Geographical genotypes (geotypes) of poliovirus case isolates from the former Soviet Union: relatedness to other known poliovirus genotypes

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A 150 nucleotide long region corresponding to adjoining segments of the genes encoding polypeptides VP1 and 2A of 84 poliovirus strains recently isolated from patients with paralytic poliomyelitis over the territory of the former Soviet Union (FSU) were characterized by sequencing and/or PCR amplification using specially designed primers. Eighteen isolates were found to be very closely related to one or another of the three Sabin vaccine strains. Three distinct classes of geographical genotypes (geotypes) were discerned among 42 wild-type (non-Sabin) strains of serotype 1. One such geotype (called A) was widely circulating in 1990–91 in the Caucasian (Azerbaijan and Georgia) as well as Asian (Kyrgyzstan and Turkmenistan) Republics; this geotype exhibited only weak relatedness to known strains isolated outside the FSU. On the other hand, a subset of strains belonging to another geotype (T) of serotype 1, which circulated in 1991 in Tajikistan, demonstrated very close relatedness to contemporaneous strains isolated in Pakistan, India and Jordan. Strains that were somewhat different, but belonging to the same T-geotype, were found also in Moldova and Georgia. Strikingly, the primary structure of the VP1/2A junction of certain T-geotype isolates differed from the corresponding region of Sabin 1 only in 13–15% of positions, thereby not reaching the upper limit accepted for a geotype. This observation raises, though does not prove, the possibility that at least the relevant segment of the T-geotype RNA originated from the vaccine strain. The third geotype of serotype 1 was represented by a single, perhaps imported, isolate. Four distinct subsets of a common geotype (C) were discerned among 24 wild-type isolates belonging to serotype 3. These strains exhibited a broad geographical distribution being found, in particular, in Armenia, Azerbaijan, Georgia, Turkmenistan and Tajikistan; on the other hand, the C-geotype strains exhibited only a relatively distant relatedness to a strain isolated outside of the FSU (in Oman).

Introduction

Poliovirus-induced poliomyelitis remains a significant health problem in many countries, despite the availability of efficient vaccines. Thus, some 130 000 cases of paralytic poliomyelitis were estimated to occur over the world in 1991 (World Health Organization, 1991). Although a tiny proportion of them could be linked to the use of the live Sabin poliovaccine (Assaad et al., 1982; Nkowane et al., 1987; Lipskaya et al., 1991) naturally circulating ‘wild-type’ poliovirus strains are the principal aetiological agents of the disease.

The 7·5 kb poliovirus genome appears to change, upon natural transmission among humans (the only natural host of the virus), at a rate of approximately 100 nucleotides (1–2% of the genome) per year (Nottay et al., 1981; Kew et al., 1981) or probably even faster (Kinnunen et al., 1990). Indigenous strains isolated in different geographical regions usually exhibit a significant genetic diversity (Rico-Hesse et al., 1987; Kew et al., 1990a, b). On the other hand, a high degree of similarity has been observed among strains isolated during a given epidemic (Nottay et al., 1981; Kew &
Fig. 1. For legend see opposite.
Nottay, 1984; Lipskaya et al., 1985; Magrath et al.,
1986; Kinnunen et al., 1991), and the viral genomes
demic to a territory tend to preserve some common
features for years (Rico-Hesse et al., 1987; Kew et al.,
1990a). These observations led to the concept of
genealogical genotypes of poliovirus (Rico-Hesse et al.,
1987; Kew et al., 1990b, 1993); for the sake of brevity
and to avoid possible confusion, we shall designate the
genealogical genotypes as geotypes. The geotypes could
be arbitrarily defined by the requirement to have no
more than a fixed level of divergence in a given segment
of viral RNA. Different parts of the poliovirus genome
are known to exhibit significantly different rates of
evolution; for example, the region encoding capsid
proteins evolves more rapidly compared to the genes for
nonstructural polypeptides (Toyoda et al., 1984). De-
pending on specific aims, viral RNA segments with
various levels of conservation may be sequenced for the
geotyping of wild-type polioviruses. In particular, it was
found convenient to investigate just the junction between
the structural and nonstructural regions of the genome,
which encompasses a 3′ portion of the gene for the capsid
protein VP1 and a 5′ portion of the nonstructural gene
2A (directing the synthesis of a multifunctional protein
with a protease activity) (Rico-Hesse et al., 1987; Pööry
et al., 1990); a limit of 15% divergence within this 150-
nucleotide-long segment was set for strains belonging to
the same geotype (Rico-Hesse et al., 1987).

