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Neutralization capacity of highly divergent type 2 vaccine-derived polioviruses from immunodeficient patients

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INTRODUCTION

The oral poliovirus vaccine (OPV) of Albert Sabin is nearly ideal for use in polio eradication [1]. This inexpensive vaccine is easily administered by mouth, facilitating its widespread use. OPV induces intestinal immunity, making recent OPV recipients resistant to infection by wild polioviruses, and effectively blocking wild poliovirus transmission when used in mass campaigns and routine immunizations. It provides long-term protection against poliomyelitis through durable humoral immunity. Because OPV is a live vaccine, vaccine virus can spread to and immunize unvaccinated contacts of vaccine recipients, increasing the impact of OPV beyond those actually immunized. Through effective use of this excellent vaccine, the World Health Organization's Global Polio Eradication Initiative (GPEI) has brought wild polioviruses to the threshold of eradication [2, 3].

Despite its many advantages, the continued use of trivalent OPV was identified as a threat to maintaining eradication because use of OPV carries certain liabilities arising from the genetic instability of the live, attenuated vaccine strains during replication in the human intestine [4]. In 2016, the live type 2 component of the oral vaccine was removed from OPV and replaced with bivalent OPV (types 1 and 3) to reduce the risk of type 2 vaccine-derived polioviruses (VDPVs), though a stockpile of monovalent type 2 OPV (mOPV2) is maintained for outbreak response.

OPV and inactivated poliovirus vaccine (IPV) both induce neutralizing antibodies against poliovirus, especially after multiple vaccinations. The effectiveness of both vaccines led to the elimination of wild type polio with the exception of Pakistan, Afghanistan and Nigeria [4]. In order to successfully move forward with the eradication effort, we must adhere to the so-called pillars of the global polio eradication initiative: routine immunization, supplementary immunization, acute flaccid paralysis surveillance and targeted mop-up campaigns [5]. The 'hallmark of efficacy' for the poliovirus vaccines is a robust neutralizing antibody response against the viral capsid, with a 50% endpoint serum titer greater than 1:8 corresponding to protection [6].

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While use of OPV has increased immunity to all three serotypes in populations, the attenuated vaccine virus can revert to a neurovirulent phenotype that is indistinguishable from that of wild polioviruses. VDPVs fall into three categories depending on the sampling source and epidemiologic data: iVDPV (derived from an individual with a known primary immunodeficiency) [5–8], circulating VDPV (cVDPV; at least two genetically related VDPVs derived from individuals who are not close contacts of one another, implying circulation in the community), and aVDPV (ambiguous—a single virus not genetically linked to any other VDPV, whether from a paralyzed case or an environmental source). In areas with low vaccine coverage, these VDPVs may emerge and circulate [7]. By definition, the VP1 nucleotide sequence of an OPV isolate must have diverged by more than 1% (0.6% for type 2 VDPV) from the reference OPV strain to be designated a VDPV [8].

Primary immune deficiencies affecting antibody production leave patients susceptible to infections, requiring regular treatment with intravenous immunoglobulin [9, 10]. In these patients, exposure to OPV through vaccination or contact with a vaccine recipient poses a higher risk of developing paralytic poliomyelitis [11, 12]. In patients with an antibody deficit, Sabin vaccine viruses can replicate for extended periods of time compared to immunocompetent vaccine recipients [13]. Over time, increasing numbers of nucleotide changes accumulate in the viral genome, with reversion to neurovirulence. Although extremely rare, some immunodeficient individuals can excrete VDPVs over prolonged periods (>6 months), posing a risk of infection in their community.

As these nucleotide substitutions are introduced, amino acid substitutions may accumulate in the proteins of VDPVs. These substitutions can occur in the capsid region within known antigenic sites, resulting in distinct antigenic profiles compared to the original OPV strains [14]. Where examined, sera from poliovirus recipients showed reduced neutralizing capacity against VDPVs, although titers were sufficiently high to confer protection [15–23]. However, little work has been done to determine the effect of increasing amino acid changes in iVDPVs on the neutralization capacity of sera from poliovirus vaccine recipients.

In the current study, we selected sera from a previous study investigating the effect of a combined OPV/IPV vaccine schedule in children from low income communities in the United States during the switch in immunization schedule from OPV to IPV [24]. The sera were tested for neutralizing antibodies against a panel of type 2 iVDPVs with increasing number of nucleotide changes in the viral capsid proteins. For each type 2 iVDPV, homology models of the capsid were generated for mapping antigenic sites and comparing the structures to the Sabin 2 reference.

MATERIALS AND METHODS

Patient Sera

Sera were obtained from children aged 19–35 months, who participated in a 4-year U.S. poliovirus immunity surveillance study from 1997–2001 conducted as part of the Childhood Immunization Demonstration Project of Community Health Networks [24]. The study protocol was reviewed and approved by the Centers for Disease Control and Prevention Institutional Review Board. Study participants consented to serological testing against

poliovirus antigens, allowing for use of the specimens in the current study. This study was originally designed to evaluate the effects of the change in poliovirus vaccination schedule in the United States, from OPV to a combined OPV/ IPV, and IPV-only schedule. Immunity to poliovirus was measured in these children by determining the presence of neutralizing antibodies to poliovirus types 1, 2, and 3 using the poliovirus microneutralization assay [25]. Thirty serum specimens were randomly selected from each of the three sites in the 1997–2001 serosurvey: Detroit, Michigan; Manhattan, New York and Denver, Colorado. All sera were stored at −20°C at the Centers for Disease Control and Prevention, Atlanta, Georgia, until the cross neutralization study began in July 2012.

