The Development of Polio Vaccines: the Current Update (Review)

DOI: 10.17691/stm2019.11.4.22
Received August 12, 2019

© **A.A. Ishmukhametov,** MD, DSc, Professor, Corresponding Member of the Russian Academy of Sciences, General Director[1]; Head of the Department of Organization and Technology of Immunobiological Production[2];
A.A. Siniugina, Production Manager[1];
K.M. Chumakov, DSc, Staff Member, Center for Biologics Evaluation and Research[3]

[1]Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products, Russian Academy of Sciences, Moscow Settlement, 8/1 Village of Institute of Poliomyelitis, Moscow, 108818, Russia;
[2]I.M. Sechenov First Moscow State Medical University (Sechenov University), 8/2 Trubetskaya St., Moscow, 119991, Russia;
[3]Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, Food and Drug Administration, 10903 New Hampshire Avenue, Silver Spring, MD 20993, USA

The dramatic history of the development and use of polio vaccines reflects the evolution of vaccine preparations under the influence of changing epidemiological conditions and socio-economic factors.

The invention of two polio vaccines — the inactivated Salk vaccine and the live oral vaccine from Sabin strains, each with its own advantages and disadvantages — is on the list of the most significant medical achievements of the XX century. Over the past 50 years, these vaccines were used in various modalities, schemes, and combinations. As a result, poliomyelitis has been completely eradicated in almost all countries of the world. The sustained WHO-led efforts toward full eradication of the disease are expected to result in complete cessation of the virus circulation. In this case, the poliovirus, like the smallpox virus, will remain only in laboratories. However, it would be unreasonable to stop the vaccination even after the pathogen circulation has been stopped like it was in the case with the elimination of smallpox virus. Unlike the smallpox vaccination, vaccines against poliomyelitis will not lose their relevance in the near future because these two viruses significantly differ from each other in terms of biological and epidemiological characteristics.

Key words: poliomyelitis; poliovirus; live attenuated vaccine; inactivated vaccine.

Introduction

Vaccines are of special significance among medical products. The earliest vaccines were invented centuries ago; some of them are still produced using these early methods. However, the increasing requirements of safety and efficiency of production, stimulate manufacturers to develop innovative approaches.

Polio vaccines are on the list of the most frequently used and effective ones; they serve as a reference for other vaccination products. More than 60 years ago, polio vaccines made it possible to practically get rid of this dangerous disease in most countries of the world. This overwhelming success led to the public reassessment of vaccines and the prevalence of benefits over the risk of complications. As a result, a number of important changes had been introduced in polio vaccination programs.

Today, the goal is set to completely eradicate the disease in the near future; this goal necessitates developing innovative vaccines to replace the current ones. This change in policy reflects changes in the epidemiological situation and socio-economic factors, and also rationalizes the need for continuous improvement of vaccine production.

The present review provides general information on polio and the history of vaccines against this disease.

General characteristics of poliomyelitis

Poliomyelitis (inflammation of the gray matter of the spinal cord, from the Greek. *polios* — gray and *myelos* — the spinal cord) is an acute infectious disease caused by poliomyelitis virus (poliovirus) of one of the three serological types. The clinical manifestations vary from asymptomatic infection, mild malaise to severe body paralysis if the virus enters the central nervous system (CNS). In the latter case, the infection causes irreversible damage to motor neurons of the gray matter of the anterior spinal horns and the nuclei of the cranial nerves of the brain stem.

The disease was first described in the XVIII

Corresponding author: Aidar A. Ishmukhametov, e-mail: ishmukhametov@chumakovs.su

century by the British physician Underwood [1]; yet the humanity had been confronted with polio many centuries earlier. Images of people with characteristic manifestations of polio from ancient Egypt date back to the XVI–XIV centuries BC. However, until the end of the XIX – beginning of the XX centuries, poliomyelitis was a sporadic disease affecting mainly children (it was then called "childhood paralysis") [2–5] and it did not attract much attention against the background of plague, cholera, and smallpox epidemics. In the early XX century, the epidemiological profile of polio began changing so that its outbreaks gradually grew into world-wide epidemics [6–8]. Unexpectedly, the reason for this transformation was a dramatic improvement in hygiene habits due to changes in socio-economic factors. In the past, most children had first exposure to the virus at a very young age when they were protected by mother's antibodies and, therefore, were more resistant to the virus. Because of that, before the XX, the incidence rate was low — the disease affected one of several hundred infected kids. Thanks to this early contact with the virus, the vast majority of children received immunity for life. Thus, wild strains of poliovirus themselves provided vaccination for humans and thereby limited the spread of the disease. The development of sanitary requirements and hygiene postponed the child's first contact with the virus to a later age when the child no longer had mother's antibodies. As a result, the incidence of paralysis has increased. The decrease in collective immunity against poliovirus created the conditions for a rapid spread of the virus, an increase in epidemic outbreaks and severity of the disease.

The first major polio outbreak in Europe (1031 cases) occurred in 1905 in Sweden. In the United States, the first report on multiple cases of poliomyelitis in one state was published in 1843; in 1916, a polio epidemic was declared throughout the entire country (27 thousand cases of the disease, 6 thousand cases with fatal outcome). In Russia, reports on limited outbreaks of poliomyelitis (several dozen cases) began to appear from 1905. Prior to the vaccination campaign in the USSR, a steady increase in the polio incidence was observed; it reached its peak in 1958: more than 13 thousand cases (10.66 per 100 thousand population) [9].

Etiology of poliomyelitis. In 1909, the Austrian researchers Landsteiner and Popper [2] reported for the first time on successful isolation of poliovirus. At the same time, Flexner and Lewis [10] showed that primates were also susceptible to poliovirus and able to develop immunity after both passive and active immunization [11]. Further studies revealed the existence of three distinct poliovirus serotypes [12–14].

The "poliomyelitis virus (poliovirus)" entity combines three antigenically different viruses (types 1, 2, 3) belonging to the *Enterovirus* genus [15] of the *Picornaviridae* family [16].

This small viral particle (27–30 nm in diameter) has no lipoprotein membrane; its genome is represented by single-stranded RNA of about 7500 nucleotides confined inside a capsid of icosahedral symmetry. The capsid consists of 60 copies of each of the four capsid proteins (VP1–VP4) that determine the antigenic specificity of the poliovirus particle. The poliovirus reproduction cycle in a cell takes about 7 h. The penetration of poliovirus into a living cell occurs with the help of a specific receptor CD155 — a glycoprotein (belonging to the superfamily of immunoglobulins), which is located on the cytoplasmic membrane of human and monkey cells. Attachment to the receptor causes changes in the capsid structure and thus allows the virus to enter the cytoplasm. No other picornavirus can use this protein as a cell receptor. After the penetration, the viral genome is translated and replicated, the viral particles mature and leave the cell; the host cell then dies [17–19]. Type 1 poliovirus is the most neurovirulent of all three serotypes.

The current classification of polioviruses (regardless of serotype) subdivides them by the genetic similarity to the Sabin vaccine strain, i.e., by the number of nucleotide substitutions in the genome fragment encoding for VP1 protein [20–24].

Poliovirus is highly resistant to external factors (temperature, pH, disinfectants). Under laboratory conditions, it remains viable for many years at –20°C, and for many months at 2–4°C. For several months, the virus can persist in the environment contaminated by human feces (soil, sewage, surface water). Poliovirus is not destroyed by digestive juice. When heated to 50°C, it dies within 30 min. It is quickly destroyed by boiling, ultraviolet radiation or drying. It can also be inactivated by disinfectants [25].

Pathogenesis. All three types of poliovirus cause acute infection. The primary reproduction of the virus in the human body occurs in the tonsils, intestinal M-cells, Peyer's plaques of the intestine, or mesenteric lymph nodes. The virus enters the bloodstream, and (in some cases) the CNS, where it spreads along the nerve fibers and can damage or completely destroy nerve cells. The virus multiplies mainly in motor neurons of the anterior horns of the spinal cord; cell death leads to the development of flaccid muscle paralysis. Sometimes, the virus settles in the brain stem cells innervating the respiratory muscles; as a result, breathing is affected and bulbar paralysis develops [25].

Reservoir and source of the infection. The reservoir and source of polio infection in nature is either an infected person or a carrier [25, 26]. During the first days of infection, before the appearance of the severe clinical symptoms, the virus can be detected in the blood and pharyngeal mucus, and also in feces. For virological diagnosis, massive ($10 \cdot 10^6$ virus particles per 1 g) virus excretion with feces for 3–4 weeks (up to 2 months) is essential regardless of the form of infection. Excretion of the virus can be intermittent, and its amount is gradually reduced with time.

Transmission of the pathogen occurs via the fecal-oral (main) or aerosol (probable) mechanisms, as well

as via vertical transmission (possible). The routes of transmission include water, food, contact, or air.

The natural susceptibility of humans is high, but a clinically pronounced infection is much less common than the carrier state: one case with clinical manifestation accounts for 100 to 1000 cases of asymptomatic carriage of poliovirus. Therefore, for the epidemic significance, cases of asymptomatic carriage or asymptomatic infection pose a greater risk.