Sequencing of even limited regions of the wild-type
poliovirus RNA has obvious epidemiological impli-
cations; the characterization of the geotype-specific
RNA segments is also important for understanding
certain regularities of poliovirus evolution (Rico-Hesse
et al., 1987; Kew et al., 1990a, b).

Once the primary structures of an appropriate RNA
segment of representatives of distinct poliovirus geotypes
are known, geotyping can be carried out by indirect
methods, such as oligonucleotide hybridization (Pööry
et al., 1990; da Silva et al., 1991) or PCR (Yang et al.,
1992; Zheng et al., 1993). In both cases, different levels of
variability within the chosen genetic segment should be
taken into account, and specific oligonucleotides (hybrid-
ization probes or primers) should be targeted to the
most constant, for a given geotype, stretches of the
segment.

The incidence of paralytic poliomyelitis in some
territories formerly belonging to the USSR has been, and
continues to be, relatively high; official statistics reported
several hundred such cases per year in 1990–91 (World
Health Organization, 1992). In regions with a higher
standard of public health services, e.g., Byelorussia and
Russia, most, if not all, instances of the disease could be
linked to vaccination with the attenuated Sabin strains
(Lipskaya et al., 1987; Kutitova et al., 1990). In other
regions, however, the incidence of poliomyelitis was
largely due to wild-type strains, whose circulation did
not appear to be suppressed because of deficient
immunization programmes (cf. Lipskaya et al., 1985).

In this paper, the genomes of 84 strains isolated in
different parts of the former Soviet Union (FSU) from
cases of paralytic poliomyelitis were characterized by
sequencing the junction of the VP1/2A genes and/or
geneotype-specific PCR analysis. Type 1 strains were
represented by three different geotypes. Five distinct
branches of a single type 3 geotype could be identified.

Methods

Viruses. Poliovirus vaccine strains of type 1 (Lsc 2ab), type 2 (P712
ch 2ab) and type 3 (Leon 12 a,b) were from the collection of the
Institute of Poliomyelitis and Viral Encephalitides (Moscow Region);
wild isolates had been sent to that Institute by local virological
laboratories of the Republics of the FSU. The viruses were grown in
RD or Hep2 cells and were characterized in a microneutralization assay
with type-specific sera.

Preparation of RNA. For PCR amplification, either total RNA
extracted from infected cells, or RNA present in tissue culture fluid
after complete CPE of the infected cells were used (Yang et al., 1991).
RNAs used for sequence analysis were extracted from purified virions
(Lipskaya et al., 1991).

Nucleic acid sequencing. Partial genomic RNA sequences were
determined either by the dideoxy chain termination method (Sanger
et al., 1977; Zimmern & Kaesberg, 1978), or by Taq DyeDeoxy
 Terminator Cycle Sequencing of the purified DNA product of PCR-
 amplified viral RNA (2 x Taq New Protocol, Applied Biosystems,
1993).

Oligonucleotide primers. Synthetic oligodeoxynucleotides were pre-
pared, purified and analysed as described by Yang et al. (1991). The
following previously described primers were used: (i) the polio/2A
primer (corresponding to positions 3508–3527) for RNA sequencing
(Rico-Hesse et al., 1987); (ii) the PCR primer pair, EV/PCR1 (539–565)
and EV/PCR2 (452–476), matching highly conserved sites within the
5′-noncoding regions of enterovirus genomes (da Silva et al., 1991); (iii)