Poliovirus Isolates

The seven isolates used in this study are listed in Table 1. Isolates 18277 and 18278 were collected from the same patient. Viral stocks were grown in HEp2-C cells and incubated at 35°C with 5% CO_2, in a humidified atmosphere, until full cytopathic effect (CPE) was observed. The virus was then harvested by performing three freeze-thaw cycles at −70°C, centrifugation to remove cell debris, and collection of the supernatant, which was stored in aliquots at −20°C until used in the assay.

Each virus was titrated on HEp2-C cells, as described previously [25], to ensure that all the antigens used had the same starting concentrations, expressed in 50% cell culture infectious dose ($CCID_{50}$).

Polio neutralization assay

All sera had been previously randomized using a balanced block scheme. Since results from previous tests run on these sera were readily available, 30 specimens having positive titers for Sabin 1, 2, or 3 were chosen from the final year of each study[25]. The existing randomization scheme was followed, but specimens with volumes less than 200 μl were excluded. The sera were tested in multiple runs, with sera from all three sites being tested against the same two or three polioviruses each day. Therefore, each run contained 90 test sera plus an internal reference standard of pooled sera, used 10 times. All sera were randomly assigned to positions on 96-well cell culture plates (Corning Inc., Corning, NY). Each test plate contained four sera in triplicate in two-fold dilution increments ranging from 1:8 to 1:1024.

A virus titration control plate was prepared for each antigen to ensure that test plates were inoculated with the correct concentration of antigen. In addition, a cell control plate consisting only of cells and media was used to monitor cell viability. Serum dilutions were incubated with virus for 3 hours at 35°C before the addition of 25 μl of HEp2-C cell suspension at a concentration of 3×10^5 cells/ml to each well of the test plates, back titration plates, and cell control plate.

After incubation for five days, the plates were stained with 0.05% crystal violet solution and the optical density measured at 570 nm by plate reader. Endpoint titers were calculated using the Spearman-Kärber formula [26]. Seropositivity was defined as the detection of neutralizing antibody at a titer of 1:8 or higher ($\log(\text{titer}) \geq 3$).

Sequence analysis

Complete capsid sequence analysis was performed as described previously from cell culture isolates [27, 28]. Sequences have been deposited in GenBank (Table 1).

Structural modeling and antigenic site sequence comparisons

Polio protomer homology models were generated for each strain using the SWISS-model server (http://swissmodel.expasy.org), using the template PDB file 1EAH (MEF-1) as a reference, with amino acid similarity ranged from 94.3% for the 10 nt change isolate to 90.2% for the 115 nt change isolate. Amino acid changes and antigenic sites were highlighted using UCSF Chimera (Version 1.6.1). Each protomer was assembled into a pentamer configuration using the biological assembly data in the 1EAH crystal structure metadata file.

Protomer models for each iVDPV capsid (P1 region) were compared to the Sabin 2 capsid model using the MatchMaker tool in UCSF Chimera. The root mean square deviation (RMSD) was calculated across the entire capsid sequence for each iVDPV capsid.

To determine if there was a correlation between the degree of amino acid conservation across an antigenic site and the median neutralization titer for each iVDPV isolate, a substitution score was assigned to each amino acid identified as a key residue in each antigenic site by monoclonal antibody escape [21, 29–31]. For each antigenic site, the rate of substitutions per site was calculated using the JTT model of peptide substitution and then plotted versus the corresponding median neutralizing antibody titer for each virus isolate [32].

Data Analysis

All statistical analyses were conducted using SAS v. 9.2 (SAS Institute, Cary, NC). The Wilcoxon signed rank test was used to compare the antibody titers for each iVDPV2 with the Sabin 2 titer, as iVDPV2 and Sabin 2 titers were assumed to be dependent variables for a given serum.

RESULTS

Seropositivity rates in vaccinated children decrease as P1 nucleotide substitutions increase in iVDPVs.

To measure the neutralization capacity of seven type 2 iVDPVs with varying degrees of nucleotide substitution in P1. Isolates ranged in divergence from 10 nt changes (6 amino acid changes) to 115 nt changes in P1 (53 amino acid changes) (Table 1). Sera from 90 low-income children in New York, Detroit, and Denver, were used in the gold-standard poliovirus microneutralization assay. Sera with a $\log_2$ neutralization titer ≥ 3 are considered seropositive and protective against paralytic disease. The rates of seropositivity for the children's sera were determined for all seven iVDPVs. We observed seropositivity rates for strains 18279, 18280, 18281 and 18282 of 98.9% each and for strains 18277 and 18278 of 94.4% each compared to 97.8% for Sabin 2 ($p>0.05$). Strain 18276, with 115 nucleotide changes from Sabin 2, was the exception to these very high seropositivity rates against the

iVDPV2 panel, with only 28.9% of sera containing antibodies capable of neutralizing this antigen ($p<0.001$) (Table 2).