Post-infectious immunity (lifelong) is specific to only one type of poliovirus, therefore a person who has been infected with one of the poliovirus serotypes remains susceptible to infection with other serotypes.

The incubation period in acute poliomyelitis ranges from 4 to 30 days, in most cases — from 6 to 21 days.

The clinical picture. The basic information and characteristics of polio infection were obtained from studies on monkeys [25–28]. Later, it was found that humans were also susceptible to the virus and that almost everyone could contract the infection. In about 85% of infected individuals, the infection is asymptomatic, about 15% develop a mild or moderate febrile illness, and only in 0.1–1% it causes CNS damage — paralysis, paresis, meningitis.

The clinical manifestations of poliomyelitis range from asymptomatic infection to severe disseminated paralysis. Four forms of poliovirus infection are defined.

1. The inapparent form (virus-carrying) is not accompanied by any clinical symptoms; the infectious process is limited to virus multiplication in the upper respiratory tract and the intestine. Diagnosis is made only by virological tests. It makes up approximately 72% of all cases of polio infection.

2. The abortive form (minor illness) is characterized by general infection symptoms with no signs of CNS damage: mild febrile events, malaise, headache, nausea, sometimes catarrhal symptoms or intestinal dysfunction. With the abortive form, viremia is usually detected. It makes up about 24% of infections.

3. The meningeal form develops due to the virus entry into the CNS and the inflammatory response of the meninges. This form is characterized by an acute onset and accompanied with serous meningitis syndrome, fever, severe headache, stiff neck, and vomiting; sometimes pain in the limbs, neck, and back is present. It makes up 4% of all polio cases.

4. Paralysis — the consequence of the viral invasion into the CNS — develops in less than 1% of infected individuals.

The course of paralytic polio is usually divided into 4 periods: preparative, paralytic, restorative, and residual. The pre-paralytic period begins after the incubation period; its duration varies from several hours to 3–6 days. It begins acutely, with fever, symptoms of intoxication, catarrhal manifestations in the oropharynx, and sometimes, dyspepsia. The paralytic period is characterized by apparent motor impairment (most often in the morning), which aggravates within a few hours, but not longer than 3 days. After 2–3 weeks, a recovery period begins (6–12 months), during which the restoration of impaired motor functions develops. In the least affected muscles, only partial recovery can be achieved; severely affected muscles remain completely paralyzed. Paralysis that remains after the recovery period is attributed to the residual effects of paralytic polio; they remain with the patient for life. About 20–25% of patients who underwent paralytic poliomyelitis develop a condition referred to as post-poliomyelitis syndrome — B91 according to ICD-10, 20–50 years after the paralytic phase. This post-polio syndrome is a slowly progressing illness characterized by muscle weakness, fatigue, myalgia, arthralgia, and respiratory disorders. It affects both atrophied and previously unaffected muscles.

Depending on the anatomical location of the affected motor neurons in the spinal cord or brain stem, the syndrome can have spinal, bulbar, pontine or mixed (pontospinal or bulbospinal) clinical forms.

The variety of clinical manifestations of the infectious process and the predominance of asymptomatic infection that drives the steadily developing epidemic process are the main factors complicating the eradication of poliomyelitis.

Specific laboratory diagnostics. In the cases of acute flaccid paralysis (AFP), the diagnostic procedure includes virological and serological analyses, instrumental and clinical examinations, and neurological tests 60 days after the onset of the disease [25, 26, 29]. The acute onset (from several hours to 1–2 days), peripheral paresis and paralysis, proximal limb involvement, and asymmetric paralysis — all those indicate the diagnosis of polio. At the same time, sensitivity is preserved, and pelvic organ functions are not impaired.

Since AFP syndrome can have a different etiology (infectious, toxic, neurological, traumatic), confirmation of its viral nature is crucial in making the right diagnosis. The final diagnosis is based on a virological examination of stool samples (two samples of feces are taken within 24–48 h). Samples should be taken no later than the 14 days from the onset of paralysis. The virological test involves using a cell culture to grow the virus followed by the virus identification using RT-PCR, and further genotyping, i.e., partial sequencing of the VP1 genome. The serological tests (probing anti-VP antibodies in the blood) are carried out at the onset of the disease and 3 weeks later. A 4-fold increase in the antibody titer is considered diagnostically significant [30].

Re-examining the patient, re-sampling the feces, and testing for vaccine-associated paralytic polio (VAPP), is carried out on the 60^{th} and 90^{th} days upon the onset of paralysis.

In fatal outcome, autopsy material is examined (cervical and lumbar spinal cord tissue, *medulla oblongata*, the pons Varolii, descending colon, intestinal contents).

In the Russian Federation, laboratory examinations of materials from patients with polio/AFP are performed by institutions designated by the Ministry of Health and accredited by the WHO. They are part of the WHO Global Polio Laboratory Network.

Differential diagnosis. The DD analysis is usually made versus polyradiculoneuropathy, Guillain-Barré syndrome, acute myelitis, serous meningitis, facial nerve neuritis, bone-articular pathology, botulism, tick-borne viral encephalitis, and acute poliomyelitis of other etiology (caused by Coxsackie viruses, ECHO, enterovirus 71) [27].

Treatment. The clinical symptoms suspicious of acute polio requires urgent hospitalization and strict bed rest [27, 28]. Physical rest is of great importance in the preparative phase in order to reduce the severity of upcoming paralysis and hopefully prevent its development. It is recommended to minimize any manipulations, including intravenous and intramuscular injections. There is no specific treatment for blocking the poliovirus. The administration of human immunoglobulin in high doses is ineffective. Therefore, the preventive measures like the routine immunoprophylaxis, are even more important.

In the paralytic period, the physical comfort of a patient lying in the bed is of great importance: it helps minimize the negative consequences of paralysis. Treatment of severe generalized polio is carried out in the intensive care unit. After the acute period ends, exercises, massage, and physiotherapy are recommended. For a later period, spa treatment is indicated.

Prognosis. With the inapparent, abortive, and pontine forms, the prognosis is favorable. The outcome of the paralytic form depends on the severity of the CNS lesion [25]. In mild forms, complete recovery of motor functions occurs within 2–3 months. Bulbar and bulbar-spinal forms of polio are the most severe ones: they are accompanied by deep paresis and paralysis of the trunk and limb muscles. Motor functions recover extremely slowly so that the first movements begin on the 4–5th month of the disease; further restoration of movements can be only partial. Persistent paresis and paralysis with no signs of recovery are typical residual effects of poliomyelitis and have a significant differential diagnostic value.

Before the mass vaccination began, the death toll among unvaccinated populations reached 5–7%. In most cases, death occurred during the first two weeks from the onset of the disease; the mortality and disability rates were highest in older children and adolescents [25].

Complications. Polio complications include pneumonia, pulmonary atelectasis, and myocarditis; with the bulbar form, acute stomach extension, gastrointestinal disturbances with bleeding, ulcers, and bowel obstruction can develop.

The severe course of spinal polio is accompanied by complete paralysis. The disease ends with leaving residual phenomena such as gross dysfunctions, atrophies, bone deformities, and contractures. Having been ill with polio, a person remains disabled for life [25, 27].

Risk factors. Today, the greatest risk of contracting polio (caused either by a wild poliovirus or vaccine-related polioviruses) remains in children who have not been vaccinated or received less than 3 vaccinations or missed a due vaccination time-point. The factors that increase the risk of paralysis in polio infection include intramuscular injections, exercise, injuries, and pregnancy.

Manifestations of the epidemic process. In the pre-vaccination times, polio infection was spreading on the epidemic scale. In a temperate climate, the summer-autumn polio outbreaks were typical. In countries with a tropical climate, newborns and infants were most vulnerable to polio infection; in countries with a temperate climate, those were school-age children. However, outbreaks in isolated populated ion groups can cause paralytic forms in older people as well. Thus, the largest outbreaks of polio with a large proportion of paralytic cases were noted among island residents or isolated populations (for example, Eskimos, religious sects). During outbreaks, unvaccinated or incompletely vaccinated populations were affected. The disease was most often caused (74%) by type 1 poliovirus. In developing countries, children under 2 years of age were most affected; in developed countries, those were older people who remained susceptible to poliovirus. In the post-vaccination period, a sharp decrease in the incidence of poliomyelitis was observed [31].

After the eradication of poliomyelitis in the European Region (2002), including Russia was confirmed and certified, imported cases of wild poliovirus from endemic countries remains the mail threat; in addition, vaccine-related polioviruses (VRPV) can initiate an infection process. In 2010, a large outbreak of poliomyelitis caused by wild poliovirus was recorded in the Republic of Tajikistan. Over 700 people fell ill, and for the first time since 1996, the virus was brought into Russia by labor migrants.

In the absence of active polio diseases caused by a wild strain of poliovirus, the problem of VRPV came to first place in Russia. All such cases have been identified as part of epidemiological surveillance of AFP. In order to eliminate cases of VRPV, vaccination with the inactivated (killed) Salk polio vaccine (IPV) was introduced into the vaccination calendar.