Fig. 1. Comparison of nucleotide sequences at the VP1/2A junction region of wild-type 1 (a) and type 3 (b) isolates obtained on the
territory of the FSU in 1982–91. Sequencing was performed as described in Methods. Nucleotide differences from the respective Sabin
strains are shown; dashes indicate identities. The sequences of the strains labelled with an asterisk (*) were used for the primer design.
The sequences of the A-, T-, G- and C-geotype primer pairs are also presented (the left-hand primer corresponds to the sense polarity
and the right-hand primer corresponds to the antisense polarity). Each strain is designated by the serotype, number of isolate as given
in the laboratory where isolation was performed, place and year of the corresponding polio case. Geographical abbreviations are: AZB,
Azerbaijan; ARM, Armenia; CHE, Chechen-Ingush; GEO, Georgia; KRG, Kyrgyzstan; MOL, Moldova; RUS, Russia; TAJ, Tajikistan; TRK, Turkmenistan.
the Sabin vaccine strain-specific primer pairs, SAB1/PCR1 (2584–2601) and SAB1/PCR2 (2505–2523), SAB2/PCR1 (2580–2595) and SAB2/PCR2 (2525–2544), SAB3/PCR1 (2562–2580) and SAB3/PCR2 (2537–2553) (Yang et al., 1991). Sequences of newly designed PCR primer pairs specific for A, G, T and C geotypes corresponded, respectively, to the positions given in parentheses: A/PCR1 (3441–3462) and A/PCR2 (3322–3342); G/PCR1 (3396–3418) and G/PCR2 (3319–3339); T/PCR1 (3411–3429) and T/PCR2 (3327–3348); C/PCR1 (3412–3456) and C/PCR2 (3348–3369) (Fig. 1 a, b). The odd- and even-numbered primers corresponded to antisense and sense polarity, respectively. Positions in the poliovirus genome for production of specific amplicons by in vitro amplification by PCR was modified from the method of Yang et al. (1991). The 50 μl reaction mixtures contained 50 mm-Tris–HCl (pH 8.3), 70 mm-KCl, 5 mm-MgCl₂, 10 mm-dithiothreitol, one or more primer pairs (10 pmol each), 200 μM each of dATP, dCTP, dGTP, dTTP (Pharmacia), 5 U placental ribonuclease inhibitor (Boehringer Mannheim), 2.5 U avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim), 2.5 U Taq DNA polymerase (Perkin Elmer Cetus), and the RNA template. The mixtures were overlaid with mineral oil and incubated at 42 °C for 30 min before 25 cycles of amplification (95 °C, 30 s; 62 °C, 45 s; 72 °C, 1 min) in a DNA thermal cycler (Perkin Elmer Cetus). Polyacrylamide gel electrophoresis and detection of amplified products by ethidium bromide staining were as described previously (Yang et al., 1991).

Construction of the dendrograms of sequence similarity. Dendrograms were generated from the nucleotide sequence data by cluster analysis using the GENEBEE package (Brodsky et al., 1992).

Results

Preliminary characterization of wild poliovirus geotypes found in the former Soviet Union

Eighty-four poliovirus isolates (43 type 1, 10 type 2 and 31 type 3) from individual paralytic cases were analysed for production of specific amplicons by in vitro amplification with the enterovirus group-specific primers and the Sabin strain-specific PCR primers. RNAs of all 84 isolates produced the expected 114 bp amplicons when analysis with primers specific to the enterovirus group was modified from the method of Yang et al. (1991). The 50 μl reaction mixtures contained 50 mm-Tris–HCl (pH 8.3), 70 mm-KCl, 5 mm-MgCl₂, 10 mm-dithiothreitol, one or more primer pairs (10 pmol each), 200 μM each of dATP, dCTP, dGTP, dTTP (Pharmacia), 5 U placental ribonuclease inhibitor (Boehringer Mannheim), 2.5 U avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim), 2.5 U Taq DNA polymerase (Perkin Elmer Cetus), and the RNA template. The mixtures were overlaid with mineral oil and incubated at 42 °C for 30 min before 25 cycles of amplification (95 °C, 30 s; 62 °C, 45 s; 72 °C, 1 min) in a DNA thermal cycler (Perkin Elmer Cetus). Polyacrylamide gel electrophoresis and detection of amplified products by ethidium bromide staining were as described previously (Yang et al., 1991).

Construction of the dendrograms of sequence similarity. Dendrograms were generated from the nucleotide sequence data by cluster analysis using the GENEBEE package (Brodsky et al., 1992).

