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A mucosal adjuvant for the inactivated poliovirus vaccine

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ABSTRACT

The eradication of poliovirus from the majority of the world has been achieved through the use of two vaccines: the inactivated poliovirus vaccine (IPV) and the live-attenuated oral poliovirus vaccine (OPV). Both vaccines are effective at preventing paralytic poliomyelitis, however, they also have significant differences. Most importantly for this work is the risk of revertant virus from OPV, the greater cost of IPV, and the low mucosal immunity induced by IPV. We and others have previously described the use of an alphavirus-based adjuvant that can induce a mucosal immune response to a co-administered antigen even when delivered at a non-mucosal site. In this report, we describe the use of an alphavirus-based adjuvant (GVI3000) with IPV. The IPV-GVI3000 vaccine significantly increased systemic IgG, mucosal IgG and mucosal IgA antibody responses to all three poliovirus serotypes in mice even when administered intramuscularly. Furthermore, GVI3000 significantly increased the potency of IPV in rat potency tests as measured by poliovirus neutralizing antibodies in serum. Thus, an IPV-GVI3000 vaccine would reduce the dose of IPV needed and provide significantly improved mucosal immunity. This vaccine could be an effective tool to use in the poliovirus eradication campaign without risking the re-introduction of revertant poliovirus derived from OPV.

1. Introduction

The Global Poliovirus Eradication Initiative (GPEI) has reduced poliovirus cases by more than 99% worldwide since it was initiated in 1988 by the World Health Organization (WHO) [1]. To highlight a recent milestone by GPEI, wildtype poliovirus cases in India have not been reported for over 2 years [2]. Currently, however, the risk of wildtype poliovirus spreading from the endemic countries of Afghanistan, Pakistan, and Nigeria to polio-free countries continues to require vaccination coverage worldwide.

Poliovirus infects the gut and is transmitted primarily through shedding in feces by the fecal–oral route, but can also be transmitted by the oral–oral route [3]. In <1% of cases [4], acute flaccid paralysis occurs when the virus spreads to the central nervous system (CNS) [3]. Two vaccines are in use to protect against poliovirus: the inactivated poliovirus vaccine (IPV) and the live-attenuated oral poliovirus vaccine (OPV), with each containing the three poliovirus serotypes. Both IPV and OPV induce serum antibodies that prevent poliovirus spread to the CNS, but OPV is superior at inducing mucosal immunity, shortening the period of poliovirus replication in the gut and subsequent duration of shedding (after ≥2 doses OPV) [5–7]. OPV is also thought to reduce transmission in this manner, but the induction of mucosal immunity can be incomplete and the relationship between the level of mucosal immunity and likelihood of transmission is unknown [8–10]. Nevertheless, OPV use has led to the eradication of poliovirus in several countries.

One significant disadvantage of OPV, however, is that in rare cases (about 1 in 0.9 million vaccinees, [11]), an attenuated strain in OPV can revert to virulence and cause vaccine-associated paralytic poliomyelitis (VAPP). The use of OPV may also lead to vaccine-derived polioviruses (VDPVs) capable of spread between individuals [12–16]. Another disadvantage of OPV, is that in its trivalent form the three vaccine strains compete with one another to infect the gut, resulting in a stronger immune response to type 2 versus types 1 and 3 [17]. More recently, the use of monovalent and bivalent OPV has helped to overcome this issue, but still relies on infection of the gut which can lower vaccine efficacy when there are intercurrent infections [18]. Use of IPV avoids these issues since it lacks replicating virus and uses a different route of administration (intramuscular). OPV was selected over IPV as the vaccine for

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worldwide eradication due to its ability to induce mucosal immunity, its lower production cost, and ease of administration [1,19]. If a new IPV vaccine formulation had a lower cost and induced mucosal immunity this would be a significant asset to the GPEI. Such a vaccine could be used after cessation of OPV use in the post-eradication era or in mop-up campaigns where wildtype poliovirus has been introduced into a polio-free country [20].

Currently, IPV is not used with an adjuvant and an adjuvant that induces a mucosal immune response by a non-mucosal intramuscular route like that used for IPV would be advantageous. Without inducing mucosal immunity, IPV can prevent symptomatic poliomyelitis but may not reduce infection and asymptomatic excretion of wildtype poliovirus [21]. Previously, the adjuvant 1,25-dihydroxyvitamin D3 was shown to enhance the mucosal IgA immune response to IPV in mice, but the fold increase was very small [22]. An IPV adjuvant that allows for dose-sparing to lower cost and improves the mucosal immune response would greatly improve this vaccine.

A promising mucosal adjuvant for IPV is a novel alphavirus-based adjuvant. This adjuvant enhances humoral, cellular and mucosal immunity to antigens, even when delivered at a non-mucosal site [23–25]. The alphavirus-based adjuvant is a disarmed RNA virus particle which targets inflammatory dendritic cells in the draining lymph node and mimics the earliest stages of viral infection [26]. The disarmed virus cannot propagate as the RNA genome lacks the structural genes of the virus. Inside the cell, replication of the RNA genome induces an antiviral innate immune response. When this adjuvant is co-administered with an antigen, the adaptive immune system sees this antigen as if it was the product of a viral infection. Accordingly, the resulting humoral, cellular and mucosal immune responses are significantly improved relative to antigen alone. An IPV vaccine that includes an alphavirus-based adjuvant could allow for dose-sparing to reduce cost and also induce mucosal immunity that would increase protection against poliovirus replication in the gut, reduce poliovirus excretion into the environment and induce serum antibodies that would prevent spread of poliovirus to the CNS. Such a vaccine could help break transmission cycles during a poliovirus outbreak in a previously poliovirus-free country.