Preventive actions. The major preventive measure is routine immunization of children. In Russia, vaccination and revaccination against polio are carried out in accordance with the National calendar of preventive vaccinations with vaccines approved for use in the Russian Federation [32].

For this purpose, two types of vaccines are used — the live oral poliovirus vaccine prepared from the Sabin strains and the inactivated Salk vaccine.

History of polio vaccines

In the XX century, the growing danger of polio outbreaks attracted a lot of public and medical attention and stimulated the search for ways to fight the disease. The fact that US President Franklin Roosevelt contracted polio when he was 39 years old and then remained partially paralyzed for life contributed to the raising interest in and awareness of the problem. Together with his friend Basil O'Connor, he helped establish the National Foundation for Infantile Paralysis, which later became known as the March of Dimes. This charity organization raised funds for polio patients and also sponsored the research into polio prevention.

Many leading scientists have been involved in the development of polio vaccine. The research was speeded up by the data showing that serum obtained from convalescents could prevent polio, infection, and that monkeys could be vaccinated with an inactivated virus [33, 34].

In 1949, John Enders, Thomas Weller, and Fred Robbins made a breakthrough in their work on the vaccine. They obtained *in vitro* cell cultures that supported the growth of poliovirus under laboratory conditions [35]. For this discovery, which allowed scientists to work with poliovirus in the laboratory, and for the development of vaccines, they were awarded the Nobel Prize in physiology and medicine in 1954.

Subsequent important studies were conducted by William Hammond and other scientists: they tested the possibility of using immune serum to protect potentially susceptible individuals. In a major clinical trial, serum gamma globulin was found to provide 100% protection against polio paralysis [36]. This result was considered the ultimate proof of the sufficiency of humoral immunity for protection against polio and the possibility of creating a vaccine, which would induce this kind of immune response.

The manufacturing technology for vaccine production was developed in two ways.

A group of scientists led by Salk succeeded in creating a trivalent formalin-inactivated vaccine based on three types of polioviruses grown in cell cultures [37]. In the presence of formalin, the virus lost its infectivity, but at the same time retained its immunogenic properties. The Salk vaccine began to be widely used in the United States in 1954; by 1957, the incidence of paralytic polio decreased several-fold, which confirmed the high prophylactic efficacy of this product. IPV production was launched in several countries, and the vaccine was commonly used until a live poliovirus vaccine was created.

It should be noted that in the USSR in 1956, the Salk vaccine production was mastered at the Institute for Poliomyelitis, and by 1958, the volume of IPV produced at the Institute reached 5 million doses. That amount was, however, still insufficient to cover the entire country. A serious disadvantage of the Salk vaccine was its high cost and the need for re-vaccinations [38].

Other groups of scientists were also busy with developing vaccines based on attenuated strains of poliovirus. Such strains were expected to mimic the natural infection (but without paralysis) and establish a sustained long-term immunity against a virulent wild strain [38].

After the discovery of Enders et al. [35] about the ability of poliovirus to multiply in cell cultures, a number of virus titration methods were developed; those made it possible to quantify the attenuation of poliovirus by measuring its titers in cell culture and after intracerebral inoculation in monkeys. It became possible to identify strains with reduced neurovirulence in monkeys and also conduct extensive research into variability of the virus and conversion of highly or moderately neurovirulent strains into mildly neurovirulent strains suitable for vaccine development [38]. Thus, Koprovsky and his collaborators developed a technology for manufacturing a live vaccine using polio strains adapted to mice [39, 40]. About 20 million children were immunized with the Koprovsky vaccine; its use in a number of countries continued until the middle 70s when they switched to the Sabin live vaccine, better studied by that time.

A detailed description of the origin of attenuated Sabin vaccine strains was published in 1973 [41]. Sabin live attenuated vaccine, an oral poliovirus vaccine (OPV), was administered orally in the form of a drop on a small cube of sugar.

The introduction of OPV into healthcare practice was slowed for some time due to competition with the Salk vaccine that was widely used at that time and, most importantly, because of doubts about the safety of using live (albeit attenuated) poliovirus.

At the beginning of 1959, several batches of experimental OPV based on attenuated Sabin strains were produced at the Polio Institute; using these products, 13.5 million people under the age of 20, received vaccination [42]. The polio incidence rate, having an epidemic proportion at that time, was reduced 3-5-fold. The results provided the ultimate evidence for the high efficacy and safety of the oral polio vaccine.

In addition, the positive attitude of both the US administration and the general public towards OPV has grown significantly due to the incident with IPV in 1955 at the Cutter Laboratories pharmaceutical company [43]. Due to errors in the IPV manufacturing process, some batches of the vaccine probably contained an incompletely inactivated live poliovirus. As a result of this accident, 79 cases of poliomyelitis among vaccinated children were recorded, 105 cases among members of their families and 20 cases among people contacted with them; a total of 204 cases of the disease were recorded, of which 11 were fatal [44]. This incident forced a change in the rules guiding the production and use of the vaccines, and in addition, it removed all barriers to the widespread use of OPV.

Replacing the oral polio vaccine with the inactivated polio vaccine: problems and solutions

The inactivated polio vaccine was licensed on April 12, 1955, exactly ten years after the death of the most famous polio patient, President Roosevelt. In the United States and European countries, the use of the Salk vaccine had led to a marked decrease in the incidence of acute paralytic polio. However, the vaccination with IPV does not cause sterile immunity. In other words, despite full protection against the paralytic form of the disease, vaccinated individuals can be infected with poliovirus and infect others. Thus, the IPV cannot be considered efficient from the epidemiological point of view: this vaccine is not able to stop the spread of the virus and break its transmission. In contrast, OPV protects the tissues of the gastrointestinal tract from the infection and thereby prevents virus replication and excretion with feces. OPV has an additional advantage: after vaccination, some collective immunity is formed. The virus administered to a vaccinated child is transmitted in a contact way to relatives, friends, and other people in the close circle, which leads to their passive immunization. This is perhaps the biggest advantage of the live vaccine over the inactivated one, along with a lower cost of production and ease of use. As a result, after licensing the OPV in the early 1960s, the vast majority of countries (except for three Scandinavian countries) replaced the IPV with the OPV in their national vaccination programs.

As already mentioned, the production of OPV is cheaper, and its common use does not require much effort. IPV is administered by intramuscular injection, which requires qualified medical personnel. OPV is administered orally with a drop of liquid vaccine, which does not require trained health personnel. This is a significant advantage, especially for poor countries. The transition from IPV to OPV was also facilitated by the fact that Dr. Albert Sabin provided a free license for the use of his attenuated poliovirus strains to companies, which would comply with his advice on the OPV production process. In 1972, he transferred his strains of poliovirus to the WHO and granted it the right to control their use.

Despite a number of obvious advantages of OPV, its global use was not without unpleasant surprises. The first problem emerged shortly after reports of sporadic acute paralytic polio cases in vaccinated children appeared [20–22, 45]. Researchers have long suspected a relationship between very rare cases of paralytic polio and OPV, but could not prove it. Only with the introduction of molecular genetic research and sequencing methods [23] was it possible to find conclusive evidence that the emerging VAPP was caused by a mutated vaccine virus that re-acquired neurovirulent properties. The number of VAPP cases varied in different countries. According to one of the most representative studies conducted in the USA, during primary vaccination, VAPP developed in one of 600,000 vaccinated individuals [24]. Thus, in the United States, 5–10 cases of VAPP were recorded per year, which at first did not attract much attention, since the mortality caused by a wild strain of the virus was significantly higher. However, in 1990s, VAPP became the leading form of polio in the country; due to that, health authorities faced a difficult question on the ethical aspects of continued vaccination with OPV. Then a new generation of IPV became available; some countries began using a vaccination regimen when the IPV was administered first and then followed by OPV. Subsequently, the health systems of several countries completely abandoned the use of OPV in favor of the inactivated vaccine.

Later, another unpleasant discovery was made: a mutated vaccine poliovirus may not only lead to paralysis in vaccinated individuals but also spread in the population, causing outbreaks of acute paralytic polio. The discovery of circulating VAPP was made in the island of Haiti in 2000 [46]. Since then, dozens of outbreaks of acute paralytic polio caused by VAPP of all three types have been recorded [47, 48]. Most often, such outbreaks were due to type 2 VAPP.

Doubts about the continued use of OPV increased even more with the discovery of other types of vaccine-associated polioviruses isolated from individuals chronically infected with poliovirus [49, 50]. Patients with some types of primary immunodeficiency characterized by impaired antibody production (agammaglobulinemia) can become chronic carriers of poliovirus after the vaccination and release it into the environment for a long time (often for many years). Long-term release of poliovirus was also observed in healthy individuals [51]. The discovery of the three types of VAPP finally disapproved of the accepted concept that Sabin strains were not capable of fully restoring their virulence, and made scientists aware of the serious danger posed by this phenomenon. It is now generally accepted that the virulence of VAPP can be comparable to the virulence of wild strains of poliovirus. The inevitability of their appearance in regions where OPV is used for vaccination has become a serious reason for the transition from OPV to IPV, especially in countries where the circulation of wild poliovirus strains had been stopped.