Table 1. Geotyping of wild-type 1 polioviruses

<table>
<thead>
<tr>
<th>Isolate*</th>
<th>Sequence analysis†</th>
<th>Geotype identification by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A-geotype</td>
</tr>
<tr>
<td>PV1/4080/AZB90</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
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<td>A</td>
<td>+</td>
</tr>
<tr>
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<td>–</td>
</tr>
<tr>
<td>PV1/269/AZB90</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>PV1/274/AZB90</td>
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<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>PV1/4008/TRK90</td>
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<td>+</td>
</tr>
<tr>
<td>PV1/4023/TRK91</td>
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<td>+</td>
</tr>
<tr>
<td>PV1/4026/TRK91</td>
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<td>+</td>
</tr>
<tr>
<td>PV1/4081/GEO90</td>
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<td>+</td>
</tr>
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<td>PV1/4082/GEO90</td>
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<td>+</td>
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<tr>
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<td>–</td>
</tr>
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<td>PV1/889/GEO90†</td>
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<td>+</td>
</tr>
<tr>
<td>PV1/891/GEO90†</td>
<td>A</td>
<td>+</td>
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<tr>
<td>PV1/906/GEO90†</td>
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</tr>
<tr>
<td>PV1/827/GEO85</td>
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<td>–</td>
</tr>
<tr>
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<td>–</td>
</tr>
<tr>
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<td>–</td>
</tr>
<tr>
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<td>T1</td>
<td>–</td>
</tr>
<tr>
<td>PV1/5/TAJ91</td>
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<td>T1</td>
<td>–</td>
</tr>
<tr>
<td>PV1/7/TAJ91†</td>
<td>T1</td>
<td>–</td>
</tr>
<tr>
<td>PV1/8/TAJ91</td>
<td>T1</td>
<td>–</td>
</tr>
<tr>
<td>PV1/9/TAJ91†</td>
<td>T1</td>
<td>–</td>
</tr>
<tr>
<td>PV1/434/MOL91</td>
<td>T3</td>
<td>–</td>
</tr>
</tbody>
</table>

* All isolates were positive by PCR with the enterovirus-specific primer pair and negative with the Sabin-1-specific primer pair.
† Isolates initially sequenced for design of geotype-specific PCR primers.
‡ See Fig. 3(a) and Table 3 for criteria for intrageotypic classifications; ND, not determined.

were from cases that occurred in 1990–91 in four southern Republics (Georgia and Armenia in the Caucasus; Kyrgyzstan and Tajikistan in Central Asia). The 12 type 1 isolates formed three distinct geotypes, which we designated A (isolates from Kyrgyzstan and all but one from Georgia), T (from Tajikistan) and G (the remaining isolate from Georgia). Sequence differences across the geotypes were 19–26% (A/T), 17–19% (A/G) and 23–26% (T/G). All seven type 3 isolates initially
sequenced (four from Armenia and three from Georgia) were very similar, differing by no more than 5 nucleotide positions (Fig. 1b; isolates labelled *). These isolates were assigned to one geotype, which we named C.

Design of the geotype-specific PCR primers

Referring to the sequences of the 19 wild isolates initially compared, we designed four sets of oligodeoxynucleotide primers (19–25 nt in length) for rapid, specific identification of each geotype by PCR (see Methods). The specific amplification products for each geotype were predicted to have the following chain lengths: geotype A, 141 nt (positions 3322–3462); geotype T, 103 nt (3327–3429); geotype G, 100 nt (3319–3418); type 3 geotype C, 103 nt (3351–3456). For all but one pair, at least one (sense or antisense) primer had a minimum of five mismatches with the relevant segments of the non-cognate geotype and a minimum of four mismatches with the genomes of any of the three Sabin strains. Consequently, under our PCR conditions we did not expect any cross-amplification among the representatives of the different wild genotypes or between wild poliovirus and vaccine-strain templates. One template/primer combination [C-geotype (type 3) isolates/T-geotype (type 1) primer pair] might potentially yield false-positive signals under amplification conditions of low stringency, because only two mismatches were predicted for one of the primers. However, we encountered no analytical ambiguities under the amplification conditions described here.

