Abstract—The study of cytokine expression in mice under the influence of inactivated poliovirus and Imovaks polio vaccine in combination with derivatives of chitosan shows various kinds of processes. There is a significant increase in IL-12 in the serum of immunized animals, which should stimulate the production of IFN-γ NK-cells and T-cells and polarize the immune response to Th1 type. Thus, the derivatives of chitosan can promote cell component of the immune response, providing a full antiviral immunity.

Keywords—Poliovirus, chitosan, cytokine expression, antiviral immunity.

I. INTRODUCTION

POLIO vaccination is carried out in most countries with the live polio vaccine (LPV). However, the application of the vaccine due to the possibility of post-vaccination for polio reversion of attenuated vaccine strains. In this regard, the use of inactivated polio vaccine (IPV), does not result in such complications is more appropriate. Taking into account the weak immunogenicity of IPV is very important inclusion adjuvants in to the vaccine that enhance the immunogenicity and protective efficacy of polio vaccines [1-4].

The purpose of research is the study of immune response during parenteral immunization of mice with IPV, including chitosan derivatives as adjuvants.

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II. MATERIALS AND METHODS

The paper used the polio vaccine inactivated "Imovaks Polio" (Sanofi-Pasteur, France) containing polioviruses 1, 2, Type 3, and live polio vaccine for oral use 1, 2, 3 types (FGU "PIPVE" named M.P. Chumakov RAMS). As an adjuvant we used chitosan, derived from the shells of shrimp, by a single crumb shell deproteinization in 5% solution of sodium hydroxide and re-demineralization in 2.5% hydrochloric acid, followed by deacetylation of 40% sodium hydroxide.

In the experiments we used two types of drugs - a micro/nanoparticles (MNP) of sulfate chitosan (SC) and glutamate chitosan (GC). Experiments were carried out on CBA mice and C57/B16 weighing 10-12 g (females) obtained from the nursery of Biomedical Technology Research Center of RAMS "Andreyevka". Mice were injected intramuscularly (twice with an interval of 21 days) with 0.2 mL formulation containing 3 mg for each component of the vaccine, in combination with 0.5% chitosan glumatate with different pH, or buffer.

10 days after the second immunization, the animals were taken blood in accordance with the "Rules of the work from the experimental animals."

Assessment of spleen lymphocyte subpopulation was performed by flow cytometry with monoclonal antibodies (Caltag Laboratories, USA) against relevant antigens of various lymphocyte populations.

Cytokine levels were determined in serum/plasma of mice by ELISA using test kits (Biosource, Austria) in the range of detectable concentrations of 1 to 13 pg/ml.

Splenocytes from immunized mice were pre-incubated for 24 h in growth medium RPMI 1640 + PHA (5 µg/ml), then examined induced production of cytokines in the supernatants. Cytokine levels were determined by flow cytometry FacsCalibur (Becton Dickinson, USA) using a test system FlowCytomix Mouse Th/Th2 10 plex (Bender MedSystems, Austria) according to the manufacturer's instructions. Statistical analyzes were conducted using the software package Excel (Microsoft Corporation, USA), an integrated statistical package Statgraphics Plus v5.0 (Manugistics Group, Inc., USA) using parametric and nonparametric methods.

III. RESULTS AND DISCUSSION

In the study of levels of IgG in sera of mice immunized with inactivated Sabin strains of poliovirus types 1, 2, 3 in...
Inactivated poliovirus type 1 has a negligible effect on the lymphocytes (CTL) after a single and double immunization. The mechanism of enhancing the immune response in combination of poliovirus or polio vaccine with chitosan may be connected with the conduct of signals through Toll-receptors. This paper investigated the effect of chitosan products, inactivated poliovirus and polio vaccines, as well as the combined effect of inactivated poliovirus, and poliomyelitis vaccine in conjunction with preparations for a group of chitosan Toll-like receptors: TLR2, TLR4, TLR9. Chitosan derivatives activate these receptors to different degrees. The most active of chitosan glutamate and micro/nanoparticles of chitosan sulfate activate TLR9. To a lesser degree of chitosan derivatives activate TLR2. When intramuscular administration of inactivated poliovirus observed activation only TLR9. Introduction of polio vaccine was accompanied by activation of all three studied TLRs. As chitosan glutamate and micro/nanoparticles of chitosan sulfate showed adjuvant effect in combination with inactivated poliovirus, increasing the expression of these receptors and increasing the relative number of cells expressing them. A similar pronounced adjuvant effect of chitosan glutamate was observed in combination of this drug with polio Immovaks.

Parenteral administration to mice and inactivated poliovirus Polio Immovaks accompanied by an increase in the serum of mice a number of cytokines, including INF-γ, IL-17, TNF-α, IL-5, TGF-β, IL-6, IL-10. There was a significant increase in TNF-α (10-fold), TGF-β (8-10 times), IL-6 and IL-10 (2-4).

In control experiments, injection of chitosan glutamate (CG) and micro/nanoparticles of chitosan sulfate (CS) led to the appearance in the serum of mice, a significant amount of IL-6 (2-4), IL-10 and moderate amounts of IL-5. There was a sharp increase in IL-12 during immunization poliovirus in conjunction with micro/nanoparticles of CS (in 2-4, 9, 6 times in comparison with poliovirus) and polio vaccine in combination with both the GC and the CS (in 3, 4, 4 times after 8 h compared with polio Immovaks). On the other hand there was a moderate increase in INF-γ at all stages of follow-up (2-24 h) and the reduction of TNF-α with the introduction of poliovirus with chitosan derivatives in 8.5 (GC) and 4.6 times (CS)) and 24 hours (2.2-fold (CG) and 4 times (CS)) and barely noticeable decrease with the introduction of polio vaccine with chitosan.

The combined injection of poliovirus with chitosan increased the expression of TGF-β (in 5, 6-1, 6 times in comparison with poliovirus) at 8 and 24h and the expression of IL-6 (2.5-5, 3 times in comparison with poliovirus), followed by decline in the same period. Administration as inactivated poliovirus type 1, and trivalent inactivated polio vaccine to the CG lead to an increase subpopulations CD3+,
NK, CD3/NK, CD8+ and γδT cells and their activation (expression of CD25+ and MHC II). Significantly increases the number of CD3/NK cells (5.8-6.8%), with intramuscular administration of polio vaccine to the CG. After immunization of mice with inactivated polio vaccine or drugs poliovirus in combination with a suspension micro/nanoparticles of chitosan is growing in number these subpopulations, T-helper cells (CD4+) and B cells (CD19+). This fact indicates that the use of micro/nanoparticles of chitosan as an adjuvant induces a connection mechanisms of activation of both cellular and humoral immunity and that is an important factor for the formation of a full immune response to innate and adaptive components.

Chitosan derivatives without antigen can activate receptors TLR2 and TLR9, and in combination with inactivated polio vaccine preparation of poliovirus and they exhibit a pronounced adjuvant effect, increasing the expression of these receptors and increasing the content of expressing cells.

The study of cytokine expression in mice under the influence of inactivated poliovirus and Imovaks polio vaccine in combination with derivatives of chitosan shows various kinds of processes. There is a significant increase in IL-12 in the serum of immunized animals, which should stimulate the production of IFN-γ NK-cells and T-cells and polarize the immune response to Th1 type. Thus, the derivatives of chitosan can promote cell component of the immune response, providing a full antiviral immunity.

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