Because the sera used for analysis were collected from multiple sites, we determined the seropositivity rates for each site (n=30 per site) (Table 2). For strains 18279, 18280, 18281, and 18282, the seropositivity rates for each strain were 100%, 100%, and 96.7% for New York City, Denver and Detroit, respectively ($p > 0.05$, compared to Sabin 2). For strains 18277 and 18278, we observed a reduced seropositivity rate compared to Sabin 2 for New York City, Denver, and Detroit of 96.7%, 93.3%, and 93.3%, respectively ($p > 0.05$). The lowest seropositivity rates were seen against strain 18276 in Denver and Detroit (20% and 16.7%, respectively) ($p < 0.001$). Sera from the New York City site had the highest seropositivity against 18276 compared to Sabin 2, at 50% ($p < 0.001$).

Highly diverged type 2 iVDPVs are poorly neutralized

iVDPV2 isolates 18282, 18281, 18280 and 18279 were fully neutralized, with median titers of 9.34, 9.17, 8.50 and 8.50, respectively, compared to a median titer of 8.17 to the Sabin 2 control ($p = 0.0561$ for all compared to Sabin 2). The data for the iVDPV isolates 18278, 18277 and 18276 showed median titers of 7.17, 7.00 and 2.50, respectively, indicating a significant drop in neutralizing capacity at the 98 nucleotide substitution threshold (Figure 1) ($p < 0.001$ for all compared to Sabin 2). Interestingly, Sabin 2 did not always appear to correspond to the highest titers. iVDPVs with 10, 14, 16 and 20 nucleotide substitutions showed higher median neutralizing capability than Sabin 2 in this study.

For a given serum, the neutralizing titer for Sabin 2 was compared to that of each iVDPV. To quantify that statistical relationship, we conducted a Spearman correlation test (Figure 2). The highest coefficients (0.95, 0.93, 0.95, and 0.93) were seen in the iVDPV2 variants with 10, 14, 20 and 26 changes (18282, 18281, 18280 and 18279, respectively). There was a noticeable reduction in the value of the statistic as the number of mutations increased. Isolate 18276, with 115 nucleotide changes in VP1 showed the weakest correlation coefficient value of 0.59. Therefore, as the number of nucleotide and amino acid changes in VP1 increases, the less Sabin-like the VDPV becomes in terms of neutralizing properties.

Highly diverged iVDPVs accumulate a higher number of amino acid changes in neutralizing antigenic sites

Because we observed a trend of decreasing neutralization titers against iVDPVs with increasing VP1 nucleotide substitutions, we performed multiple sequence alignments of the neutralizing antigenic sites in the translated capsid proteins (Figure 3). The alignment of the neutralizing antigenic sites indicated that as nucleotide changes accumulate in P1, there is a corresponding increase in amino acid changes in the capsid neutralizing antigenic sites. For iVDPVs with fewer than 98 nucleotide changes in VP1, we observed that the majority of amino acid substitutions occurred in the NAg3a and NAg3b. In the isolate with 10 nt changes (18282), we observed non-conservative substitutions at VP3-$T_{73}\rightarrow$A (symbolized as T3073A),. For the isolate with 14 nt change (18281), there were two non-conservative substitutions at S3058G and K3061M. The sequence data also indicated a mixed base at amino acid residue 1221 of VP1 for the isolate with 14 nt changes (18281), resulting in

either a Sabin-like Ala or a Thr substitution. In the isolate with 16 nt changes (18280), we identified two non-conservative mutations at Q3059K and T3073A. In the isolate with 20 nt change (18279), we observed a non-conservative substitution at T3073M.

As the number of accumulated nucleotide substitutions in VP1 reached 98 or more, we observed a higher frequency of non-conservative substitutions in all neutralizing antigenic sites. In the isolate with 98 nt change (18278), we observed substitutions in NAg2 at A1221T, T1223A, A2170E, and N2172K. We also observed non-conservative substitutions in NAg3a/b at Q3059G, E3069M, R2070S, H3075P and T3078E. In the 107 nt change isolate (18277), we observed non conservative substitutions in NAg2 at N2172E. We also observed a non-conservative substitution in NAg3a/b at H3075P.

The isolate with 115 nt change (18276) had 13 non-conservative amino acid substitutions, distributed among all antigenic sites. For NAg3b and NAg2, we observed substitutions at R2070S, T2164K, A2166T, T2167D, and N2172K. For NAg3a/3b, substitutions occurred at T3073A, H3075N, S3076L, and T3078E. For NAg1, NAg2 and NAg3a, substitutions occurred at K1099E, S1102D, T1223A, and G1228E. Most notably, the alignment of the NAg1 site indicates that the A1101 residue was deleted in this virus.

Linear regression analysis was used to determine whether a correlation exists between the rate of substitution per site and the neutralization titer; a negative slope and a high R^2 would suggest that the substitutions in a given antigenic site correlate with the neutralization titers. The linear regression analysis suggests that the neutralization titers have the strongest correlation with the rate of substitution per site for NAg1 (slope = −0.030, R^2 = 0.866) (Figure 4A). In addition, NAg2 (Figure 4B) had a good correlation between the rate of substitution per site and neutralizing antibody titers (slope = −0.069, R^2 = 0.600), while only a weak correlation exists for NAg3b (slope = −0.129, R^2 = 0.513) (Figure 4D). No correlation was observed between neutralization titers and rate of substitutions per site for NAg3a (Figure 4C). These data suggests that NAg1, 2, and 3b might play a role in the reduced neutralization capacity for the highly divergent iVDPV2 isolates.