The transition to IPV was made possible thanks to a significant improvement made by scientists from the National Institute of Public Health and Environment (the Netherlands): they mastered the production of IPV with increased specific activity [52]. In contrast to the classical Salk method that used formalin for the inactivation of virus from infected cell cultures, the Dutch group proposed, firstly, to cultivate the infected cells in bioreactors (and not in monolayer cultures), where the cells grew suspended on special microbeads. This method provided a much higher cell density and, accordingly, a larger number of viral particles. Secondly, the scientists recommended that the virus be purified

using a combination of gel filtration and ion-exchange chromatography, which made it possible to effectively remove most cellular elements from the final product. As a result, each dose of IPV contained a greater amount of antigen, which determined its higher activity. A similar technology is currently used by IPV manufacturers.

The process of gradually replacing OPV with IPV is still ongoing. With the improvement of the economic situation in a particular country, they begin to use more expensive methods of prevention. The substitution of OPV for IPV was facilitated by the introduction of combined vaccines, in which other vaccine preparations were added to the IPV. For example, IPV has been used in combination with diphtheria, tetanus, hepatitis B, hemophilia, and whooping cough vaccines. This approach helped to ease the overload in the National vaccination calendars.

There are a number of serious problems with replacing the OPV with IPV; the most important of them are the high cost of the vaccine and the need to employ qualified medical personnel for intramuscular injections. Another problem is the low ability of IPV to boost the local immunity in the mucous membranes; this type of immunity blocks the virus transmission. Finally, the IPV used today is made of highly virulent strains, so the production process poses a serious risk for people working with these strains. To solve the above problems attempts to develop a new generation of vaccines are under way.

New vaccine preparations have to meet the following requirements: low cost, increased ability to induce an immune response in the mucous membranes and compliance with biosafety criteria [53].

To create new live vaccines, it is necessary to use viruses with increased genetic stability in order to rule out the possibility of virulence reversal. Genetic stability is assessed *in vitro* (in cell cultures) and *in vivo* (in animals), but ultimately, the safety of a vaccine can only be judged by its use in humans.

Despite all efforts to completely stop the spread of poliovirus, there is always a small chance of accidental or deliberate release of live virus into the environment that may have catastrophic consequences. This was the reason behind the renewed search for the way to manufacture IPV without compromising biological safety of work personnel.

There are several ways to accomplish these goals. One obvious solution is to create an IPV based on attenuated Sabin strains (sIPV). It should be noted that the immunogenicity of sIPV type 1 was not less than that of IPV obtained from the wild Mahoney strain. However, the immunogenicity of IPV made from two other serotypes of Sabin viruses, especially type 2, was lower than that of IPV from wild strains [54–57]. As a result, the optimal composition of trivalent sIPV is different from that of IPV from wild strains. Currently, sIPV is licensed in Japan and China [58]. In Japan, the drug is made for subcutaneous administration in the form of a combined vaccine against poliomyelitis, diphtheria, tetanus, and pertussis (DTP). Since polio is absent in China and Japan, the expected outcome of sIPV clinical trials is evaluated by seroconversion. The Institute of Translational Vaccinology in the Netherlands, with the support of the WHO, has developed its own sIPV vaccine technology [59] and granted manufacturing licenses to a number of companies in developing countries.

In Russia, using the long-time experience in the creation of antiviral vaccines (oral polio, anti-rabies, against tick-borne encephalitis and yellow fever), scientists from the Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products developed an original technology for manufacturing the inactivated cultured concentrated purified polio vaccine based on attenuated Sabin strains [60]. This vaccine is currently undergoing clinical trials and, in the long term, could become the first domestic inactivated vaccine for the prevention of polio, which will replace the expensive imported vaccine products currently used.

Problems and prospects of creating new poliovirus vaccines

The sIPV vaccine is the first of the new generation of IPV. A number of important unresolved issues remain regarding this vaccine preparation. Some of them relate to the standardization of this new class of IPV and the selection of appropriate methods for testing its efficacy as well as and the choice of the reference reagents. Other aspects that need further study relate to biosafety of the production process. For example, due to the eradication of polio and cessation of its circulation in the environment, the laboratory Sabin strains must be kept under strictly the same conditions as wild strains to preserve their immunogenicity. In this case, bio-factories manufacturing sIPV must meet the BSL3/polio safety requirement, which is inevitably associated with extra costs and may impact the manufacture and sales of the product. In other words, sIPV is a step in the right direction, but this vaccine is not able to solve all the problems, so in the future, we will have to develop a more advanced vaccine preparation.

After the molecular mechanisms of attenuation and reversal of virulence in polioviruses were elucidated in the 1980s and 1990s, several attempts were made to create attenuated strains with increased genetic stability. Basically, these attempts were aimed at limiting the occurrence and accumulation of point mutations that lead to virulence restoration. Most VRPV strains were obtained by recombining the Sabin ains with other non-polio enterovirus strains; due to that, it has been suggested that this recombination may play a role in the reversal of virulence. Genetic stability is assessed *in vitro* (in cell cultures) and *in vivo* (in animals), but ultimately, vaccine safety can only be judged by its results in humans. A number of researchers have

been able to achieve increased stability in experiments *in vitro*, but confirming this fact in clinical trials will not be easy. Given the relatively low incidence of vaccine-associated complications (approximately 1 in the 600,000 first doses), achieving the statistical significance needed to draw final conclusions on the superiority of the new strain will require a colossal clinical trial.

Another problem complicating the development of a more stable attenuated strain is the lack of reliable biomarkers of safety for *in vitro* and *in vivo* models. For this reason, a large number of studies were not conducted until a group of laboratories funded by the Bill and Melinda Gates Foundation was set up and tasked with developing a strain of OPV type 2, with a greater genetic stability. Although this study has not been completed by now, we can describe the general principles of their work.

One of the determinants of virulence and attenuation are mutations in a hairpin-type structure (denoted as the "F domain of hairpin VI") in the 5′-untranslated region. This domain is part of the internal ribosome entry site (IRES); it is believed to be involved in the interactions of translation initiation factors with ribosomes and the viral RNA molecule [61, 62]. There is evidence in the literature that some of these factors are tissue-specific; therefore, mutations in this area can alter the viral tropism to certain tissues and thus limit its propagation in neurons. It was found that recombinants in which this region was replaced by a homologous element of human rhinovirus had a very low virulence [63, 64]. Currently, these chimeric rhinoviruses and polioviruses are studied as oncolytic agents acting against gliomas [65]. Theoretically, such chimeric viruses can be used as the basis for vaccines with increased stability.

Another approach (based on the same determinant of attenuation) attempts to destabilize the hairpin and thus attenuate the virulence. For example, attenuation of type 3 poliovirus was achieved by replacing the stable G:C pair with the unstable G:U pair, which led to destabilization of the entire hairpin. If the virulence gets reversed, this G:U pair is replaced by the original G:C pair. The A:U pair is intermediate between the G:C and G:U in stability, therefore, when the RNA hairpin is rearranged by replacing the G:C and G:U pairs with the A:U pairs, the overall structural stability and virulence of this virus remain almost unchanged. However, the genetic stability is higher in this case, since two mutations are required to convert the A:U pair to a more stable G:C pair, and the intermediate pairs (G:U and A:C) in this process have lower structural stability and therefore, adversely affect the replicative ability of the virus. A number of genetic constructs created by this principle do have increased genetic stability. At present, they are supposed to be used to create virus-based vaccines with increased genetic stability [66–68].

Another way to weaken the IRES function is to delete nucleotides or insert additional nucleotides, which would alter the conformation of the entire structure. However, such manipulations do not increase genetic stability, since the virus can easily restore its replicative potential by cutting out the inserted fragments or filling the deleted fragments by using fragments of RNA from other sources. Toyoda and his colleagues tried to overcome this problem of instability [69]. They proposed a method based on using the cis-acting replicative element in viral RNA. Normally, this element is located in the center of the RNA molecule and is extremely important for the initiation of RNA replication. Transferring the cis-element from its normal position to the IRES region of the 5′-untranslated region leads to significant attenuation. Since the cis-element plays an important role in RNA replication, the virus cannot cut it off. As a result, this weakened construct is genetically stable.

Viral RNA replicases are known for their ability to make errors during the process of replication, which leads to a large number of mutations. This feature is one of the reasons for the genetic instability of viral RNA genomes. The high mutation rate, on the one hand, creates obvious problems, but on the other hand, gives viruses a number of advantages that allow them to quickly adapt to a new or changing environment. Thus, the accuracy of viral replicases is tailored to the needs of the viruses: it is neither high nor low. This was proven by the discovery in the polymerase encoding gene of mutations that increased the replicase accuracy [70] and reduce the ability of the virus to infect animals [71, 72]. This phenomenon can be used to create high precision mutant polymerases in order to reduce the reversal of virulence and facilitate attenuation.

