INTRODUCTION

Sepsis caused by gram-negative bacteria is known to induce severe complications after surgery, traumas, and burns. It remains a severe pathological state characterized by high lethality, which may reach 50% at septic (endotoxic) shock in spite of application of antibiotics of the last generation, which are characterized by a wide spectrum of activities, and state-of-the-art methods of treatment [1]. Ineffectiveness of immune therapy of septic states after surgery makes it necessary to develop new approaches not only to treatment of sepsis, but also to prophylaxis and prevention of septic states.

Endotoxic shock may occur after introduction of a bacterial endotoxin which is lipopolysaccharide (LPS), which is known to be the component of cellular wall of gram-negative bacteria, to the organism in a pure form. The destructive role of LPS is caused by massive up-regulation of proinflammatory cytokines release, such as TNF-α and IL-1β [2, 3]. Overproduction of proinflammatory cytokines triggers a series of pathological processes associated with sepsis: generalized inflammation, damage of vessel endothelium, activation of coagulation and inhibition of fibrinolysis. These disorders may lead to disseminated intravascular clotting (DIC), tissue hypoxia, and, eventually, to damage of tissues and dysfunction of organs [4]. Together with stable hypotension, these events lead to endotoxic (septic) shock.

Recently, a conception of immune prophylaxis of endotoxic shock aimed at the development of anti-shock vaccines on the basis of carbohydrate biopolymers, which would provide resistance of an organism to further massive contamination with bacterial endotoxin, has been proposed. Despite the importance of the problem, the number of studies aimed at prophylaxis of endotoxic shock is rather limited. These include studies of lipopolysaccharides, which endotoxicity does not allow their use in clinical practice [5].

In the present study, we have tried to assess the basic possibility to prevent endotoxic shock by prophylactic immunization of mice (CBA × C57B1/6)F1 with a new apyrogenic, low-endotoxic complex preparation on the basis of carbohydrate biopolymers of S. sonnei, such as exopolysaccharide (EPS) and native lipopolysaccharide, which possessed full endotoxic activity, using experimental model of endotoxic shock.

EXPERIMENTAL

Experimental Modeling of Endotoxic Shock in Mice (CBA × C57B1/6)F1 Sensibilized with D-galactosamine

Mice (CBA × C57B1/6)F1 were obtained from the National Center for Biomedical Technologies (Andreevka, Russia). The average weight of the animals was 20–22 g. Complex preparation (EPS + LPS) S. sonnei, was introduced intraperitoneally (i/p) in the doses 0.1, 1, 10, or 25 μg/mouse 12 h prior to injection of standard endotoxin of E. coli O:55 (Sigma-Aldrich, United States) together with 15 mg of D-galactosamine (Alfa Aesar, Germany) dissolved in 0.5 mL
of 0.9% sodium chloride (saline). The control group consisted of animals that received i/p injections of 0.5 mL saline 12 h prior to introduction of D-galactosamine and LPS of *E. coli* O:55 in the same doses as in the experimental group.

**Direct Model of Endotoxic Shock**

Mice (CBA × C57B1/6)F1 i/p received lethal dose of LPS *E. coli* O:55 (2 mg per mouse) without sensitizers 72 h after i/p treatment with (EPS + LPS) *S. sonnei* in the doses of 100, 200, or 400 μg per mouse in 0.5 mL of saline. The control group consisted of animals that received intraarterially of 0.5 mL saline 72 h prior introduction of 2 mg lipopolysaccharide of *E. coli* O:55.

Concentration of TNF-α in serum of intact animals and animals immunized with (EPS + LPS) *S. sonnei* was measured using Quatikine Mouse TNF-α/TNFSF1A testing system (R&D Systems, United States) by the method of solid-phase ELISA according to the manufacturer’s protocol. Blood samples were collected 90 min [6] after induction of endotoxic shock.

