Genetic Basis for Immunological Aberrations in Poliovirus Sabin Serotype 3 Strains Imported in The Netherlands

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During the characterization of poliovirus type 3 strains imported in The Netherlands, Sabin serotype 3 strains that reacted with both specific antisera against Sabin-like (vaccine) and non-Sabin-like (wild-type) strains by the intratypic strain differentiation assay have been found. The present study was done to determine the pathogenic potential of these virus strains for humans. Characterization of these so-called double-reactive strains with neutralizing monoclonal antibodies (MAbs) against the major antigenic sites of serotype 3 Sabin virus led to the identification of two groups with different antigenic properties. Six of the seven strains were resistant to neutralization with MAbs against sites 2B and 3B and one strain was neutralized by all the MAbs in a manner similar to that for the Sabin serotype 3 virus. Partial sequencing of the coding regions confirmed the antigenic changes for all six antigenically distinct strains. By inoculation of these viruses into transgenic mice which express the human poliovirus receptor, one strain was identified as highly neurovirulent, three were identified as intermediate, and three were identified as attenuated. Sera from vaccinated persons efficiently neutralized the mutants. Our data suggest that some double-reactive strains are a potential risk to the unvaccinated community but not to the vaccinated population.

The three serotypes of poliovirus cause acute flaccid paralysis in 0.1 to 1% of infected persons, resulting in death or a lifelong debilitating condition called poliomyelitis. In 1988, the World Health Assembly committed the World Health Organization (WHO) to the global eradication of this disease and its cause (the wild-type polioviruses) by the year 2000 (26). To reach this goal, both vaccination of susceptible individuals and virological surveillance are essential. Since 1988, a substantial increase in the vaccination coverage rate and a decrease in the number of cases of poliomyelitis have been found. Nevertheless, epidemics continue to occur, even in countries with high rates of vaccination coverage, such as The Netherlands. In The Netherlands, the main reason for this is the existence of closely knit, orthodox religious communities which refuse vaccination. These religious objectors live in an area that stretches as a belt from the southwest to the northeast of the country, where there are several villages with vaccination coverage rates below 90% and with some even having vaccination coverage rates as low as 60% (20). These communities are at continued risk for introduction of wild-type and vaccine-derived polioviruses.

Several assays for the detection and serotyping of poliovirus and for discrimination of wild-type from vaccine polioviruses (intratypic strain differentiation [ITSD]) have been developed. A variety of molecular and immunological methods are available, but for the eradication program two methods have been chosen as standards for the ITSD: an enzyme-linked immunosorbent assay (ELISA) with cross-absorbed antisera (ITSD-E) and a hybridization assay with specific RNA probes (5, 24). For ITSD-E, sera from rabbits immunized with purified virus were made strain specific by cross adsorption with the heterologous strain; e.g., rabbit antisera to poliovirus serotype 1 absorbed by prototype wild-type virus strains specifically react with type 1 Sabin-like (SL) strains but no longer react with wild-type viruses in the ITSD-E. The assay is used by numerous laboratories in the WHO poliovirus laboratory network.

For about 1 to 2% of patients, poliovirus strains react both with SL reference sera and with the non-Sabin-like (NSL) reference sera (17). In order to address the pathogenic potential of these so-called double-reactive (DR) strains, we characterized the genetic, antigenic, and biological properties of DR strains that were found in The Netherlands. In addition, we studied humoral immunity to these strains in the population by neutralization assays with sera from persons vaccinated with inactivated polio vaccine (IPV) or live attenuated oral polio vaccine (OPV).

MATERIALS AND METHODS

Virus isolates, sera, and MAbs. The seven serotype 3 polioviruses were isolated from stool specimens that had been collected from healthy adoptive children upon arrival in The Netherlands (17). Ten Sabin serotype 3 vaccine viruses that had been isolated from OPV-vaccinated persons in Pakistan were used as controls for Sabin virus-specific characteristics. All viruses were grown in HEp-2c or RD cells and were typed by standard protocols by neutralization assays. DR virus isolates were cloned by plaque purification and were retested by ITSD-E to exclude the possibility of mixtures of an SL virus and an NSL virus.

Sera for the neutralization assays with DR isolates as the challenge virus had been collected from healthy blood donors from Belgium and The Netherlands who had received a complete set of vaccinations either with OPV or with IPV (9). Mutant virus 4022, a Sabin serotype 3 virus mutated at site 3B (7), and a panel of monoclonal antibodies (MAbs) to poliovirus serotypes 1, 2, and 3 were kindly provided by M. Ferguson (National Institute for Biological Standards and Control, London, United Kingdom).