All organisms, including polioviruses, have the codon preference mechanism (using only one of the synonymous codons in the coding regions of the genome). This phenomenon has found its use in the field of biotechnology, when a foreign protein is expressed in a heterologous system. To maximize the protein production, the gene encoding for the target protein is transcoded so that the codons are replaced by others that are most often used in the expression system. This process is called the codon optimization. In experiments with polioviruses, it was found that the reverse process — codon de-optimization (i.e., introduction of rare codons into the poliovirus genome) reduced the replicative ability of the virus and the yield of infectious virions [73]. As a result, this "weakened" virus cannot easily restore its virulence and replicative ability, because its genome has been changed by multiple mutations.

It is possible that the mechanism by which de-optimization of codons reduces the replicative ability is more complex than just using rare codons. In addition to the phenomenon of codon preference, most organisms also show a preference for using certain pairs of codons [74]. This means that there is a preference for selecting a codon for encoding neighboring amino acids: some pairs of codons are used more often than others. If

this preference is changed by replacing codons with synonymic ones, the result will be similar to the codon de-optimization, even though the total number of codons remains unchanged [75]. The reason for the preference for certain codon pairs is not known yet. To complicate the situation, the frequencies of the CpG dinucleotide and the UpA dinucleotide are lower than could be expected in a random sequence. When the poliovirus RNA is re-coded into a sequence with a larger number of CpG and UpA, the size of the sterile spots formed by the virus in a cell culture decreases according to the number of introduced changes [76]. Viruses obtained after such "genome scrambling" significantly decrease the yield of infectious virions, while the total production of viral particles changes a little. The biological mechanisms underlying these phenomena remain unknown. In addition, it is unclear whether all these phenomena are the result of one or more unrelated causes. Nevertheless, "rearrangement of genome elements" can provide important clues to the development of attenuated and inactivated vaccines [77].

So far, we have paid attention only to a few rational methods for attenuating the virus while maintaining genetic stability and limiting the reversal of virulence by preventing point mutations. Another approach to the development of a more genetically resistant poliovirus is an attempt to limit its ability to recombine with other viruses. Poliovirus and enteroviruses are generally characterized by recombination, the frequency of which is extremely high [78–81]. This characteristic is valuable because it allows them to propagate quickly and neutralize the effect of point mutations by replacing the damaged parts of their genome with the genetic material obtained from other viruses. In all likelihood, recombination facilitates the replacement of genome elements damaged during attenuation and, as a result, restore some of the virus replication ability. Thus, restricting the recombination frequency can be considered as a possible option for perfecting the vaccine strain.

Research into this direction is complicated by the limited knowledge of recombination mechanisms. Homologous recombination is believed to be an important property of poliovirus; therefore, recoding certain parts of the vaccine poliovirus genome and minimize the genetic homology with other viruses can reduce the frequency of recombination. In addition, it may be useful to limit the ability of viruses to exchange parts of their genome by detecting mutations in polymerase-encoding genes, leading to a decrease in the base recombination frequency [82]. However, to date, the feasibility of such an approach remains unknown. It is still unclear which factor limits the incidence of recombinant viruses: is it the very fact of recombination or the selection based on replicative ability. Studies on this subject continue in a hope to shed light on this interesting aspect of poliovirus biology.

A few research groups are working to create other, even safer, strains that can be used to produce IPV. The main requirement for such attenuated strains is their complete non-pathogenicity and resistance both *in vitro* and *in vivo*, in order to avoid reversal of virulence and resumption of circulation, even when the virus enters the environment. One approach used to produce such stable attenuated viruses is by replacing Sabin polioviruses that are susceptible to reversing IRES elements with homologous regions of other viruses that do not have tropism to neural tissue, such as human rhinoviruses [63–65]. Other approaches involve the stabilization of the weakening domains in IRES by modifying the hairpin F domains using A:U pairs [73]; displacing the cis-element toward the 5′-untranslated region [69]; inducing high-precision mutations in the polymerase-encoding gene [72]; rearranging the coding elements of the genome in order to change the preference for codons, the preference for the use of pairs of codons [69] or the number of CpG and UpA dinucleotides [76]. Preliminary studies on the clinical efficacy of each of these approaches *in vitro* have shown that the resulting virus is likely to have higher genetic stability. However, it remains to be determined whether these methods can be used to produce amounts of poliovirus antigen sufficient for IPV production. In addition, it is not known whether the products will be more stable *in vivo* (and therefore safer), which is extremely difficult to test due to the lack of a preclinical (animal) model suitable for studying the transmission and genetic stability of poliovirus *in vivo*.

An ideal solution to the biosafety problem would be a production process that does not utilize a virus with an infectious potential. Antigens for many other vaccines can be successfully obtained from various expression systems (baculovirus, yeast, etc.). In the case of polio vaccine, the difficulty of using this approach is that most of its immunogenic epitopes (if not all) are formed by secondary or even tertiary rearrangement between segments of amino acids from different polypeptide chains. Their immunogenicity is extremely sensitive to polypeptide conformational changes, and, therefore, only native viral particles can produce immunity. At the moment, there is no effective algorithm for poliovirus assembling *in vitro* that could be used to obtain the required amount of poliovirus particles for vaccine production. The assembly process of poliovirus capsid is rather complicated and has not been fully studied. Nevertheless, it is known that it includes the autoproteolytic cleavage of one of the precursor proteins, which occurs only after RNA gets encapsulated and "fixes" the correct conformation of the entire structure. "Empty" particles that do not contain poliovirus RNA are characterized by instability. This problem can theoretically be solved by protein engineered stabilization [83]. If successful this method may open the way to the creation of empty capsids with immunogenic properties. These capsids can be used as vaccines, the production of which does not require a live poliovirus.

Another research approach to the creation of new inactivated polio vaccines is an attempt to reduce the cost and/or increase their immunogenicity (which will reduce the dose of antigen needed to develop immunity). Cost reduction can be achieved by increasing the yield of viral particles by introducing new production processes and cell substrates. According to published data, the use of PerC6 cell suspensions cultured in a serum-free nutrient medium allows the cells to grow to much higher density and provide a higher yield of poliovirus [84].

Another way to reduce the cost of vaccine production is to use alternative routes of administration, which will increase immunogenicity and reduce the administered dose. A common way to increase immunogenicity and lower the dose of a vaccine is to add adjuvants. To date, a number of research groups are actively exploring the possibility of using various standard and new adjuvants in combination with poliovirus vaccines. Among the usual adjuvants, aluminum hydroxide enhances the immunogenicity [85, 86]. New adjuvants are also being studied, such as oil-in-water emulsions [87], toll-like receptor agonists and other elements of non-specific immune defense. In addition, there is evidence that some adjuvants, when administered intramuscularly, increase the mucosal immune response [88].

The skin is the first "line of defense" against pathogens and, therefore, contains elements of the immune system, including dendritic cells and macrophages, which prevent the invasion of pathogenic microorganisms. This fact underlies the assumption that the intradermal administration of antigens can be more effective than the intramuscular route. In clinical trials of intradermal administration of a fractional dose of IPV [89, 90–92], the assumption was confirmed; however, the minimum dose needed to create immunity was higher than the estimated intramuscular dose. The efficacy of the primary immunization with a single intradermal dose of IPV has been proven by the presence of a secondary immune response to the booster dose of the vaccine [93]. Thus, intradermal administration is an acceptable route of vaccination, and it can be made using devices for needleless injections. An alternative option for intradermal administration is the use of "microneedle patches" [94–97]. These small devices contain multiple antigen-coated plastic microneedles for intradermal delivery. They can be applied to the skin painlessly, like a patch. The practical feasibility and the efficacy of this technology are presently under study.

All of the above approaches concern the development of an inactivated polio vaccine, able to facilitate the transition from OPV to IPV and contribute to the final phase of the polio eradicating campaign. Nevertheless, in the long run, IPV will be used in combination with other antigens in the form of a tetravalent, pentavalent or hexavalent vaccine. The use of combined vaccines can simultaneously provide maximum benefits for the healthcare system and reduce the cost and number of injections required for a successful vaccination.

Conclusion

The history of polio vaccines is a captivating tale about two highly effective vaccine preparations, each with its own advantages and disadvantages. The first of the two, the inactivated polio vaccine, clearly demonstrated both the ability to prevent polio infections and the danger of multiple side effects of vaccination, which in turn led to the emergence of the modern regulatory framework for the development and use of vaccines. It also eliminated all the barriers to the oral poliovirus vaccine, which has been the vaccine of choice for many years and has contributed to significant progress in the fight against polio. Subsequently, this success led to a gradual return to the inactivated polio vaccine and the need to completely replace the oral poliovirus vaccine with a safer inactivated vaccine. However, the inactivated polio vaccine of the future is likely to be different from the current product. Thus, the constantly changing epidemiological situation and socio-economic factors motivate us to continuously improve the existing vaccines and introduce innovative products that meet the new requirements.

Research funding. The study was not funded by any sources.

Conflict of interest. The authors declare no conflicts of interest to be reported.

References

1. Underwood M. *A treatise on the diseases of children with general directions for the management of infants from the birth*. London: J. Mathews; 1789.

2. Badham J. Paralysis in childhood: four remarkable cases of suddenly induced paralysis in the extremities, occurring in children, without any apparent cerebral or cerebro-spinal lesion. *London Med Gazzette* 1834; 17: 215.

