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Long-term evaluation of mucosal and systemic immunity and protection conferred by different polio booster vaccines

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ABSTRACT

Oral polio vaccine (OPV) and Inactivated Polio Vaccine (IPV) have distinct advantages and limitations. IPV does not provide mucosal immunity and introduction of IPV to mitigate consequences of circulating vaccine-derived polio virus from OPV has very limited effect on transmission and OPV campaigns are essential for interrupting wild polio virus transmission, even in developed countries with a high coverage of IPV and protected sewer systems. The problem is magnified in many countries with limited resources. Requirement of refrigeration for storage and transportation for both IPV and OPV is also a major challenge in developing countries. Therefore, we present here long-term studies on comparison of a plant-based booster vaccine, which is free of virus and cold chain with IPV boosters and provide data on mucosal and systemic immunity and protection conferred by neutralizing antibodies.

Mice were primed subcutaneously with IPV and boosted orally with lyophilized plant cells containing 1 μg or 25 μg polio viral protein 1 (VP1), once a month for three months or a single booster one year after the first prime. Our results show that VP1-IgG1 titers in single or double dose IPV dropped to background levels after one year of immunization. This decrease correlated with >50% reduction in seropositivity in double dose and <10% seropositivity in single dose IPV against serotype 1. Single dose IPV offered no or minimal protection against serotype 1 and 2 but conferred protection against serotype 3. VP1-IgA titers were negligible in IPV single or double dose vaccinated mice. VP1 antigen with two plant-derived adjuvants induced significantly high level and long lasting VP1-IgG1, IgA and neutralizing antibody titers (average 4.3–6.8 log2 titers). Plant boosters with VP1 and plant derived adjuvants maintained the same level titers from 29 to 400 days and conferred the same level of protection against all three serotypes throughout the duration of this study. Even during period, when no plant booster was given (~260 days), VP1-IgG1 titers were maintained at high levels. Lyophilized plant cells expressing VP1 can be stored without losing efficacy, eliminating cold chain. Virus-free, cold-chain free vaccine is ready for further clinical development.

1. Introduction

Poliovirus, the causative agent of poliomyelitis, is a human enterovirus with an RNA genome (7.5 kbp) and a capsid protein. Because of its smaller size (30 nm diameter) and simple structure, it has been studied extensively. Poliovirus enters human cells by binding to CD15, an immunoglobulin like receptor and endocytosis [1,2]. Because poliovirus is a positive stranded RNA virus, upon entry into human cells, it is readily translated. Poliovirus hijacks the cell by producing a protease that destroys the cap binding proteins; because translation of poliovirus mRNAs is cap-independent, host cell translational machinery becomes totally dedicated for production of viral proteins. Inhibition of host translational system in favor of virus specific protein synthesis results in production of a single long protein, which is cleaved into ten viral proteins by internal proteases.

Poliovirus enters human body through the fecal-oral route and the virus is shed in the feces of infected individuals, posing a major problem in eradication of this disease. Even in countries where public sewer system is well protected, silent polio outbreaks have been detected. Upon careful environmental monitoring a silent polio outbreak was recently reported in Israel [3,4] but most countries including the United States such monitoring is not done. In a large majority of infected patients poliovirus is detected in the bloodstream and such infections are asymptomatic. However, in some cases the virus spreads, replicates leading to minor symp-

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toms including fever, headache and sore throat. Paralytic poliomyelitis occurs when poliovirus enters the central nervous system crossing the blood brain barrier [5] and replicates within the spinal cord or brain, causing destruction of motor neuron leading to temporary or permanent paralysis. There are three known serotypes of poliovirus (type 1 – Mahoney, type 2 – Lansing, type 3 – Leon), each with a slightly different capsid protein and all three forms are highly infectious. The outer surface of capsid contains viral protein 1 (VP1), which is the same protein in all poliovirus serotypes and is therefore an ideal antigen for development of vaccines.

Two different polio vaccines were developed sixty years ago. The oral polio vaccine (OPV) contains a mixture of three different polioviruses with mutations to decrease their virulence. There are 57 nucleotide substitutions in the Sabin 1, two in Sabin 2 and ten in Sabin 3 stains that distinguish attenuated strains from virulent strains and reduce ability of poliovirus to translate in the host cell. Attenuated strains escape the acid and enzymes in the human gut and replicate efficiently but are unable to replicate in the central nervous system. OPV eliminated the need for sterile syringes required by IPV and generated mucosal immunity, protecting the primary site of poliovirus entry making this an ideal vaccine for global regions where this virus is endemic and reinfection is more common. Unfortunately, genetic stability of Sabin strains has been a major problem. Vaccine associated paralytic poliomyelitis among recipients of OPV was observed in several outbreak areas in the USA [6,7], Haiti [8], Dominican Republic [8], India [9], Phillipines [10], African continent [11,12] and many other global regions. In order to control polio outbreaks, several doses of OPV (as many as 13 doses) were administered [9] but resulted in several cases of vaccine induced polio [13]. In order to address these concerns, WHO Strategic Advisory Group of Experts (SAGE) recommended withdrawal of OPV 2 and the Global Polio Eradication Initiative is facilitating the switching of bivalent OPV from trivalent OPV in summer 2016 in many countries around the globe.