Predicted structural displacement within NAg1 of highly diverged iVDPVs containing an amino acid deletion in the BC loop of VP1

We sought to investigate further the alterations in the NAg sites that may have contributed to the reduced capacity of vaccine-induced antibodies to neutralize isolate 18276. Therefore, we generated models of the capsid protomer, assembled five copies into the pentameric structure for the 18276 isolate, and compared it with the structure of Sabin 2. The structure similarity was determined using the root mean square difference (RMSD) method and visualized on the pentameric unit (Figure 5). Not surprisingly, the highest degree of structure variation occurred near the deletion site in the BC loop of VP1 (which corresponds to NAg1) and also at NAg2 in VP2. The structure comparison of the homology models suggests a role for both NAg sites in the reduced capacities of sera from vaccinated individuals to neutralize the highly divergent iVDPV.

Discussion

The primary drawback to OPV is the genetic instability of the vaccine strains, leading to reversion from an attenuated phenotype to the neurovirulent, wild phenotype [33, 34]. These vaccine-derived polioviruses have the capacity to transmit from person-to-person in regions with poor vaccine coverage and have led to outbreaks in Africa, Asia, and the Caribbean [35]. As a result, the Global Polio Eradication Initiative Strategic Plan 2013–2018 called for a switch from trivalent OPV to bivalent OPV and IPV during the pre-eradication phase in an effort to reduce the emergence of VDPVs [36] and the global switch occurred during April 2016.

The ability of OPV and IPV to induce neutralizing antibodies to VDPVs has not been investigated extensively. Previous studies have investigated the antigenic properties of circulating vaccine derived polioviruses (cVDPV) and iVDPV isolates using antigenic site-specific monoclonal antibodies or polyclonal sera from OPV or IPV recipients [17, 19, 21, 22, 28]. These approaches were limited by the number and divergence of VDPV isolates as well as the source and number of sera tested. In the present study, we have demonstrated that nucleotide substitutions in the capsid correlate with reduced neutralizing capacity of sera from immunized children. Furthermore, the substitutions were mapped to sites that are predicted to result in significant structural changes to important neutralization epitopes based on comparisons of pentamer structure models. It is expected that these structural changes contribute to reduced neutralization capacity.

The data from isolate 18276 show some regional differences in seropositivity rates for immunized children, with low rates observed in all three sites included in this study. Interestingly, the study reported that vaccine coverage for at least three doses was 93% in New York, 79% in Detroit and 88% in Denver [17]. However, the proportion of sera with a protective titer against isolate 18276 was 50% in the sera from New York and only 16.7% and 20% in the sera from Detroit and Denver, respectively. This is consistent with a relationship between high vaccine coverage rates and high seropositivity rates against these particular highly diverged iVDPVs. Data from a Sabin-based IPV clinical trial in China support the antigenic divergence of this isolate with an average seropositivity rate of ~24% [37].

The potential for iVDPVs to spread in a population with either high or low OPV/IPV coverage is unclear but wide circulation is considered unlikely. Two documented cases of iVDPVs suggest that it is possible for a VDPV from an immunocompromised individual to be transmitted to unvaccinated subjects. In a 2002 iVDPV case in Russia, a hospitalized infant exhibiting symptoms of acute flaccid paralysis transmitted a VDPV to contact children sharing a hospital room [15]. In a 2005 iVDPV event in the United States, the evidence did not support a clear source of the virus; however, the circulating VDPV was hypothesized to originate from another immunodeficient individual previously exposed to OPV outside the United States [38]. In the case report for isolate 18276 used in this study, there was no indication that household or hospital contacts were tested for poliovirus or for antibodies against the iVDPV, although it was noted that none exhibited neurologic symptoms of poliomyelitis [39]. VDPVs resembling strains isolated from immunodeficient

excretors have been detected in environmental samples from countries that have not reported cases of poliomyelitis since 1985 [40–42].

The sequence and structural analysis of isolate 18276 showed that an amino acid deletion occurred at position 101 in the VP1-NAg1 region, resulting in prediction of a significant structural change in the BC loop of VP1. The antigenicity of this site and the flexibility of the BC loop has been proposed for the generation of chimeric vaccine virus, suggesting an important role for NAg1 in the immunogenicity of polioviruses [43]. Mulders et al. have reported a type 1 VDPV isolate with a two-amino acid deletion in the BC loop of VP1 which exhibited a unique neutralization pattern with antigenic-site-specific monoclonal antibodies [19]. However, sera from IPV and OPV vaccine recipients were able to neutralize the VDPV strain with titers against Sabin 1 equal to those of the Mahoney wild reference strain [19]. The BC loop of VP1, which makes up part of NAg1, clearly plays an important role in antibody binding and neutralization although other sites also influence the neutralization capacity of highly diverged VDPV isolates. VDPV type 2 isolates shed from a patient in the United Kingdom for over 30 years have been shown to have reduced binding with antigenic site specific monoclonal antibodies and sera from UK adults, although the most diverged isolate tested did not have a deletion in the BC loop as with isolate 18276 [13]. Due to the significant gap between the number of nucleotide changes in isolate 18279 (20 changes) and isolate 18278 (98 changes), it is not possible to know whether there is a sudden or gradual decrease in neutralizing capacity as the number of mutations increases.