Geotype-specific identification of wild poliovirus isolates by PCR

All isolates were retested in PCR assays using the wild geotype-specific primer sets. None of the RNAs of isolates previously identified as Sabin vaccine-related served as templates for production of specific amplicons. The majority of serotype 1 isolates (30 of 42; including those sequenced for the design of the primers) could be assigned by this assay to the A geotype (Table 1; Fig. 2a). They originated from the Caucasus Republics of Georgia (18 isolates) and Azerbaijan (six isolates), as well as from the Asian Republics [Turkmenistan (three isolates) and Kyrgyzstan (two isolates)]; one strain was from Russia.
Table 2. Geotyping of wild-type 3 polioviruses

<table>
<thead>
<tr>
<th>Isolate*</th>
<th>Geotype identification by:</th>
<th>PCR analysis with primers</th>
<th>C-geotype</th>
</tr>
</thead>
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<tr>
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<td>PCR analysis</td>
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</tr>
<tr>
<td></td>
<td>with primers</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>to the sequence</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>analysis‡</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Eleven isolates gave a positive signal with the geotype T-specific primers (two strains from Georgia, eight from Tajikistan and one from Moldova) (Table 1; Fig. 2b). No G-geotype isolates other than those already described in Fig. 1 were found. Thus, all of the type 1 strains in our collection could be assigned to one or another class defined by the PCR primers used.

When the 24 non-Sabin-related type 3 isolates were similarly analysed, the majority (21) were found to be positive with the geotype C-specific primers. However, no amplifications were obtained with the RNAs of three isolates (Table 2).

Partial sequencing of the PCR-positive and PCR-negative isolate genomes

To assess the validity of the assignments of isolates to specific genetic groupings on the basis of the PCR results, the VP1/2A region sequences were determined for several additional wild isolates. The PCR-based geotype assignments were found to be fully consistent with the sequence data (Tables 1 and 2; Fig. 1). All isolates of the type 1 A geotype were closely related [maximum pairwise difference 7% (10/150 positions); Table 3], despite their isolation from widely separate regions. These isolates appear to have been derived from a recent common progenitor.

The group of type 1 isolates recognized by the T geotype-specific primers, though comprising a single cluster, was more heterogeneous (Figs 1a and 3a; Table 3). The 1991 isolates from Tajikistan exhibited an obvious relatedness to each other (maximum pairwise difference 7%); we called the corresponding branch of the dendrogram T1. The two 1985 isolates from Georgia were nearly identical in VP1/2A sequence (one difference), and formed a separate branch (T2) which differed by 15% (22/150 nt) from the T1 group. The 1991 Moldovan isolate was a representative of a third branch (T3) within the T geotype cluster, differing from the T1 isolates by 13-16% (20-24 differences) and the T2 isolates by 12-13% (18-19 differences). Overall, the T geotype is quite heterogeneous, with some pairwise differences (T3/T1) exceeding 15%, the defined upper limit for divergence within a geotype (Rico-Hesse et al., 1987).

The type 3 isolates were also heterogeneous in their VP1/2A sequences (Figs 1b and 3b; Table 3). The 1991 isolates from Tajikistan exhibited an obvious relatedness to each other (maximum pairwise difference 3%); two contemporaneous isolates from Turkmenistan (3991 and 4020) were closely related (maximum pairwise difference 3%), forming a cluster we called C1. Two 1982 isolates from the Chechen-Ingush Region of Russia (Northern Caucasus) and from geographically separate Moldova were very similar (3% nt differences), constituting a second branch (C2) 10-11% divergent from the C1 cluster. Another 1990 isolate from Turkmenistan (4002) was the sole representative of a third branch (C3), almost equally divergent (~12%) from clusters C1 and C2. Two other 1990 Turkmenistan isolates (3988 and 3997) and a 1991 Tajikistan isolate formed a fourth cluster (C4). Although
Table 4. Intrageotypic divergence of type 3 wild strains*

<table>
<thead>
<tr>
<th>Geotype</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C'</th>
<th>S3†</th>
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<tr>
<td>C1</td>
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<td>C3</td>
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<tr>
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<td>12-15</td>
<td>12-16</td>
<td>14-16</td>
<td>1-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C'</td>
<td>12-16</td>
<td>8-14</td>
<td>10-12</td>
<td>16-20</td>
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<td>20-22</td>
<td>22-24</td>
<td>25</td>
<td>24-27</td>
<td>23-24</td>
<td></td>
</tr>
</tbody>
</table>

* Values represent minimal and maximal percentage of divergence.
† Sabin-3.