Intratypic differentiation by ELISA. Intratypic differentiation of the serotype 3 poliovirus isolates was carried out by ITSD-E (24). After each incubation step the microtiter plates were washed four times with wash buffer (phosphate-buffered saline plus 0.05% Tween 20). Briefly, the microtiter plates (Nalgene Nunc International, Roskilde, Denmark) were coated with hyperimmune bovine immunoglobulin G to poliovirus serotype 3 (Saukett strain; National Institute of Public Health and the Environment [RIVM] batch 90-1079-893) in coating buffer (0.05 M sodium carbonate buffer [pH 9.6]). After an overnight incubation at 4°C, the microtiter plates were washed four times with wash buffer (phosphate-buffered saline plus 0.05% Tween 20). Then, the microtiter plates were incubated with horseradish peroxidase-labeled MAbs against specific viral proteins of serotype 3 (6, 14, 21, 23, 24). After each incubation step the microtiter plates were washed four times with wash buffer (phosphate-buffered saline plus 0.05% Tween 20). Finally, the microtiter plates were washed four times with wash buffer (phosphate-buffered saline plus 0.05% Tween 20) and incubated with orthophenylene diamine substrate solution (25). After 20 min of incubation at room temperature, the plates were read at 492 nm. Absorbance was read at 492 nm using a microtiter plate reader.

The microtiter plates were washed four times with wash buffer (phosphate-buffered saline plus 0.05% Tween 20). After each incubation step the microtiter plates were washed four times with wash buffer (phosphate-buffered saline plus 0.05% Tween 20). Finally, the microtiter plates were washed four times with wash buffer (phosphate-buffered saline plus 0.05% Tween 20) and incubated with orthophenylene diamine substrate solution (25). After 30 min of incubation at room temperature, the plates were read at 492 nm. Absorbance was read at 492 nm using a microtiter plate reader.
well, which served as a serotyping control, NSL strain-specific cross-absorbed antiserum (RIVM batch 3-400-1294) was added to the second well, and SL strain-specific cross-absorbed rabbit antiserum (RIVM batch 4-1089-1294) was added to the third well. The detector, goat anti-rabbit immunoglobulin G conjugated with horseradish peroxidase (Sigma Chemical Company, St. Louis, Mo.), was added for 1 h at 37°C. Incubation with the substrate 3,3',5,5'-tetramethylbenzidine (Sigma Chemical Company) was at room temperature for 15 min. The absorption values were measured at a wavelength of 450 nm in an ELISA reader (Organon Teknika, Boxtel, The Netherlands).

The results of the ITS-DE are expressed as ratios between the optical density signal in the wells reacted with SL strain- and NSL strain-specific antibodies. A ratio above 2.5 is considered diagnostic for SL viruses. If ratios are below 2.5 and the A450 of both wells with cross-absorbed antiserum (SL and NSL viruses) are higher than 0.400, the virus is labeled DR.

**Protein expression and purification of cRNA probes.** Dot blot hybridization was performed with crude RNA extracts from cultures with a fully developed cytopathic effect as template, as previously described for the assay protocol (5). For detection of the cRNA-RNA hybrids, the chemiluminescent ECL detection reagents (Amersham International, Buckinghamshire, United Kingdom) were used.

**Analysis of the VP1-2A junction region.** Sequencing of the VP1-2A junction was carried out after amplification by reverse transcription-PCR as described previously (16). For automated sequencing analysis, dye-labeled dideoxynucleotides were used with primers M6s and M3s or M3sB (16) according to the manufacturer's instructions (Applied Biosystems, Inc.). After completion of the sequencing reactions, the products were electrophoresed in an automated DNA sequencer (model 373A; Applied Biosystems, Inc.). Each PCR product was sequenced in both directions to resolve possible ambiguous nucleotides.