Conclusions

Maintenance of population immunity to poliovirus through high vaccine coverage will prevent cVDPV emergence and spread. Apart from possible limited transmission within an unimmunized community of a VDPV that may have originated with an infection of an immunodeficient person [44], evidence is lacking for spread of iVDPVs in the general population. This observation suggests that other factors may limit transmission of even the most antigenically divergent iVDPVs. Possible contributing factors include: 1) residual cross-neutralizing immunity from immunization may protect immunocompetent individuals before induction of antigen-specific neutralizing antibodies; 2) improved hygiene of older individuals may reduce exposure of contacts to excreted virus; and 3) the highly divergent iVDPVs have distinctive biological properties (such as unusual antigenic structures) that may impair replicative fitness. However, to minimize the potential risks of iVDPV spread, it is important to maintain high levels of population immunity and sensitive poliovirus surveillance now and into the future. In addition, surveillance of immunodeficient patients exposed to OPV should be conducted to identify chronic or long-term excretors of iVDPVs [45]. Although no current treatment options exist, new antiviral drugs and monoclonal antibody treatments show some promise as treatments for these patients with the added benefit of reducing the risk of re-introduction of poliovirus post-eradication [46, 47].

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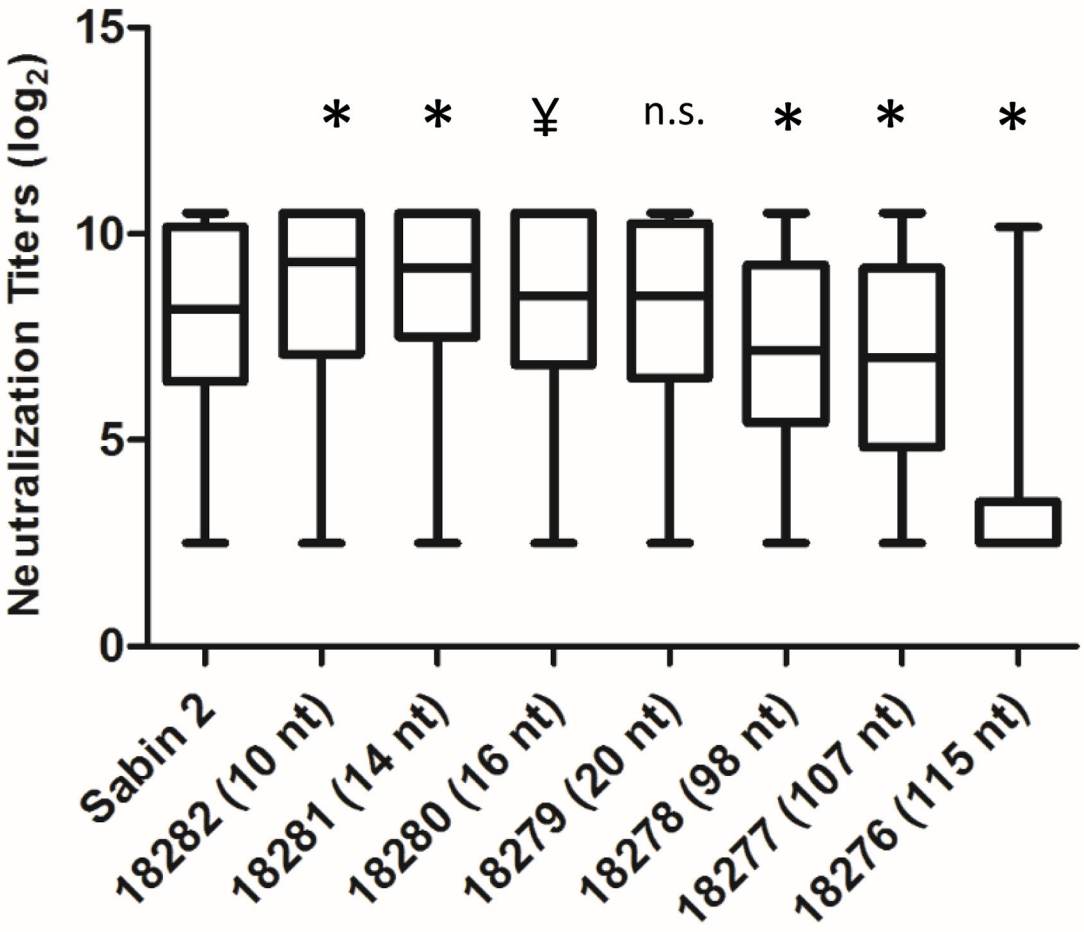

Figure 1.

Highly diverged type 2 iVDPVs are poorly neutralized by sera from OPV/IPV vaccinated children. Box plots of neutralization titers against each type 2 iVDPV and Sabin 2, indicating median, upper and lower quartiles, and minimum/maximum titers. All titers are represented in a $-\log_2$ format.