3. Heine J. *Beobachtungen über Lähmungszustände der unteren Extremitäten und deren Behandlung*. Stuttgart: Köhler; 1840.

4. Cornil V. Paralysie infantile; cancer les seins; autopsie; altérations de la moelle épinière, des nerfs et des muscles; généralisation du cancer. *C R Soc Biol (Paris)* 1863; 5: 187.

5. Jacobi M. Pathogeny of infantile paralysis. *Am J Obstet* 1875; 7: 1.

6. Putnam J.J., Taylor E.W. Is acute poliomyelitis unusually prevalent this season. *Bost Med Surg J* 1893; 129(21): 509–510, https://doi.org/10.1056/nejm189311231292103.

7. Flexner S., Clark P.F. A note on the mode of infection in epidemic poliomyelitis. *Proc Soc Exp Biol Med* 1912; 10: 1–10.

8. Frost W.H. Epidemiologic studies of acute anterior poliomyelitis. *Hyg Lab Bull* 1913; 90.

9. Chumakov M.P., Voroshilova M.K., Drozdov S.G., Dzagurov S.G., Lashkevich V.A., Mironova L.L., Ralph N.M., Gagarina A.V., Ashmarina E.E., Shirman G.A., Fleer G.P., Tolskaya E.A., Sokolova I.S., Elbert L.B., Sinyak K.M. Some results of the work on mass immunization in the Soviet Union with live poliovirus vaccine prepared from Sabin strains. *Bull World Health Organ* 1961; 25(1): 79–91.

10. Flexner S., Lewis P.A. The transmission of acute poliomyelitis to monkeys. *JAMA* 1909; 53(20): 1639, https://doi.org/10.1001/jama.1909.92550200027002g.

11. Flexner S., Lewis P.A. Experimental poliomyelitis in monkeys. Seventh note: active immunization and passive serum protection. *JAMA* 1910; 54(22): 1780, https://doi.org/10.1001/jama.1910.92550480001001i.

12. Bodian D., Morgan I.V., Howe H.A. Differentiation of types of poliomyelitis viruses: III. The grouping of fourteen strains into three basic immunological types. *Am J Hyg* 1949; 49(2): 234–245, https://doi.org/10.1093/oxfordjournals.aje.a119273.

13. Burnet F.M., Macnamara J. Immunological differences between strains of poliomyelitic virus. *Br J Exp Pathol* 1931; 12(2): 57–61.

14. Kessel J.F., Pait C.F. Differentiation of three groups of poliomyelitis virus. *Proc Soc Exp Biol Med* 1949; 70(2): 315–316, https://doi.org/10.3181/00379727-70-16911.

15. Pallansch M.A., Oberste M.S., Whitton J.L. Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. In: Knipe D.M., Howley P.M. (editors). *Fields virology.* Vol. 1. Philadelphia: Lippincott Williams and Wilkins; 2013; p. 490–530.

16. Racaniello V.R. Picornaviridae: the viruses and their replication. In: Knipe D.M., Howley P.M. (editors). *Fields virology.* Vol. 1. Philadelphia: Lippincott Williams and Wilkins; 2013; p. 453–489.

17. Herold J., Andino R. Poliovirus RNA replication requires genome circularization through a protein–protein bridge. *Mol Cell* 2001; 7(3): 581–591, https://doi.org/10.1016/s1097-2765(01)00205-2.

18. Rossmann M.G., He Y., Kuhn R.J. Picornavirus–receptor interactions. *Trends Microbiol* 2002; 10(7): 324–331, https://doi.org/10.1016/s0966-842x(02)02383-1.

19. Stanway G. Structure, function and evolution of picornavirus. *J Gen Virol* 1990; 71(Pt 11): 2483–2501, https://doi.org/10.1099/0022-1317-71-11-2483.

20. Chang T.W., Weinstein L., Macmahon E. Paralytic poliomyelitis in a child with hypogammaglobulinemia: probable implication of type 1 vaccin strain. *Pediatrics* 1966; 37: 630–636.

21. Feigin R.D., Guggenheim M.A., Johnson S.D. Vaccine-related paralytic poliomyelitis in an immunodeficient child. *J Pediatr* 1971; 79(4): 642–647, https://doi.org/10.1016/s0022-3476(71)80313-x.

22. Wright P.F., Hatch M.H., Kasselberg A.G., Lowry S.P., Wadlington W.B., Karzon D.T. Vaccine-associated poliomyelitis in a child with sex-linked agammaglobulinemica. *J Pediatr* 1977; 91(3): 408–412, https://doi.org/10.1016/s0022-3476(77)81309-7.

23. Nottay B.K., Kew O.M., Hatch M.H., Heyward J.T., Obijeski J.F. Molecular variation of type 1 vaccine-related and wild polioviruses during replication in humans. *Virology* 1981; 108(2): 405–423, https://doi.org/10.1016/0042-6822(81)90448-7.

24. Alexander L.N., Seward J.F., Santibanez T.A., Pallansch M.A., Kew O.M., Prevots D.R., Strebel P.M., Cono J., Wharton M., Orenstein W.A., Sutter R.W. Vaccine policy changes and epidemiology of poliomyelitis in the United States. *JAMA* 2004; 292(14): 1696–1701, https://doi.org/10.1001/jama.292.14.1696.

25. Ishmukhametov A.A., Ivanova O.E., Chernyavskaya O.P. Poliomielit. V kn.: *Rukovodstvo po epidemiologii infektsionnykh bolezney.* T. 1 [Infectious disease epidemiology guide. Vol. 1]. Pod red. Briko N.I. [Briko N.I. (editor)]. Moscow; 2018; p. 313–324.

26. *MU 3.1.1.2130-06. Enterovirusnye zabolevaniya: klinika, laboratornaya diagnostika, epidemiologiya, profilaktika* [MU 3.1.1.2130-06. Enteroviral diseases: clinic, laboratory diagnostics, epidemiology, prevention]. Moscow; 2006.

27. Leshchinskaya E.V., Latysheva I.N. *Klinika, diagnostika i lechenie ostrogo poliomielita* [Clinic, diagnosis, and treatment of acute poliomyelitis]. Moscow; 1998.

28. Chumakov M.P., Prisman I.M., Zatsepin T.S. *Poliomielit, detskiy spinnomozgovoy paralich* [Poliomyelitis, childhood cerebrospinal paralysis]. Moscow: Medgiz; 1953.

29. World Health Organization. *Polio laboratory manual.* Geneva: WHO; 2004.

30. World Health Organization. *Manual for the virological investigation of polio.* Geneva: WHO; 1997.

31. World Health Organization. *Rukovodstvo po provedeniyu dopolnitel'nykh meropriyatiy, napravlennykh na likvidatsiyu poliomielita* [Guidelines for additional activities aimed at eradicating poliomyelitis]. Geneva: WHO; 1997.

32. *Prikaz Ministerstva zdravookhraneniya RF ot 21 marta 2014 g. No.125n "Ob utverzhdenii natsional'nogo kalendarya profilakticheskikh privivok i kalendarya profilakticheskikh privivok po epidemicheskim pokazaniyam"* [Order of the Ministry of Health of the Russian Federation dated March 21, 2014 No.125n "On approval of the national calendar of preventive vaccinations and the calendar of preventive vaccinations according to epidemic indications"].

33. Kramer S.D., Aycock W.L., Solomon C.I., Thenebe C.L. Convalescent serum therapy in preparalytic poliomyelitis. *N Engl J Med* 1932; 206(9): 432–435, https://doi.org/10.1056/nejm193203032060902.

34. Brodie M. Active immunization in monkeys against poliomyelitis with germicidally inactivated virus. *Science* 1934; 79(2061): 594–595, https://doi.org/10.1126/science.79.2061.594.

35. Enders J.F., Weller T.H., Robbins F.C. Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues. *Science* 1949; 109(2822): 85–87, https://doi.org/10.1126/science.109.2822.85.

36. Hammon W.M., Coriell L.L., Stokes J. Jr. Evaluation of Red Cross gamma globulin as a prophylactic agent for poliomyelitis. 2. Conduct and early follow-up of 1952 Texas and Iowa-Nebraska studies. *J Am Med Assoc* 1952; 150(8): 750–756, https://doi.org/10.1001/jama.1952.03680080012002.

37. Salk J.E., Bennet B.L., Lewis L.J., Ward E.N., Youngner J.S. Studies in human subjects on active immunization against poliomyelitis. *JAMA* 1953; 151(13): 1081–1098, https://doi.org/10.1001/jama.1953.13.1081.

38. Lashkevich V.A. History of development of the live poliomyelitis vaccine from Sabin attenuated strains in 1959 and idea of poliomyelitis eradication. *Voprosy virusologii* 2013; 58(1): 4–10.

39. Koprowski H., Jervis G.A., Norton T.W. Immune responses in human volunteers upon oral administration of a rodent-adapted strain of poliomyelitis virus. *Am J Hyg* 1952; 55(1): 108–126, https://doi.org/10.1093/oxfordjournals.aje.a119499.