The RNAs of the cluster C4 isolates served as templates for specific amplification with the C geotype-specific primer pairs, cluster C4 had the lowest degree of VP1/2A sequence similarity with the other clusters (Table 4). Under the moderate stringency conditions used for our PCR assays, the mismatches between the C-geotype primers and the C4 templates (three to four with C/PCR1; two to three with C/PCR2; Fig. 1b) did not prevent amplification.

No amplifications were observed when RNAs of the three 1987 type 3 Georgia isolates were tested in PCR assays using the C-geotype-specific primers. These isolates formed a separate, relatively homogeneous cluster, designated C'. Cluster C' was more closely related to clusters C1, C2 and C3 than was cluster C4 (Table 4). The absence of template activity of C' genomes in our PCR assays appears to be the result of localized mismatch with the C-geotype primer pairs (eight with C/PCR1; two to three mismatches at the 3'-donor end of the primer; one to two mismatches with C/PCR2; Fig. 1b).

Taken together, the pattern of reactivity of the type 3 C-geotype isolates with the C-geotype primer pairs was in reasonable agreement with the VP1/2A sequence relationships among the isolates. Primers C/PCR1 and C/PCR2, designed to match the consensus sequence of the C1 cluster, primed PCR amplifications of sequences of all C1 isolates, as well as sequences of C2 and C3 isolates, which were most closely related to the C1 cluster. However, when these primers were tested in PCR assays with templates approaching (or exceeding) 15% VP1/2A sequence differences from the C1 cluster, the patterns of reactivity did not strictly reflect the observed sequence relationships.

Amino acid substitutions in the VP1/2A junction of the polyprotein

The deduced amino acid sequences of the VP1/2A junction in the polyproteins of the type 1 and type 3 strains are presented in Fig. 4. Although the total number of amino acid substitutions is too small to provide further insights into the genetic relationships among isolates, some of the observed substitutions warrant brief description.

The VP1/2A polyprotein junction varied by zero to three amino acids among the type 1 isolates (Fig. 4a). When compared to Sabin-1, the difference was again zero to three amino acids. The corresponding region of the two 1985 Georgia isolates was identical to that of Sabin-1. Some substitutions in both VP1 and 2A polypeptides were shared by large subsets of the isolates belonging to one or more geotypes. Nonconservation was found, among the T-geotype strains, of a residue immediately downstream of the VP1/2A cleavage site, Y/G.

The maximal interstrain divergence, in the region studied, among the wild isolates of type 3 was four amino acids. In contrast, the maximal difference from the Sabin-3 genome was seven amino acids. Interestingly, the characteristic pattern of amino acids in the VP1 region with coordinates 285–290 appears to be present in nearly all wild-type 3 strains sequenced thus far (Kew et al., 1990a; O.M. Kew, unpublished data). It should be noted that tripeptide KDG/E at positions 285–288 shared by most type 3 isolates is identical to that present in the VP1 of Sabin-1 and Sabin-2 as well as in wild-type 1 and 2 isolates we analysed (Fig. 4b).

Discussion

No exhaustive analysis of the strains circulating within the borders of the FSU is possible due to the political situation and shortcomings of the local public health services. Nevertheless, an analysis of the available strains isolated in different regions of this territory in 1990–1991 demonstrated the coexistence of a variety of geotypes. This is especially true of type 1 isolates, which fall into at least three clearly distinct groups, i.e. A, T and G. Geotype A comprises a set of closely related strains, which undoubtedly originated from a common predecessor quite recently. In spite of that, A-geotype strains have been isolated in 1990–91 from regions as separate as the Caucasian (Azerbaijan, Georgia) and Asian (Kyrgyzstan, Turkmenistan) Republics (Fig. 5). Interestingly, an isolate of a quite distinct geotype, G, was isolated simultaneously in Georgia. Strains belonging to still another geotype, T, were repeatedly isolated in Tajikistan in 1991.