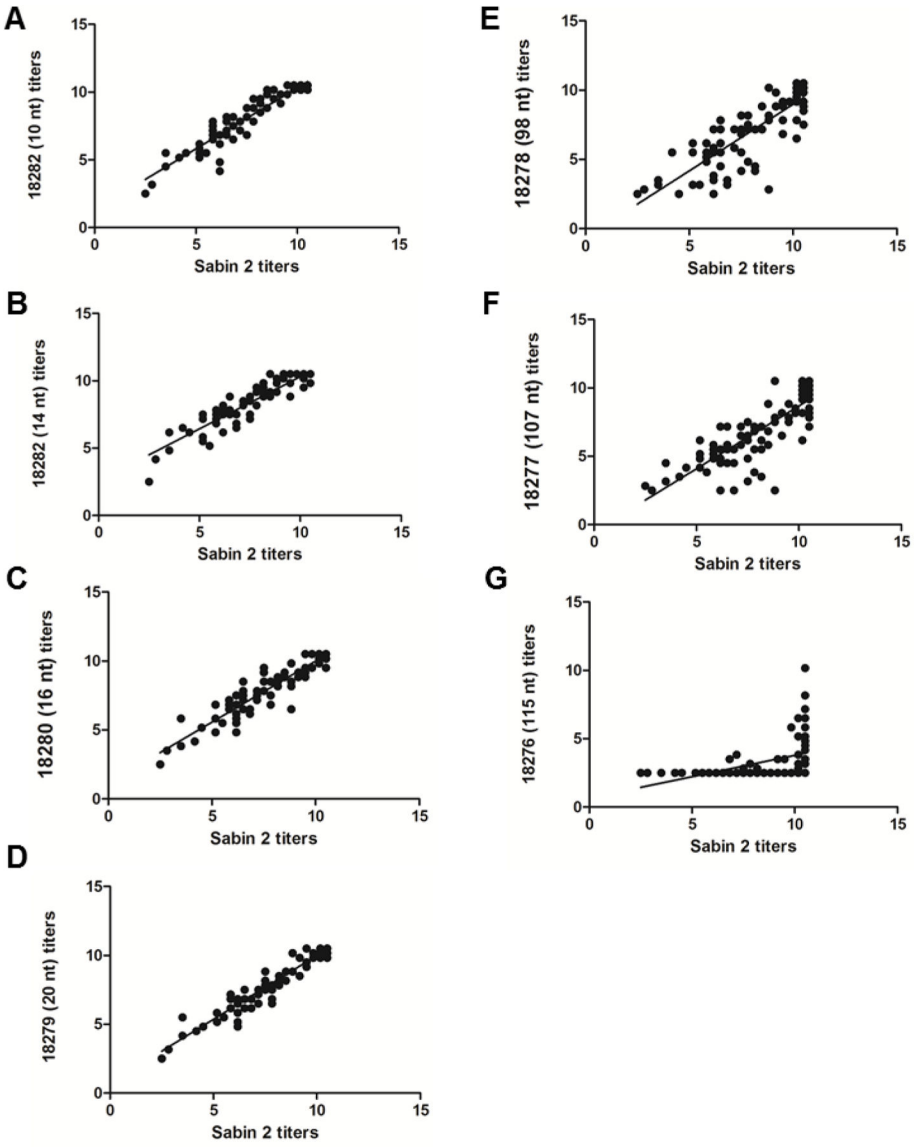

Figure 2.

Neutralizing antibody titers to highly divergent type 2 iVDPVs do not correlate with Sabin 2 titers. The neutralization titers of 90 sera from children vaccinated using a combined OPV/IPV schedule against iVDPV2 isolates with increasing numbers of nt substitutions were correlated with the neutralization titers against Sabin 2. All titers are represented in a $-\log_2$ format. (**A**) 10 nt changes, 18282; (**B**) 14 nt changes, 18281; (**C**) 16 nt changes, 18280; (**D**) 20 nt changes, 18279; (**E**) 98 nt changes, 18278; (**F**) 107 nt changes, 18277; (**G**) 115 nt changes, 18276.

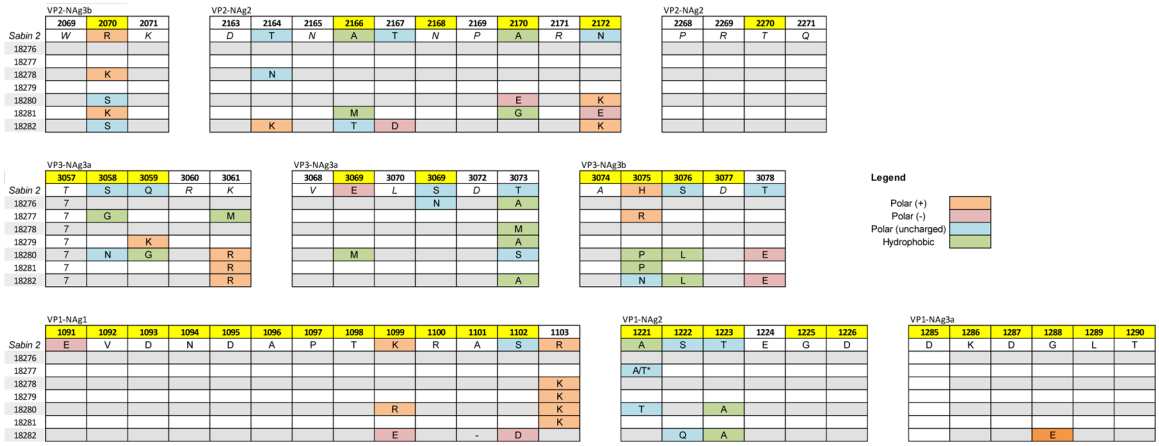

Figure 3.

