

# Features of the Immune Response in Mice were Immunized with Polio Vaccine in Combination with Chitosan Preparations as Adjuvants

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**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- $\gamma$  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

**P**OLIO 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/Bl6 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 glutamate 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  $\mu$ g/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 Th1/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

combination with derivatives of chitosan (GC and SH), after double immunization IgG titer was at 1:3200, 1: 6400 and 1:12400, respectively, and after the third immunization - 1:12800, 1:12400 and 1:6400, respectively. However, the introduction glutamate chitosan in to the drug inactivated poliovirus types 1, 2, 3, increased serum IgG titers after twice immunization to level 1:152000, 1:152000 and 1:102000, respectively, after three immunizations - to the level of 1:102400, 1:102400 and 1:409600 respectively.

Similarly, the introduction suspensions of chitosan micro/nanoparticles sulfate in to the drug inactivated poliovirus types 1, 2, 3 was an increase of serum antibody titer after twice immunization to level 1:102400, 1:102400 and 1:51200, respectively, after three immunizations before level 1:204800, 1:102400, 1:102400, respectively. The results indicate a pronounced adjuvant effect of chitosan derivatives with respect to inactivated poliovirus Sabin all types.

Inactivated poliovirus type 1 has a negligible effect on the cellular part of the immune response in mice, reflected in the increasing numbers of NK, NKT (CD3/NK) and T-reg (CD4/CD25/Foxp3+) cells on the seventh day after a single immunization and reducing the number of cytotoxic lymphocytes (CTL) after a single and double immunization. Polio vaccine immunization Immovaks resulted in moderate decreases in the number CD3+ T lymphocytes (from 24.4 to 15.5%) for all periods of observation, NKT cells on the first day of immunization and CTL (CD8+) - on the seventh day after the first and second immunization, and as CD19 (B lymphocytes) after the second immunization week. This fact indicates the suppressive effect of inactivated vaccines on the immune system.

Intramuscular injection only glutamate chitosan mice increased the number of NK cells (from 2.0 to 6.7%) on the first day, the expression of MHC II (c 20 to 37%) on day 7 after the second immunization. Parenteral administration of micro/nanoparticles chitosan sulfate led to an increase in the number of NK cells (from 2.0 to 7,2-6,5%), CD3/NK cells (from 0.5% to 2.3%) and CD4/CD25/Foxp3+ (from 0,8 to 2,4%) on the first day after the first and second immunization, as well as expression of MHC II (from 20 to 40%) on the seventh day after the first immunization.

The combined administration of inactivated poliovirus together with chitosan glutamate increased significantly subpopulations CD3/NK (NKT), CD4/CD25/Foxp3+ and  $\gamma\delta$ T cells. Parenteral administration of poliovirus in combination with micro/nanoparticles of chitosan sulfate significantly increased the percentage of cells CD3/NK (from 2.7% to 9.6%) on the seventh day the first immunization, and on the seventh day CD8+ (from 4,6 to 17% after the first immunization), MHC II (from 22 to 42.5%, after the second immunization),  $\gamma\delta$ T and CD19+ (from 16.3 to 56.4% after the second immunization).

Administration Polio Immovaks with chitosan glutamate to mice led to an increase the subpopulations: CD3+, CD3/NK, CD4/CD25/Foxp3+ at all stages of immunization, CD8+,  $\gamma\delta$ T, CD19+ - on the seventh day of the first and second immunization, CD5+ - on the seventh day the second

immunization. However, intramuscular immunization with polio vaccine containing adjuvant micro/nanoparticles of chitosan sulfate was associated with increased levels of CD3+, NK, CD3/NK, CD8+,  $\gamma\delta$ T, CD19+ at all stages of monitoring and CD4/CD25/Foxp3+ - on the first and seventh days after the first immunization.

Thus, the effect of chitosan glutamate and micro/nanoparticles of chitosan sulfate found immunotherapy to the effect of inactivated polio vaccine.

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 Imovaks.

Parenteral administration to mice and inactivated poliovirus Polio Imovaks accompanied by an increase in the serum of mice a number of cytokines, including INF- $\gamma$ , IL-17, TNF- $\alpha$ , IL-5, TGF- $\beta$ , IL-6, IL-10. There was a significant increase in TNF- $\alpha$  (10-fold), TGF- $\beta$  (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, 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 4,3-4,4 times after 8 h compared with polio Immovaks). On the other hand there was a moderate increase in INF- $\gamma$  at all stages of follow-up (2-24 h) and the reduction of TNF- $\alpha$  with the introduction of poliovirus with chitosan derivatives in 8 (5.3 (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- $\beta$  (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 polio virus type 1, and trivalent inactivated polio vaccine to the CG lead to an increase subpopulations CD3+,

NK, CD3/NK, CD8+ and  $\gamma\delta$ T 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- $\gamma$  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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