**Analysis of antigenic sites.** (i) Neutralization with site-specific MAbs. Virus isolates were tested in neutralization assays with site-specific MAbs as described previously (6, 13), with some modifications. The site specificities of the MAbs had been determined by analysis of nonneutralizable escape mutants (12, 13). We used MAbs 31 (site 1), 35 (site 1), 32 (site 2), and 33 (site 3). Serial twofold dilutions of the four MAbs were made in duplicate in Eagle's minimal essential medium supplemented with 10% fetal calf serum. The infected cells were grown in a 96-well plate. One hundred 50% cell culture infectious doses (CCID50s) of the virus to be tested were added. After 2 h at 37°C, 50 μl of a cell suspension containing 3 × 10^6 HeLa cells was added to the plate, which was then sealed with adhesive film. Cytopathic effect as template, as previously described for the assay protocol (5).

(ii) Sequence analysis of antigenic sites 1, 2B, and 3B. For the sequencing of site 1, a region of 480 bp that included site 1 was amplified with primers PV1.1 and PV1.2 as described previously (8). For the sequencing of sites 2B and 3B, a 378-bp fragment that enclosed site 2B was amplified with primers JR1 (AAA GGT ACA CTA GGT ATG CA) and JR2 (GGT GCA GTC ACC TCA CCA AG), and a 374-bp fragment that included site 3B was amplified with primers JR3 (AAA TTC CAA TTA CTG TGA CA) and JR4 (GGT GTG TGA CAA GCC CCA AAT). The fragments overlapped by 70 nucleotides (Fig. 1; Table 1) (19). The reverse transcription-PCR and sequencing protocols were as described above except that primers EV1.1 and EV1.2 were used and the following PCR cycling program was used: denaturation for 30 s at 94°C, annealing for 45 s at 54°C, and extension for 1 min at 60°C (40 cycles), with a final extension of 7 min at 60°C. To analyze the reversion of the nucleotide at position 2034 (3), we used the segment of 374 bp amplified with primers JR3 and JR4.

**Neurovirulence assay with transgenic mice.** The neurovirulence of the DR isolates in vivo was assessed by intraperitoneal injection-mean healthy time [IP-MHT] test) by using transgenic mice (PVR-Tg mice) that express the human poliovirus receptor. The experiments were carried out at the Institute Pasteur (Paris, France), as described previously (5). Briefly, six mice (three males and three females) per virus were inoculated intraperitoneally with 10^7 CCID50 of poliovirus per mouse. The transgenic mice were observed for clinical signs of weakness, paraparesis, paralysis, and death (1). The mean healthy time (MHT) was determined by calculating the average number of symptom-free days for all inoculated mice during an observation period of 14 days at 24-h intervals.

![Image](https://example.com/image.png)

**FIG. 1.** Locations of sequenced regions on the poliovirus type 3 genome. Seq., sequence; *, antigenic sites according to Minor et al. (14).
TABLE 2. Reactivities of DR strains and prototype strains by ITSD-E and probe hybridization assay, VP1-2A sequence type, sequence at neurovirulence markers 472 and 2034, MHT, and neurovirulence score in transgenic mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>ITSD-E result</th>
<th>SL strain/NSL strain ELISA reaction ratio</th>
<th>Probe hybridization result</th>
<th>VP1-2A sequence type</th>
<th>Result with the following Neurovirulence marker&lt;sup&gt;a&lt;/sup&gt;</th>
<th>MHT (days)</th>
<th>Neurovirulence score&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SL strain/NSL strain ELISA reaction ratio</td>
<td></td>
<td></td>
<td>5’ NC (472)</td>
<td>VP3 (2034)</td>
<td></td>
</tr>
<tr>
<td>Saukett</td>
<td>NSL 0.2</td>
<td>NSL</td>
<td>NSL</td>
<td>U</td>
<td></td>
<td></td>
<td>Attenuated</td>
</tr>
<tr>
<td>Sabin 3</td>
<td>SL 5.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>SL</td>
<td>C</td>
<td>Ser</td>
<td></td>
<td>14</td>
<td>Attenuated</td>
</tr>
<tr>
<td>Leon/37</td>
<td>NSL 0.2</td>
<td>NSL</td>
<td>NSL</td>
<td>U</td>
<td></td>
<td>14</td>
<td>Attenuated</td>
</tr>
<tr>
<td>81-16685</td>
<td>DR 0.9</td>
<td>SL</td>
<td>C</td>
<td>Ser</td>
<td></td>
<td>14</td>
<td>Attenuated</td>
</tr>
<tr>
<td>88-8877</td>
<td>DR 0.9</td>
<td>SL</td>
<td>C</td>
<td>Phe</td>
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<td>10.16</td>
<td>Intermediate</td>
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<tr>
<td>87-10657</td>
<td>DR 1.1</td>
<td>SL</td>
<td>C</td>
<td>Phe</td>
<td></td>
<td>7.5</td>
<td>Intermediate</td>
</tr>
<tr>
<td>85-11064</td>
<td>DR 0.9</td>
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<td>C</td>
<td>Ser</td>
<td></td>
<td>8.16</td>
<td>Intermediate</td>
</tr>
<tr>
<td>85-1470</td>
<td>DR 0.5</td>
<td>SL</td>
<td>C</td>
<td>Ser</td>
<td></td>
<td>13.66</td>
<td>Attenuated</td>
</tr>
<tr>
<td>82-21507</td>
<td>DR 1.0</td>
<td>SL</td>
<td>C</td>
<td>Phe</td>
<td></td>
<td>14</td>
<td>Attenuated</td>
</tr>
<tr>
<td>88-8058</td>
<td>DR 0.6</td>
<td>SL</td>
<td>C</td>
<td>Phe</td>
<td></td>
<td>14</td>
<td>Attenuated</td>
</tr>
</tbody>
</table>