Amino acid substitutions in neutralizing antigenic (NAg) sites occur more frequently in highly diverged type 2 iVDPVs. Multiple sequence alignments for each neutralizing antigenic site (NAg1, NAg2, NAg3a, and NAg3b) were generated for the type 2 iVDPVs using the Sabin 2 sequence as a reference. Numbering system begins with each chain of the poliovirus capsid (VP4, VP2, VP3, or VP1), e.g. 2069 is VP2 residue 69. Residues numbers highlighted in yellow are known to be involved in recognition of the epitope by antigenic-site-specific monoclonal antibodies.

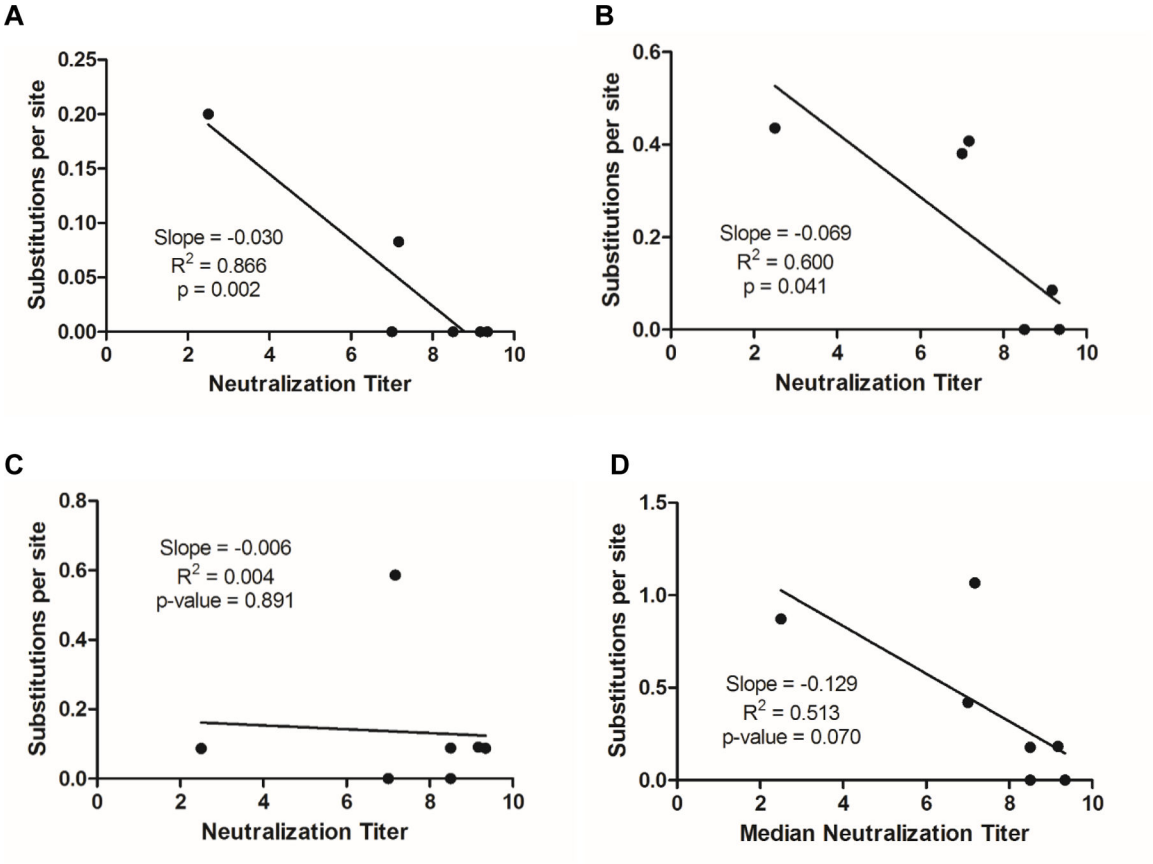

Figure 4.

Correlation between increased amino acid substitutions in NAg1 and decreasing median neutralization titers for highly divergent type 2 iVDPVs. A substitution matrix based on the JTT model was used to compare amino acid residues mapped to (**A**) NAg1, (**B**) NAg2, (**C**) NAg3a, and (**D**) NAg3b.