A previous study demonstrated that GVI3000, an adjuvant derived from the alphavirus Venezuelan equine encephalitis virus (VEE), increased the potency of IPV made from inactivated Sabin strains (sIPV), as measured by neutralizing antibody titers in rats [27]. Since the protective efficacy of sIPV in humans has not been evaluated, in the work described herein we further investigated the ability of GVI3000 to increase the potency of IPV in rats and mice, and to determine whether GVI3000 can induce a mucosal immune response to poliovirus antigens. The ability of GVI3000 to allow dose-sparing and to enhance mucosal immune responses to IPV is important for its utility as an adjuvant and whether an IPV-GVI3000 vaccine would be useful to the GPEI after global use of OPV has ceased in the post-eradication era.

2. Materials and methods

2.1. GVI3000 replicon particles

Production of GVI3000 has been described [28,29]. Briefly, GVI3000 replicon RNA and two helper RNAs expressing either capsid or glycoproteins are electroporated into BHK-21 cells. The helper RNAs produce the structural proteins in trans but lack the cis-acting packaging sequence, so that only the replicon RNA containing the packaging sequence is incorporated into the adjuvant particles. GVI3000 replicon particles are packaged in the wild-type (V3000) VEE envelope [30]. After purification, the absence of detectable propagation-competent virus is confirmed by cytopathic effect assay which can detect 1 PFU VEE. GVI3000 is titered by immunofluorescent staining of VEE non-structural proteins in infected BHK-21 cells.

2.2. Mice and immunizations

Six to eight week old female BALB/c mice were purchased from Charles River and housed at the University of North Carolina Division of Laboratory Animal Medicine animal facility according to protocols approved by the Institutional Animal Care and Use Committee and the Institutional Biosafety Committee. 6–10 mice per group were used in these studies. Mice were injected intramuscularly (i.m.) in the gastrocnemius at weeks 0 and 4 with IPV from Intravacc. All animals received 0.8/0.2/0.6 D antigen units IPV, types 1, 2, and 3, respectively with or without 10^5 infectious units (IU) of GVI3000. This IPV dose was 50-fold lower than the dose used in humans (40/8/32 DU). The dose was chosen based on a previous experiment that showed GVI3000 has its maximum adjuvant effect and antibody titers with IPV at this dose and further increases in dose did not increase titers (data not shown). This dose was a 1:5 dilution of 10 μL IPV stock (80/16/64 D antigen units per mL) into 40 μL PBS. See Supplementary Materials and Methods for analysis of poliovirus-specific IgG and IgA by ELISA.

2.3. Potency test in rats

In collaboration with Intravacc in the Netherlands, we evaluated the efficacy of the GVI3000 adjuvant combined with IPV in a potency test in outbred Rivm:TOXrats [27]. On day 0 and 28, 15 rats per group were immunized by i.m. injection with 1.5/0.3/1.2 D antigen units of IPV with or without 10^5 IU of GVI3000. All rats were bled on day 21 (3 weeks post-prime) and day 49 (3 weeks post-boost) and sera were analyzed for neutralizing antibodies to all three poliovirus serotypes [31]. The rat immunogenicity tests were approved by an Animal Welfare Committee.

2.4. Statistical analysis

For mouse experiments, the means of reciprocal endpoint dilutions between groups with and without GVI3000 were compared by Student's *t*-test as indicated by horizontal bars in the figures. The same test was performed on the endpoint dilutions of the rat neutralization titers. Statistical analyses were performed using GraphPad Prism 5® software.

3. Results

3.1. GVI3000 potentiated humoral and mucosal immune responses to IPV

Previous reports have shown that peripherally inoculated GVI3000 induced a significant increase in humoral and mucosal antibodies to co-delivered antigens [23,32]. To determine if GVI3000 could potentiate immune responses to IPV, groups of six mice were immunized i.m. with IPV alone (0.8/0.2/0.6 DU) or in combination with GVI3000. Serotype-specific antibody levels in vaccinated animals were detected 3 weeks post-boost by ELISA. GVI3000 significantly increased the serum IgG titers to all three PV serotypes compared to IPV alone (Fig. 1A). Within IgG subclasses, GVI3000 induced 6.5–30 times more IgG2a than IPV alone (Fig. 1B) and comparable IgG1 levels (Fig. 1C). IgG2a and IgG1 are markers that correlate with the induction of Th1 and Th2 responses, respectively [33–35]. An IgG2a/IgG1 ratio close to 1 (Fig. 1D) indicated that GVI3000 increased the Th1 response leading to a more balanced Th1/Th2 response compared to IPV alone.