<sup>a</sup> According to Almond et al. (3).

<sup>b</sup> Neurovirulence in transgenic mice with the human poliovirus receptor.

<sup>c</sup> Average ratio for 10 Sabin serotype 3 strains isolated from healthy OPV-vaccinated persons and the prototype Sabin strain.

isolates were classified as highly neurovirulent (MHT, <8 days), intermediate (MHT, between 8 and 14 days), or attenuated (MHT, 14 days) (6).

Neutralization assays with serum from OPV- and IPV-vaccinated persons. To address the pathogenic potential of the DR strains for the general population, a neutralization assay was performed with sera collected from healthy blood donors vaccinated either with OPV or with IPV. Details about the population have been described elsewhere (9). Two strains (87-10657 and 81-16685) were used in this neutralization assay in parallel with the prototype poliovirus serotype 3 strains Sabin and Saukett.

Replication kinetics of DR strains in HEp-2c cells at 37 and 40°C. Wild-type and SL strains can also be discriminated by their ability to replicate in cell culture at the restrictive temperature of 40°C (15). The thermal profiles of the DR strains were determined with one-step growth curves in HEp-2c cells at 37 and 40°C as described previously (5). Briefly, confluent monolayers of HEp-2c cells in six-well plates were infected with the DR strains or the prototype poliovirus serotype 3 strains Sabin and Saukett at a multiplicity of infection of 20. After preincubation for 1 h at 37 or 40°C, the plates were washed twice with phosphate-buffered saline to remove unadsorbed virus. After the addition of Eagle’s minimal essential medium without serum, the plates were incubated at 37 or 40°C. The contents of the wells were harvested after 4 h. After three rounds of freezing-thawing, the virus titer was determined by virus titration assays.

Statistical analysis. Differences between average ITSD-E ratios for Sabin serotype 3 poliovirus and the site 3B escape mutant (strain 4022) were analyzed by the t test. P values of <$0.05 were considered significant.

RESULTS

Intratypic differentiation of isolates. The virus strains tested in the present study were isolated during the interepidemic period from 1979 to 1992, during which a total of 5,868 fecal samples from adopted children, 46,000 fecal samples from Dutch patients, and 58 river water samples were tested. In total, 899 poliovirus strains were isolated, and of these, 13 (1.4%) had the DR phenotype (17, 20). In this study, seven serotype 3 DR poliovirus strains were analyzed. Their reactivities by ITSD-E are shown in Table 2.

Sequence analysis of VP1-2A region and probe hybridization. The nucleotide sequence of a 150-nucleotide stretch in the VP1-VP2A junction region of the DR serotype 3 strains was determined and compared with that of the prototype serotype 3 strains Sabin (PV3/Leon 12a,b/USA56) and Saukett (PV3/Saukett G/USA50) (Table 2). The sequences of the VP1-2A junction region of the poliovirus serotype 3 DR viruses showed a high degree of similarity (>98%) to that of the Sabin strain. This was confirmed by hybridization with Sabin strain-specific probes for the 5’NC region (Table 2).