The contemporaneous (1990–91) wild-type isolates of serotype 3 also exhibited a marked, although perhaps somewhat lesser variability. Again appropriate strains were circulating in both Caucasian (Armenia, Azerbaijan, Georgia) and Asian (Tajikistan, Turkmenistan) regions (Fig. 5). All of them could be assigned to the same geotype (C), but the level of relatedness between
Fig. 3. Genetic relatedness of the VP1/2A region of the RNAs of wild poliovirus type 1 (a) and type 3 (b) strains. Geographical abbreviations not already given in the legend to Fig. 1 are: BUL, Bulgaria; CHN, China; COL, Colombia; CYP, Cyprus; EGY, Egypt; HON, Honduras; JOR, Jordan; IND, India; INO, Indonesia; ISR, Israel; MEX, Mexico; NEP, Nepal; PAK, Pakistan; ROM, Romania; RSA, Republic of South Africa; SAA, Saudi Arabia; SEN, Senegal; SIN, Singapore; TAI, Thailand; TUN, Tunisia; TUR, Turkey; VEN, Venezuela; VTN, Vietnam; FRA, France; NL, The Netherlands; OMA, Oman; PER, Peru.
some representatives approached, or even slightly exceeded, the limit of 15% set for a geotype.

Simultaneous co-circulation of different poliovirus geotypes has also been noted in other regions with poor health services (Kew et al., 1990b; Zheng et al., 1993).

In several cases, more or less close relationships (judging by the primary structure of the VP1/2A junction) could be established between the strains circulating in the FSU and in other, primarily neighbouring, countries (Fig. 3). Thus, the A-geotype viruses and a Bulgarian strain of 1991 appear to be related enough to suggest that they had a common recent progenitor, or the Bulgarian strain was simply imported from the FSU. Otherwise, the A-geotype strains are obviously separate from other wild-type isolates analysed thus far.

The T-geotype isolates from Tajikistan are remarkably similar to the strains circulating at the same time in Pakistan (a bordering state) and India as well as in Jordan. [Closely related strains have also been isolated during a recent (1992–93) outbreak in Ukraine.] There is little doubt that all these strains belong to the same family that is endemic for this huge geographical area. Remarkably, all these strains as well as other T-geotype representatives (Moldova-91 and two Georgia-85 strains) exhibit some relatedness to Sabin-1. The extent of their divergence from the vaccine strain (13–17%) is sometimes even lower than the 15% limit accepted for a geotype (Table 3). No other known group of wild-type viruses is as close to a vaccine strain as the T-geotype isolates are. Obviously, more definitive conclusions about the possible origin of the genome of the T-geotype viruses (or at least some of its segments) from the vaccine strain could only be possible after comparative sequencing of other portions of the viral RNA; however, the data that exist already make such a comparison very important.

The type 3 strains circulating in the FSU exhibit a moderate relatedness to strains isolated in Turkey (1990) and Oman (1991). The extent of relatedness is compatible with the notion that all these strains have had a relatively recent common predecessor, but it should be taken into account that, for example, the Moldova-82 and Chechen-Ingush-82 (C2) strains appear to be closer to the C1 isolates than the Turkey or Oman strains are. On the other hand, some Turkmenian strains (of the C4 branch) are even more distantly related to the contemporaneous C1 strains from the same region. Thus, it can be assumed that several diverging lineages of the same geotype are independently evolving in the region.
Fig. 4. Deduced amino acid sequences of the VP1/2A junction in the polyproteins of the wild poliovirus type 1 (a) and type 3 (b) isolates. In (b), amino acid sequences of the same region for Sabin 1 and Sabin 2 as well as the consensus for wild-type 1 (this study) and type 2 strains (Kew et al., 1990a; O. M. Kew and others, unpublished data) are presented, for comparison. Asterisks (*) denote gaps introduced for alignment purposes.
It should perhaps be noted that, with respect to the amino acid sequence of the C-terminal portion of VP1, all the known wild-type 3 strains are closer to Sabin-1 and Sabin-2 (or to wild-type strains of these serotypes) than to Sabin-3. Although the evolutionary significance of this observation is uncertain, it is obvious that the appropriate segment of the Sabin-3 polyprotein (and RNA) is in a sense ‘anomalous’.