40. Koprowski H. Immunization against poliomyelitis with living attenuated virus. *Am J Trop Med Hyg* 1956; 5(3): 440–452, https://doi.org/10.4269/ajtmh.1956.5.440.

41. Sabin A.B., Boulgar L.R. History of Sabin attenuated poliovirus oral live vaccine strains. *J Biol Stand* 1973; 1(2): 115–118, https://doi.org/10.1016/0092-1157(73)90048-6.

42. Chumakov M.P., Voroshilova M.K., Vasil'eva K.A., et al. Preliminary report on massive immunization of the population with a live oral polio vaccine from attenuated Sabin virus. *Voprosy virusologii* 1959; 5: 520–533.

43. Nathanson N., Langmuir A.D. The Cutter incident. Poliomyelitis following formaldehyde-inactivated poliovirus vaccination in the United States during the Spring of 1955: II. Relationship of poliomyelitis to Cutter vaccine. *Am J Epidemiol* 1995; 142(2): 109–148, https://doi.org/10.1093/oxfordjournals.aje.a117611.

44. *Zhivaya vaktsina protiv poliomielita* [Live polio vaccine]. Pod red. Smorodintseva A.A. [Smorodintsev A.A. (editor)]. Leningrad; 1960.

45. Bartoshevich E.N., Tsuker M.B., Leshchinskaya E.V., Sokolova I.S., Martynenko I.N., Andreeva L.S., Ashmarina E.E. Polio-like paralytic diseases in children vaccinated with the Sabin live vaccine. *Vestnik AMN SSSR* 1963; 6: 16–21.

46. Kew O., Morris-Glasgow V., Landaverde M., Burns C., Shaw J., Garib Z., André J., Blackman E., Freeman C.J., Jorba J., Sutter R., Tambini G., Venczel L., Pedreira C., Laender F., Shimizu H., Yoneyama T., Miyamura T., van Der Avoort H., Oberste M.S., Kilpatrick D., Cochi S., Pallansch M., de Quadros C. Outbreak of poliomyelitis in Hispaniola associated with circulating type 1 vaccine-derived poliovirus. *Science* 2002; 296(5566): 356–359, https://doi.org/10.1126/science.1068284.

47. Kew O.M., Wright P.F., Agol V.I., Delpeyroux F., Shimizu H., Nathanson N., Pallansch M.A. Circulating vaccine-derived polioviruses: current state of knowledge. *Bull World Health Organ* 2004; 82(1): 16–23.

48. Centers for Disease Control and Prevention. Update on vaccine-derived polioviruses — worldwide, April 2011–June 2012. *MMWR Morb Mortal Wkly Rep* 2012; 61: 741–746.

49. Lopez C., Biggar W.D., Park B.H., Good R.A. Nonparalytic poliovirus infections in patients with severe combined immunodeficiency disease. *J Pediatr* 1974; 84(4): 497–502, https://doi.org/10.1016/s0022-3476(74)80667-0.

50. Davis L.E., Bodian D., Price D., Butler I.J., Vickers J.H. Chronic progressive poliomyelitis secondary to vaccination of an immunodeficient child. *N Engl J Med* 1977; 297(5): 241–245, https://doi.org/10.1056/nejm197708042970503.

51. Martín J., Odoom K., Tuite G., Dunn G., Hopewell N., Cooper G., Fitzharris C., Butler K., Hall W.W., Minor P.D. Long-term excretion of vaccinederived poliovirus by a healthy child. *J Virol* 2004; 78(24): 13839–13847, https://doi.org/10.1128/jvi.78.24.13839-13847.2004.

52. van Wezel A.L., van Steenis G., van der Marel P., Osterhaus A.D. Inactivated poliovirus vaccine: current production methods and new developments. *Rev Infect Dis* 1984; 6(Suppl 2): S335–S340, https://doi.org/10.1093/clinids/6.supplement_2.s335.

53. Ehrenfeld E., Modlin J., Chumakov K. Future of polio vaccines. *Expert Rev Vaccines* 2009; 8(7): 899–905, https://doi.org/10.1586/erv.09.49.

54. Doi Y., Abe S., Yamamoto H., Horie H., Ohyama H., Satoh K., Tano Y., Ota Y., Miyazawa M., Wakabayashi K., Hashizume S. Progress with inactivated poliovirus vaccines derived from the Sabin strains. *Dev Biol (Basel)* 2001; 105: 163–169.

55. Dragunsky E.M., Ivanov A.P., Wells V.R., Ivshina A.V., Rezapkin G.V., Abe S., Potapova S.G., Enterline J.C., Hashizume S., Chumakov K.M. Evaluation of immunogenicity and protective properties of inactivated poliovirus vaccines: a new surrogate method for predicting vaccine efficacy. *J Infect Dis* 2004; 190(8): 1404–1412, https://doi.org/10.1086/424524.

56. Dragunsky E.M., Ivanov A.P., Abe S., Potapova S.G., Enterline J.C., Hashizume S., Chumakov K.M. Further development of a new transgenic mouse test for the evaluation of the immunogenicity and protective properties of inactivated poliovirus vaccine. *J Infect Dis* 2006; 194(6): 804–807, https://doi.org/10.1086/506949.

57. Tano Y., Shimizu H., Martin J., Nishimura Y., Simizu B., Miyamura T. Antigenic characterization of a formalin-inactivated poliovirus vaccine derived from live-attenuated Sabin strains. *Vaccine* 2007; 25(41): 7041–7046, https://doi.org/10.1016/j.vaccine.2007.07.060.

58. Shimizu H. Poliovirus vaccine. *Uirusu* 2012; 62(1): 57–65, https://doi.org/10.2222/jsv.62.57.

59. Verdijk P., Rots N.Y., Bakker W.A. Clinical development of a novel inactivated poliomyelitis vaccine based on attenuated Sabin poliovirus strains. *Expert Rev Vaccines* 2011; 10(5): 635–644, https://doi.org/10.1586/erv.11.51.

60. Ivanov A.P., Klebleeva T.D., Ivanova O.E., Ipatova E.G., Gmyl L.V., Ishmuhametov A.A. Experimental approaches to the development of inactivated poliovirus vaccine based on sabin strains. *Ehpidemiologiya i vaktsinoprofilaktika* 2016; 15(4): 59–64, https://doi.org/10.31631/2073-3046-2016-15-4-59-64.

61. Guest S., Pilipenko E., Sharma K., Chumakov K., Roos R.P. Molecular mechanisms of attenuation of the Sabin strain of poliovirus type 3. *J Virol* 2004; 78(20): 11097–11107, https://doi.org/10.1128/jvi.78.20.11097-11107.2004.

62. Kauder S.E., Racaniello V.R. Poliovirus tropism and attenuation are determined after internal ribosome entry. *J Clin Invest* 2004; 113(12): 1743–1753, https://doi.org/10.1172/jci200421323.

63. Gromeier M., Alexander L., Wimmer E. Internal ribosomal entry site substitution eliminates neurovirulence in intergeneric poliovirus recombinants. *Proc Natl Acad Sci U S A* 1996; 93(6): 2370–2375, https://doi.org/10.1073/pnas.93.6.2370.

64. Chumakov K., Dragunsky E., Ivshina A., Enterline J., Wells V., Nomura T., Gromeier M., Wimmer E. Inactivated vaccines based on alternatives to wild-type seed virus. *Dev Biol (Basel)* 2001; 105: 171–177.

65. Dobrikova E.Y., Goetz C., Walters R.W., Lawson S.K., Peggins J.O., Muszynski K., Ruppel S., Poole K., Giardina S.L., Vela E.M., Estep J.E., Gromeier M. Attenuation of neurovirulence, biodistribution, and shedding of a poliovirus: rhinovirus chimera after intrathalamic inoculation in Macaca fascicularis. *J Virol* 2012; 86(5): 2750–2759, https://doi.org/10.1128/jvi.06427-11.

66. Macadam A.J., Ferguson G., Stone D.M., Meredith J., Almond J.W., Minor P.D. Live-attenuated strains of improved genetic stability. *Dev Biol (Basel)* 2001; 105: 179–187.

67. Macadam A.J., Ferguson G., Stone D.M., Meredith J., Knowlson S., Auda G., Almond J.W., Minor P.D. Rational design of genetically stable, live-attenuated poliovirus vaccines of all three serotypes: relevance to poliomyelitis eradication. *J Virol* 2006; 80(17): 8653–8663, https://doi.org/10.1128/jvi.00370-06.

68. Rowe A., Burlison J., Macadam A.J., Minor P.D. Functional formation of domain V of the poliovirus noncoding

region: significance of unpaired bases. *Virology* 2001; 289(1): 45–53, https://doi.org/10.1006/viro.2001.1111.

69. Toyoda H., Yin J., Mueller S., Wimmer E., Cello J. Oncolytic treatment and cure of neuroblastoma by a novel attenuated poliovirus in a novel poliovirus-susceptible animal model. *Cancer Res* 2007; 67(6): 2857–2864, https://doi.org/10.1158/0008-5472.can-06-3713.