Neutralization with site-specific MAbs. To study whether the DR phenotype could be related to changes within the antigenic sites, neutralization assays were carried out with intratypic and site-specific neutralizing MAbs to the known antigenic sites of poliovirus serotype 3 in mice. Only one DR strain (strain 88-8058) had the same pattern of reactivity by the MAb neutralization assays as was seen with the prototype Sabin strain and the 10 Sabin serotype 3 isolates from OPV-vaccinated persons. The remaining six strains were neutralized by site 1-specific MAbs 31 and 35 but were different in their site 2 and 3 reactivities (Table 3).

Sequence analysis of sites 2B and 3B. The 704-bp consensus sequences of overlapping PCR fragments, including the coding regions for antigenic sites 2B and 3B from the DR isolates, were compared with those of prototype serotype 3 strains Sabin serotype 3 (PV3/Leon 12a,b/USA56), Saukett (PV3/Saukett G/USA50), and Leon (PV3/Leon/USA37). In these regions, the sequences from the DR isolates were almost identical to that of the Sabin serotype 3 strain (>98%), showing that no intracapsid recombination took place within this region of the genome. However, in both antigenic sites mutations that resulted in codon changes occurred (Table 3). In antigenic site 3B, the six virus strains which could not be neutralized by MAb 33 showed an amino acid substitution (serine–asparagine) at position 59, which is similar to the case for the prototype Saukett strain (Table 3). Three of the six strains, in addition, had a substitution at position 77 (isapartic acid–asparagine). Strain 88-8058, which was neutralized by MAb 33, showed a substitution at position 77, similar to that for the strains described above, as well as a substitution (serine–leucine) at position 79. Antigenic site 2B showed much less variation than antigenic site 3B, and there was no correlation between amino acid changes and the results of neutralization assays with MAbs 32 (site 2). A single amino acid substitution (valine–leucine at position 166) was found in one DR strain (strain 88-8877). No amino acid substitutions were found in the sequence close to site 2B, which explains the observed lack of neutralization with MAbs to this site.

ITSD-E of escape mutant (Sabin) virus strain 4022. Since our findings suggested that antigenic changes in site 3B might be responsible for the observed reactivities of DR strains by ELISA, an ITSD-E was carried out with an escape mutant virus (strain 4022) (12, 13). This mutant virus has an amino acid substitution at position 59 in site 3B and was generated by culture in the presence of an MAb to this site. Indeed, the ELISA readings for the wells with anti-NSL-specific sera were significantly higher than those for the wells with the Sabin
prototype strain, resulting in a significantly lower ratio of anti-SL strain/anti-NSL strain reactions by ELISA, although the SL strain/NSL strain ELISA reaction ratio remained slightly higher than 2.5 (Fig. 2).

Analysis of point mutations associated with neurovirulence and neurovirulence assay with PVR-Tg mice. All DR strains had a reversion of the neurovirulence marker at nucleotide position 472 and four had reversions at position 2034 (Table 2). In order to test the neurovirulences of these strains in vivo, a neurovirulence assay with PVR-Tg mice was done. DR strain 88-8877 was classified as highly neurovirulent, three strains (strains 88-10657, 85-11064, and 85-1470) were classified as intermediate, and three strains were classified as attenuated (Table 2).

Serum neutralization assays with sera from OPV- and IPV-vaccinated persons and DR strains. In order to address the pathogenic potentials of the DR strains, a neutralization assay was performed with sera from IPV- and OPV-vaccinated persons. The neutralization assay ratios, expressed as titer of neutralizing antibodies against a Sabin serotype 3 strain or a DR strain divided by the titer against a wild-type strain, was slightly higher for the DR strains with mutations at site 3B (Fig. 3).

Replication kinetics of DR strains on HEp-2c cells at 37 and 40°C. One-step growth curves for the DR strains were determined on HEp-2c cells at 37 and 40°C and were compared with those of prototype strains to determine the thermostability of the viruses (Fig. 4). At the permissive temperature (37°C) all viruses grew to similar titers after 4 h. At the restrictive temperature (40°C), replication was limited for the prototype Sabin serotype 3 strain and for strain 87-10657. The six remaining strains yielded higher levels of progeny virus, indicating a change in the thermal profile toward a more thermostable phenotype.