The Inactivated Polio Vaccine (IPV) is safe but less efficient in inducing mucosal immunity that is needed to prevent reinfection. Moreover, IPV required multiple boosters to maintain immunogenicity against polio virus infection. It is also not affordable in many developing countries. The high cost and limited supply of IPV has led SAGE to propose that one dose of IPV is adequate to prime population immunity. One dose of IPV has been adapted into routine immunization systems to boost immunity against poliovirus types 1 and 3 and provide a baseline of immunity against type 2 in case of an outbreak of type 2 vaccine derived poliovirus. It is indeed a major challenge to supply IPV globally. However, a diluted (or fractional) dose IPV can overcome this problem. Traditionally, full dose IPV is delivered through an intramuscular injection. However, when delivered subcutaneously, only 1/5 of a full dose IPV can generate almost as much immunity as one full dose delivered into the muscle; and two fractional doses generates higher immunity than one full dose [14]. These two alternative delivery routes could reduce the cost of IPV immunization and enable wider use of the limited supply of IPV. Adding to previous studies, a new field study in Sri Lanka provided more evidence that using fractional dose IPV is as effective as using a full dose in OPV primed populations to boost mucosal immunity [15].

The Global Polio Eradication Initiative (GPEI) was established in 1988 as a public-private partnership led by national governments and spearheaded by the World Health Organization (WHO), Rotary International, the US Center for Disease Control, the United Nations Children's Fund (UNICEF) and with substantial support from the Bill & Melinda Gates Foundation [16]. GPEI brought together under one umbrella recent scientific advances on poliovirus and kept track of polio around the world. Afghanistan, Nigeria and Pakistan are still listed as endemic areas globally for poliovirus. GPEI is working hard to strengthen global surveillance and immunization systems. The final goal of polio eradication by GPEI is "the endgame strategic plan" to detect and stop all wild-type poliovirus transmissions, including withdrawal of the use of OPV2 in the oral vaccine. The GPEI is still exploring additional delivery methods to overcome potential operational challenges, such as adaptors and needle-free devices to make it easier to deliver the vaccine, especially for children.

While IPV was effective in saving lives, several recent studies show that lack of mucosal immunity is a major challenge in eradication of polio and prevention of transmission. Polio eradication efforts are hampered by reintroduction of virus in polio free countries. Recent silent polio outbreak observed in Israel, which has used IPV for many decades, is one such example. Environmental surveillance in the absence of paralytic cases in 2013 revealed the presence of wild poliovirus in sewage samples in the South, Central and northern parts of Israel [4]. Open sewer system in many developing countries renders IPV unsuitable for polio eradication and environmental surveillance is not meaningful. Therefore, finding an alternative booster vaccine to stimulate both systemic and mucosal immune response after priming with IPV is indeed necessary.

From discussions above, the advantages and limitations of both OPV and IPV are quite evident. While IPV has not resulted in vaccine derived poliomyelitis, it does not provide mucosal immunity and therefore is not suitable for polio eradication or prevention of transmission. Indeed, in depth studies show that introduction of IPV to mitigate consequences of circulating vaccine-derived polio virus will have very limited effect on transmission and OPV campaigns are essential for interrupting wild polio virus transmission, even in a developed country with a high coverage of IPV and protected sewer system [4]. These conditions are not realistic to achieve in many countries with poor resources. Furthermore, switching from trivalent OPV to bivalent OPV will reduce protection against type 2 poliovirus and could lead to reintroduction of this poliovirus [4]. Requirement of refrigeration for storage and transportation for both IPV and OPV is also a major challenge in developing countries. Therefore, we have recently developed a plant-based booster vaccine which is free of virus and cold chain [17]. In this study, we compare long-term efficacy of this booster vaccine with IPV prime/boost, evaluate mucosal and systemic immunity and protection conferred by both types of vaccines.

2. Materials and methods

2.1. Plant-made protein and vaccine formulation

As previously described [17], lyophilized plant cells containing 1 μg or 25 μg of viral protein 1 (VP1) and plant-made adjuvants, saponion and/or squalene, were used for oral boosting. Briefly, an oil/water (O/W) emulsion was made by mixing the primary oil emulsion (squalene and Span 80) with the aqueous phase (saponin and lyophilized VP1) and adjusting the total volume to 200 μl per mouse with PBS.

2.2. Mice and immunization study

Six-week-old female CD-1 mice (Charles River Laboratories, Wilmington, MA, USA) were housed in micro-isolator cages. Totally there are ten groups of mice vaccinated with various formulations (group 3–10) (Fig. 1A and B). Group 1 was untreated. Mice were subcutaneously (s.c) primed and boosted with IPV (Groups 2) or prime only (group 3). Mice were orally boosted with either 1 μg VP1 from expressing leaves (group 4–6) or 25 μg (group 7–10),

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