system of host cells [5], further triggering a cascade of immunological reactions aimed at limiting and eliminating the inflammatory process and formation of immunity.

At low concentrations, LBP was detected both in patients with viral diarrhea and in the control group. In group 2, its level was 5,372 [4,342–6,139] mg/mL, which did not differ from the norm ($p \geq 0.05$; Table 1). This is due to the fact that lipopolysaccharide is a natural antigen, and continuous contact with it occurs throughout life as a result of the widespread circulation of gram-negative bacteria in the environment, and the presence of gram-negative bacteria of the *Enterobacteriaceae* family in the distal parts of the human gastrointestinal tract.

Thus, LBP recognizes, binds, and sequentially presents bacterial endotoxin to hereditarily

Table 2. Correlation between lipopolysaccharide-binding protein (LBP) and cytokine levels ($p \leq 0.05$)

Clinical group	LBP, mg/ml	IL-1β	IL-10
Viral diarrhea (n = 29)	5372 [4342–6139]	–0.036	–0.027
Clarified bacterial diarrhea (n = 33)	5533 [4856.5–6374.5]	+0.659	+0.601

Note: r = 0–0.3 implies low correlation; r = 0.3–0.7 implies medium connection; r = 0.7–1 means strong correlation; in the case of a "+" result, the correlation is direct, while in case of a "–" result, the correlation is inverse; IL — interleukin.

encoded receptors located on leukocytes and other cells. This increases the sensitivity of the receptors to the pathogen, and enhances the signal of the risk of infection [6] when the pathogen enters the macroorganism, as well as the maintenance of normal immunogenic activity.

Considering the pronounced induction and expression of LBP in patients with bacterial intestinal infection, the next stage of our study was to establish the relationship between the level of LBP synthesis and the disease severity. As a result, it was determined that the LBP synthesis in both mild (5,533 [4,690–6,576] mg/mL) and moderate (5,351 [4,221–6,078] mg/mL) courses significantly exceeded the level of LBP in healthy people (4,177 [3,880–4,868] mg/mL; $p \leq 0.01$ and $p \leq 0.05$, respectively). At the same time, patients with moderate severity of the disease were characterized by lower LBP values in comparison with the mild course of AII ($p \leq 0.05$).

In case of viral diarrhea, regardless of the severity of the disease, the LBP values remained within the conditional norm, namely 4,675 [4,019–5,861] mg/mL for mild severity and 5,131 [4,121–5,848] mg/mL for moderate severity. The indices were comparable with the control group ($p \geq 0.05$).

It should be taken into account that the expression of LBP is regulated by inflammatory mediators, including IL-1β and IL-10, which play one of the most significant roles in the response to lipopolysaccharide [7]. A comparative analysis revealed a significant increase in the level of anti-inflammatory IL-10 in the groups with AII compared with the control. Thus, with bacterial AII, the level of IL-10 was determined within the range of 5.518 [0.345–7.931] pg/mL; with viral AII, it was 5.518 [0–11.25] pg/mL, and in the control group, it was 0 [0–0] pg/mL ($p \leq 0.01$). Indicators of pro-inflammatory IL-1β varied within the normal range and amounted to 0.534 [0–1.866] pg/mL and 0 [0–1.6] pg/mL in comparison with 1.046 [0.409–1.613] pg/mL, respectively ($p \geq 0.05$). There was no predominant synthesis of cytokines depending on the etiological factor ($p \geq 0.05$; Table 2).

Subsequently, we evaluated the interaction of IL-1β, IL-10, and LBP in the case of bacterial AIIs as a response to stimulation with lipopolysaccharide. A direct dependence of LBP synthesis on IL-1β was established (r = +0.659, $p \leq 0.05$). IL-1β, a pleiotropic cytokine, alters the response of a macroorganism to an inflammatory, infectious process [7, 8], by acting on inflammatory mediators at all levels of immunopathogenesis, including the synthesis of LBP. In turn, LBP induces the expression of IL-10 as a direct link of average strength (r=+0.601, $p \leq 0.05$; see Table 2), in contrast to lipopolysaccharide, where a feedback with IL-10 was registered [7]. It should be noted that IL-10 plays a key role in resolving infection, and the induction of this cytokine can improve significantly the host immune response [9]. Therefore, we can regard the presence of a high or low concentration of LBP in the blood serum as a direct predictor of the severity of the disease course.

In a retrospective analysis of the presence and duration of febrile intoxication syndrome in patients with AII of bacterial origin, it was found that at the time of admission, the febrile intoxication syndrome was more pronounced in moderate cases and manifested itself with fever up to 38°C [38–39°C] ($p \leq 0.05$), severe atony, headache, lack of appetite. With a mild degree, there was a subfebrile body temperature of 37.3°C [36.6–38°C], the above symptoms were mild or absent. In the hospital, in the course of therapy, this state persisted on average 3 ± 1 days with average body temperature of 37.8°C [37.2–38.4°C] for moderate forms, and 37°C [36.6–38.3°C] for mild forms ($p \geq 0.05$). As a result of the correlation analysis, the dependence of the severity and duration of febrile intoxication syndrome on the level of LBP expression was not established ($p \geq 0.05$).

CONCLUSIONS

1. Lipopolysaccharide-binding protein is present in the body of both patients with acute intestinal infections and healthy people. The highest level of lipopolysaccharide-binding protein was recorded in patients with acute intestinal infection of bacterial etiology, with a predominance of its synthesis in those with a mild severity of the disease.

2. A direct correlation was established between lipopolysaccharide-binding protein and interleukin-1β and interleukin-10. Interleukin-1β induces the expression of lipopolysaccharide-binding pro-

tein, which in turn stimulates the synthesis of interleukin-10.

3. Determination of the concentration of lipopolysaccharide-binding protein can be used for the primary differential diagnostics of intestinal infections caused by bacterial agents, which will narrow significantly the diagnostic search, as well as predict the severity of the disease and the possibility of an unfavorable outcome.

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