DISCUSSION

Since 1988 WHO has been committed to the global eradication of poliomyelitis by the year 2000. Two criteria must be met to achieve this goal: (i) no new cases of poliomyelitis due to wild-type poliovirus infection and (ii) no more circulation of wild-type poliovirus in humans or the environment. The Netherlands has communities of orthodox religious believers who

![ TABLE 3. Neutralization patterns of DR strains with site-specific MAbs and amino acid sequences of different antigenic sites ](http://jcm.asm.org/content/35/5/2396)

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>NIBSC titer for MAb no.:</th>
<th>Amino acid sequence*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>31 (site 1)</td>
<td>35 (site 1)</td>
</tr>
<tr>
<td>Sabin 3</td>
<td>20,480</td>
<td>20,480</td>
</tr>
<tr>
<td>Saukett G</td>
<td>5120</td>
<td>20,480</td>
</tr>
<tr>
<td>81-16685</td>
<td>&gt;20,480</td>
<td>&gt;20,480</td>
</tr>
<tr>
<td>88-8877</td>
<td>&gt;20,480</td>
<td>20,480</td>
</tr>
<tr>
<td>87-10657</td>
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</tr>
<tr>
<td>82-21507</td>
<td>&gt;20,480</td>
<td>&gt;20,480</td>
</tr>
</tbody>
</table>

* Amino acid sequences of 10 Sabin serotype 3 strains isolated from healthy OPV-vaccinated persons and of the prototype Sabin strain. Hyphens indicate the Sabin serotype 3 amino acid sequence.

![ FIG. 2. Average absorption ratios of poliovirus serotype 3 Sabin site 3B escape mutant (strain 4022), a prototype serotype 3 NSL strain (strain Saukett), and a prototype serotype 3 SL strain (strain Sabin) by ITSD-E. Virus strains with ratios of >2.5 are classified as SL strains, those with ratios of <0.5 are classified as NSL strains, and those with ratios of 0.5 and >0.5 are classified as DR. *, P < 0.05. ](http://jcm.asm.org/content/35/5/2396)

![ FIG. 3. Neutralization ratios for IPV- and OPV-vaccinated blood donors against prototype and DR strains with amino acid substitutions in antigenic site 3B. Ratios are expressed as the titer of neutralizing antibodies when Sabin serotype 3 or a DR strain was used as challenge virus divided by the titer obtained when the Saukett strain was used as the challenge virus. ](http://jcm.asm.org/content/35/5/2396)
refuse vaccination. Members of these communities are at a continuous risk of exposure to wild-type poliovirus, which has been the rationale behind our virological surveillance for polioviruses (17, 20). Between 2 and 3% of the viruses that were found had some aberrant characteristic, and half of these (1.4% of all strains) had the DR phenotype. In this study we demonstrated that the poliovirus serotype 3 DR strains are drifted poliovirus serotype 3 vaccine strains with amino acid substitutions mainly in antigenic site 3B. These changes coincide with changes in neutralization assays with a site 3B-specific MAb and are well in line with the report that a MAb 33 neutralization escape mutant (strain 4022) has a codon change in site 3B (7). Our data suggest that the site 3B changes also caused the change in ELISA reactivity since an escape mutant virus (strain 4022) which contains an amino acid substitution at position 59 of site 3B had a DR phenotype by ITSD-E as well.

It is interesting that no consistent amino acid substitutions were found at or around antigenic site 2B, even though neutralization by site 2-specific MAb 32 was clearly affected in six of the seven poliovirus serotype 3 DR strains (Table 3). It is conceivable that the changes in site 3B affected the conformation of the epitope in site 2, as has been described for poliovirus serotype 1 site 2 (18).

Next we asked ourselves whether the DR strains might pose a threat to the unvaccinated community, and such strains were tested for their neurovirulences. All DR serotype 3 strains had a reversion of the major attenuating mutation at nucleotide 472 (C→U) in the 5'NC region, and for four of the DR-3 strains the sequence had reverted to the wild-type sequence at position 2034 (2, 3, 25). When the DR strains were tested with transgenic mice, however, we saw a less clear distinction (Table 2), and three strains with both neurovirulence markers were not highly virulent in mice (two were intermediate and one was attenuated). It has been described that mutations of bases 472 and 2034 are strongly correlated with neurovirulence in vivo (3, 25). Therefore, it remains to be seen what the behavior of these strains would be in humans.

As judged from neutralization profiles with sera from vaccinated persons, there is little risk of infection in this group. However, the fact that half of the DR serotype 3 strains in this study are neurovirulent in transgenic mice suggests that in The Netherlands members of orthodox religions who refuse vaccination are at risk for the introduction of these Sabin strains with antigenic drift. Further studies will focus on the ability of aberrant strains to spread in this population.

ACKNOWLEDGMENTS

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