**RESULTS AND DISCUSSION**

**Prophylaxis of Endotoxic Shock at Preliminary Introduction of the Complex Preparation (Exopolysaccharide + Lipopolysaccharide) from *S. sonnei***

Study of protective properties of the complex preparation (EPS + LPS) *S. sonnei* against pathological effects of TNF-α in vivo was carried out on the model of endotoxic shock, using D-galactosamine (D-GalN) as an endotoxicity sensitizer. Hepatocytes are known to participate in elimination of lipopolysaccharide from the bloodstream, detoxication of lipopolysaccharide, and production of inflammatory mediators in response to endotoxin appearance in bloodstream [7]. D-GalN induces exhaustion of uridine triphosphate in liver that results in disordering of membrane glycoprotein and glycogen synthesis, and eventually death of hepatocytes and Kupffer cells. Hence, liver loses its protective function, and sensitivity threshold of an organism consequently decreases not only with respect to lipopolysaccharide but also to TNF-α [8]. The latter is known to play a key role in lethality of mice after introduction of D-GalN/LPS [9] that is due to specific apoptosis of hepatocytes and acute hepatic failure [10].

Prophylactic immunization of mice with 0.1, 1, 10, or 25 μg per mouse complex preparation (EPS + LPS) *S. sonnei* provided 100% survival of animals in which endotoxic shock was induced by introduction of D-GalN. In the control group, the lethality of animals for the first 24 h was 100%. Taking into account the mechanism of D-GalN action, it may be suggested that protective effect of the complex preparation is due to inhibition of synthesis of TNF-α in the liver.

The direct model of endotoxic shock reproduces an important pathogenetic aspect of its clinical course—massive release of bacterial endotoxin in the patient’s organism induced by introduction of lethal dose (150 mg/kg) of lipopolysaccharide of *E. coli* O:55. Primary mild activation of cells at low concentrations of endotoxin leads to formation of their resistance to secondary massive contamination of the organism with lipopolysaccharide even in lethal doses. This phenomenon is known as endotoxin tolerance. Application of lipopolysaccharide of gram-negative bacteria as an agent for correction of endotoxic shock is based on this phenomenon, which is based either on blocking of bacterial endotoxin binding with TLR4 receptors or disorganization of signal transduction from the receptor complex TLR4/MD-2 and production of proinflammatory cytokines [11]. Early endotoxic tolerance is nonspecific. It develops 3–4 days after introduction of endotoxin [5]. Therefore, tolerogenic effect of the preparation was assessed by survival of animals 3–4 days after injection of LPS *E. coli* O:55 (Table 1).

Animals immunized with 400 μg per mouse of the complex preparation demonstrated 100% survival. Immunization with 200 μg and 100 μg provided 90% and 60% survival, respectively, by the end of the third day of the experiment.

In the control group, 100% lethality was observed by the end of the second day of the experiment. The obtained data show that pretreatment with (EPS + LPS) *S. sonnei* protects animals from further introduction of lethal dose of the endotoxin in a dose-dependent manner. In other words, the increase of immmu-

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**Table 1: Survival of mice (CBA×C57B1/6)F1 in the direct model of endotoxic shock after immunization with the preparation (exopolysaccharide + lipopolysaccharide) from *S. sonnei***

<table>
<thead>
<tr>
<th>Preparation dose, μg per mouse</th>
<th>Number of mice</th>
<th>Death of mice, h</th>
<th>Survival, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0–24</td>
<td>24–48</td>
</tr>
<tr>
<td>400</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Control (0 μg)</td>
<td>10</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

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**Survival, %**

- 0–24: 100%
- 24–48: 90%
- 48–72: 60%

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**Notes:**

1. **Survival, %**
2. **Death of mice, h**
3. **Preparation dose, μg per mouse**
4. **Number of mice**

**Acronyms and Abbreviations:**

- EPS: Exopolysaccharide
- LPS: Lipopolysaccharide
- TNF-α: Tumor Necrosis Factor alpha
- TLR4: Toll-like Receptor 4
- MD-2: Myeloid Differentiation Protein 2