However limited and insufficient epidemiologic data we possess, some observations nevertheless warrant a brief discussion. The A-geotype strains of type 1, having no obvious close relatives in the neighbouring countries, appear to form a family of viruses endemic to the FSU. The spread of the relevant strains in the Caucasian region seemed to occur from Azerbaijan, where the vaccine coverage has been gradually decreasing since 1989, and where the number of paralytic polio cases (182 in 1990) has even exceeded that registered during the prevaccination period (M. Sailov, personal communication). The earliest clinical sample we possess was isolated in May 1990 in Azerbaijan. In Georgia, more than 50% of the 1990-91 cases analyses occurred in ethnic Azerbaijanians, strongly contrasting with the actual proportion of the Azerbaijani population in Georgia. The majority of cases occurred in eastern Georgia, which neighbours Azerbaijan. In 1991, the outbreak was located in a Muslim community (Saruso) in a Georgian mountain area not far from the Azerbaijani border. The available data are insufficient to suggest whether the A-geotype strains came to Turkmenistan and Kyrgyzstan from Azerbaijan or vice versa.

The single G-geotype strain (#919) isolated in Georgia in 1990 exhibits little relatedness to the A-geotype strains. It is closer to the strains isolated in Romania and Turkey in the following year. Since no evidence for the circulation of the G-geotype in the FSU is available, we may suppose that it was an imported strain.

There is an obvious epidemiologic link between the 1991 Tajikistan T-geotype strains (T1 branch), on the one hand, and strains endemic for Pakistan, India and Jordan, on the other. Epidemiologic transmission of this geotype through Pakistan and Afghanistan can be suggested. It should be mentioned that the outbreak in Tajikistan (summer 1991) preceded an outbreak in Jordan in November of the same year, where it started among Pakistani emigrants.

Several subgeotypes, geographically and/or temporarily distinct, were observed among the C-geotype strains. One subset (C1) caused numerous cases of paralytic poliomyelitis in the three Caucasian Republics (Armenia, Azerbaijan and Georgia) as well as in Turkmenistan. Another (C4) appeared to circulate...
simultaneously in Tajikistan and again in Turkmenistan. There was a third contemporaneous Turkmenistani variant (4002/TRK90, belonging to the C3 branch), only distantly related to the two above mentioned subsets. The latter isolate showed also some similarity to an Oman strain of 1991, suggesting that these viruses might share a relatively recent predecessor. Circulation of moderately related strains in Georgia (C') several years earlier (in 1987) may be interpreted to mean that the C-geotype strains are indigenous to the FSU.

The results presented above reveal the general picture of the wild-type poliovirus circulation in the FSU about 30 years since the first mass campaign of immunization. The success of that campaign in the first years of its implementation could be regarded as an indication of the strong suppression, if not elimination, of the circulation of wild polioviruses in most regions of the country. In central Russia, virtually no indigenous wild poliovirus strains have been associated with sporadic polio cases, at least until recently (the strain 422/RUS91 came most likely from an imported case). In the southern regions of the FSU, the endemicity could be reestablished by imported geotypes as soon as the effective vaccine coverage stopped to be maintained.

We thank the virologists and epidemiologists from the FSU and throughout the world who contributed poliovirus specimens for this study. We also thank Vladimir Seibl, Irina Lavrova, Galina Koroleva, Valentina Kazantzeva and Natalya Gorodkova for preparing poliovirus strains. Brian Holloway and Edwin George prepared oligodeoxynucleotides used in our study. Our thanks are also due to Alexander Gorbalenya for stimulating discussions. This work was partially supported by grants from the State Scientific and Technical Program of Russia 'Advanced Methods of Bioengineering' (branch 'Gene and Cell Engineering').

References


Poliovirus geotypes in the FSU


(Received 21 September 1994; Accepted 22 February 1995)