70. Pfeiffer J.K., Kirkegaard K. A single mutation in poliovirus RNA-dependent RNA polymerase confers resistance to mutagenic nucleotide analogs via increased fidelity. *Proc Natl Acad Sci U S A* 2003; 100(12): 7289–7294, https://doi.org/10.1073/pnas.1232294100.

71. Vignuzzi M., Stone J.K., Arnold J.J., Cameron C.E., Andino R. Quasispecies diversity determines pathogenesis through cooperative interactions in a viral population. *Nature* 2006; 439(7074): 344–348, https://doi.org/10.1038/nature04388.

72. Vignuzzi M., Wendt E., Andino R. Engineering attenuated virus vaccines by controlling replication fidelity. *Nat Med* 2008; 14(2): 154–161, https://doi.org/10.1038/nm1726.

73. Burns C.C., Shaw J., Campagnoli R., Jorba J., Vincent A., Quay J., Kew O. Modulation of poliovirus replicative fitness in HeLa cells by deoptimization of synonymous codon usage in the capsid region. *J Virol* 2006; 80(7): 3259–3272, https://doi.org/10.1128/jvi.80.7.3259-3272.2006.

74. Gutman G.A., Hatfield G.W. Nonrandom utilization of codon pairs in Escherichia coli. *Proc Natl Acad Sci U S A* 1989; 86(10): 3699–3703, https://doi.org/10.1073/pnas.86.10.3699.

75. Coleman J.R., Papamichail D., Skiena S., Futcher B., Wimmer E., Mueller S. Virus attenuation by genome-scale changes in codon pair bias. *Science* 2008; 320(5884): 1784–1787, https://doi.org/10.1126/science.1155761.

76. Burns C.C., Campagnoli R., Shaw J., Vincent A., Jorba J., Kew O. Genetic inactivation of poliovirus infectivity by increasing the frequencies of CpG and UpA dinucleotides within and across synonymous capsid region codons. *J Virol* 2009; 83(19): 9957–9969, https://doi.org/10.1128/jvi.00508-09.

77. Mueller S., Coleman J.R., Papamichail D., Ward C.B., Nimnual A., Futcher B., Skiena S., Wimmer E. Live attenuated influenza virus vaccines by computer-aided rational design. *Nat Biotechnol* 2010; 28(7): 723–726, https://doi.org/10.1038/nbt.1636.

78. Cooper P.D. Genetics of picornaviruses. In: Fraenkel-Conrat H., Wagner R. (editors). *Regulation and genetics. Comprehensive virology.* Vol 9. Springer US; 1997; p. 133–207, https://doi.org/10.1007/978-1-4684-2718-9_4.

79. Furlone M., Guillot S., Otelea D., Balanant J., Candrea A., Crainic R. Polioviruses with natural recombinant genomes isolated from vaccine-associated poliomyelitis. *Virology* 1993; 196(1): 199–208, https://doi.org/10.1006/viro.1993.1468.

80. Agol V.I. Recombination and other genomic rearrangements in picornaviruses. *Seminars in Virology* 1997; 8(2): 77–84, https://doi.org/10.1006/smvy.1997.0112.

81. Combelas N., Holmblat B., Joffret M.L., Colbère-Garapin F., Delpeyroux F. Recombination between poliovirus and coxsackie A viruses of species C: a model of viral genetic plasticity and emergence. *Viruses* 2011; 3(8): 1460–1484, https://doi.org/10.3390/v3081460.

82. Runckel C., Westesson O., Andino R., DeRisi J.L. Identification and manipulation of the molecular determinants influencing poliovirus recombination. *PLoS Pathog* 2013; 9(2): e1003164, https://doi.org/10.1371/journal.ppat.1003164.

83. Porta C., Kotecha A., Burman A., Jackson T., Ren J., Loureiro S., Jones I.M., Fry E.E., Stuart D.I., Charleston B. Rational engineering of recombinant picornavirus capsids to produce safe, protective vaccine antigen. *PLoS Pathog* 2013; 9(3): e1003255, https://doi.org/10.1371/journal.ppat.1003255.

84. Sanders B.P., Edo-Matas D., Custers J.H., Koldijk M.H., Klaren V., Turk M., Luitjens A., Bakker W.A., Uytdehaag F., Goudsmit J., Lewis J.A., Schuitemaker H. PER.C6(®) cells as a serum-free suspension cell platform for the production of high titer poliovirus: a potential low cost of goods option for world supply of inactivated poliovirus vaccine. *Vaccine* 2013; 31(5): 850–856, https://doi.org/10.1016/j.vaccine.2012.10.070.

85. Verdijk P., Rots N.Y., van Oijen M.G., Oberste M.S., Boog C.J., Okayasu H., Sutter R.W., Bakker W.A. Safety and immunogenicity of inactivated poliovirus vaccine based on Sabin strains with and without aluminum hydroxide: a phase I trial in healthy adults. *Vaccine* 2013; 31(47): 5531–5536, https://doi.org/10.1016/j.vaccine.2013.09.021.

86. Westdijk J., Koedam P., Barro M., Steil B.P., Collin N., Vedvick T.S., Bakker W.A., van der Ley P., Kersten G. Antigen sparing with adjuvanted inactivated polio vaccine based on Sabin strains. *Vaccine* 2013; 31(9): 1298–1304, https://doi.org/10.1016/j.vaccine.2012.12.076.

87. Baldwin S.L., Fox C.B., Pallansch M.A., Coler R.N., Reed S.G., Friede M. Increased potency of an inactivated trivalent polio vaccine with oil-in-water emulsions. *Vaccine* 2011; 29(4): 644–649, https://doi.org/10.1016/j.vaccine.2010.11.043.

88. Ivanov A.P., Dragunsky E.M., Chumakov K.M. 1,25-dihydroxyvitamin d3 enhances systemic and mucosal immune responses to inactivated poliovirus vaccine in mice. *J Infect Dis* 2006; 193(4): 598–600, https://doi.org/10.1086/499970.

89. Resik S., Tejeda A., Lago P.M., Diaz M., Carmenates A., Sarmiento L., Alemañi N., Galindo B., Burton A., Friede M., Landaverde M., Sutter R.W. Randomized controlled clinical trial of fractional doses of inactivated poliovirus vaccine administered intradermally by needle-free device in Cuba. *J Infect Dis* 2010; 201(9): 1344–1352, https://doi.org/10.1086/651611.

90. Cadorna-Carlos J., Vidor E., Bonnet M.C. Randomized controlled study of fractional doses of inactivated poliovirus vaccine administered intradermally with a needle in the Philippines. *Int J Infect Dis* 2012; 16(2): e110–e116, https://doi.org/10.1016/j.ijid.2011.10.002.

91. Nelson K.S., Janssen J.M., Troy S.B., Maldonado Y. Intradermal fractional dose inactivated polio vaccine: a review of the literature. *Vaccine* 2012; 30(2): 121–125, https://doi.org/10.1016/j.vaccine.2011.11.018.

92. Soonawala D., Verdijk P., Wijmenga-Monsuur A.J., Boog C.J., Koedam P., Visser L.G., Rots N.Y. Intradermal fractional booster dose of inactivated poliomyelitis vaccine with a jet injector in healthy adults. *Vaccine* 2013; 31(36): 3688–3694, https://doi.org/10.1016/j.vaccine.2013.05.104.

93. Resik S., Tejeda A., Sutter R.W., Diaz M., Sarmiento L., Alemañi N., Garcia G., Fonseca M., Hung L.H., Kahn A.L., Burton A., Landaverde J.M., Aylward R.B. Priming after a fractional dose of inactivated poliovirus vaccine. *N Engl J Med* 2013; 368(5): 416–424, https://doi.org/10.1056/nejmoa1202541.

94. Hiraishi Y., Nandakumar S., Choi S.O., Lee J.W., Kim Y.C., Posey J.E., Sable S.B., Prausnitz M.R. Bacillus Calmette-Guérin vaccination using a microneedle patch. *Vaccine* 2011; 29(14): 2626–2636, https://doi.org/10.1016/j.vaccine.2011.01.042.

95. del Pilar Martin M., Weldon W.C., Zarnitsyn V.G., Koutsonanos D.G., Akbari H., Skountzou I., Jacob J., Prausnitz M.R., Compans R.W. Local response to microneedle-based influenza immunization in the skin. *MBio* 2012; 3(2): e00012-12, https://doi.org/10.1128/mbio.00012-12.

96. Kim Y.C., Song J.M., Lipatov A.S., Choi S.O., Lee J.W., Donis R.O., Compans R.W., Kang S.M., Prausnitz M.R. Increased immunogenicity of avian influenza DNA vaccine delivered to the skin using a microneedle patch. *Eur J Pharm Biopharm* 2012 Jun; 81(2): 239–247, https://doi.org/10.1016/j.ejpb.2012.03.010.

97. Edens C., Collins M.L., Ayers J., Rota P.A., Prausnitz M.R. Measles vaccination using a microneedle patch. *Vaccine* 2013; 31(34): 3403–3409, https://doi.org/10.1016/j.vaccine.2012.09.062.