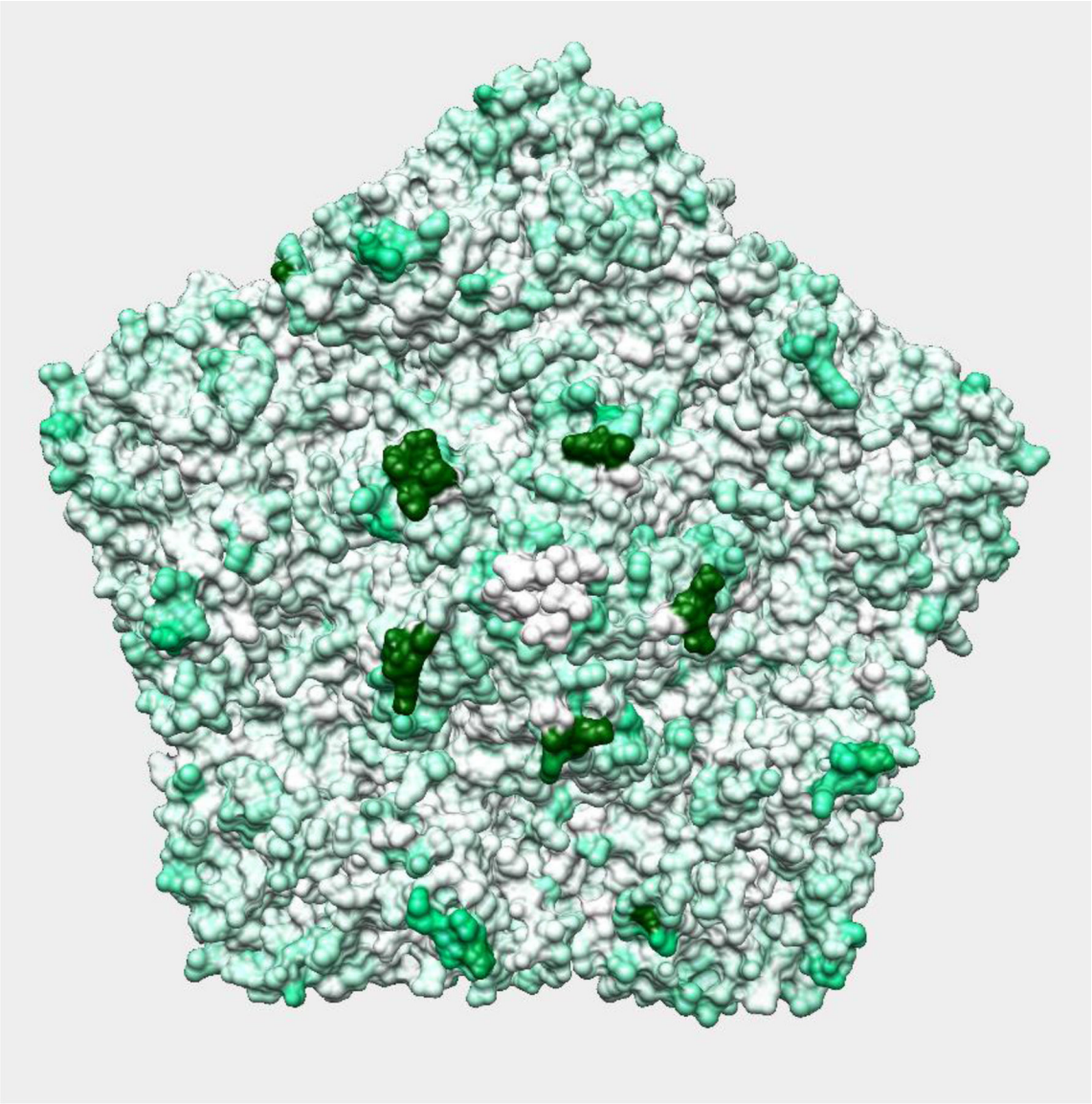

Figure 5.

Predicted alterations in the antigenic surface of highly divergent type 2 iVDPV compared to Sabin 2. Protomer capsid structures for iVDPV isolate 18276 and Sabin 2 were threaded onto the protomer X-ray crystal structure for MEF-1 (type 2) (PDB: 1EAH). Each protomer was assembled into a pentamer and aligned to the Sabin 2 pentamer structure. Root-mean square deviation (RMSD) was calculated for each amino acid residue and the relative RMSD visualized on the isolate 18276 (115 nt difference) capsid space-fill model. White areas of the pentamer indicate regions structurally similar with Sabin 2 while the intensity of green shading indicates the degree of structural displacement.

Table 1.

iVPDV isolates analyzed in this study

iVDPV2 Strain	Number of nucleotide changes in P1	% of nucleotide changes in P1	Genbank Accession Number
18276	115	11.83	GU390707
18277	107	11.1	KU878954
18278	98	10.4	GU390704
18279	20	1.71	KU878956
18280	16	1.4	KU878955
18281	14	1.5	KU878958
18282	10	0.8	KU878957

Table 2.

Seropositivity rates decrease significantly as the number of nucleotide substitutions increases in VP1 of type 2 iVDPVs. Neutralization titers ≥ 3.0 (-$\log_2$) were considered seropositive. For each study site, 30 sera were tested for neutralizing antibodies against all type 2 iVDPVs and Sabin 2.

Isolate	# nt. changes from Sabin 2	% Seropositivity at each site			
		NYC, NY	Denver, CO	Detroit, MI	Total
18276	115	50.0	20.0	16.7	28.9
18277	107	96.7	93.3	93.3	94.4
18278	98	96.7	93.3	93.3	94.4
18279	20	100	100	96.7	98.9
18280	16	100	100	96.7	98.9
18281	14	100	100	96.7	98.9
18282	10	100	100	96.7	98.9
Sabin 2	0	100	96.7	96.7	97.8