Cleavage of VP1 and Modification of Antigenic Site 1 of Type 2 Polioviruses by Intestinal Trypsin

MERJA ROIVAINEN AND TAPANI HOVI*

Enterovirus Laboratory, Department of Virology, National Public Health Institute, SF-00280 Helsinki, Finland

Received 21 December 1987/Accepted 2 June 1988

We have exposed 22 independent type 2 poliovirus isolates to human intestinal fluid and purified trypsin. In all cases the virus retained its infectivity, while polyacrylamide gel electrophoresis of viral proteins showed disappearance of the VP1 bands. Concomitantly, the viruses became resistant to antigenic site 1-specific monoclonal antibodies, indicating that the cleavage took place at the antigenic site 1. Sera from persons immunized solely with the inactivated poliovirus vaccine (IPV) neutralized intact type 2 polioviruses more readily than the corresponding trypsin-cleaved virus preparations. The ratio between the neutralization indices for the intact and trypsin-cleaved type 2 polioviruses was not significantly changed by a dose of trivalent oral poliovirus vaccine given to children previously immunized with IPV. These results indicate that while the antigenic site 1 of type 2 poliovirus is immunogenic in humans when IPV is used, the relative role of this antigenic site in human immunity appears to be less critical than that in the case of type 3 polioviruses. Before we obtained these results, only antigenic site 1 had been shown to be immunogenic in type 2 polioviruses.

Several antigenic sites involved in neutralization of polioviruses have been localized at the level of the primary structure of the virion proteins (8) and in the three-dimensional structure of virus particles (2). Antigenic site 1, corresponding to amino acids 89 to 100 in VP1, is sensitive to trypsin in most type 3 and in some type 1 poliovirus strains. No information in this regard has been published for type 2 polioviruses. The cleavage of VP1 of Sabin 1 and 3 viruses does not affect viral infectivity but results in a dramatic antigenic change preventing interactions between site 1-specific monoclonal antibodies and the virus (1, 4, 9).

We have previously shown that human intestinal fluid can bring about molecular and antigenic changes in the type 3 Sabin strain similar to those obtained with purified trypsin (10). This suggested that type 3 poliovirus replicating in the mucosa of the small intestines is modified by intestinal trypsin and that the cleaved virus might be immunogenic in humans in a way which differs from that of intact poliovirus, as was previously shown for the BALB/c mouse (8). Indeed, we found that the capacity of sera from persons immunized solely with the inactivated poliovirus vaccine (IPV) to neutralize trypsin-cleaved type 3 poliovirus, if detectable, was remarkably lower than the ability of the sera to neutralize the intact virus. In contrast, sera from most persons naturally infected with poliovirus type 3/Finnland/84 or sera from IPV-vaccinated persons after one booster dose of oral poliovirus vaccine (OPV) neutralized the two virus preparations equally (10).

These results suggested that antibodies induced by IPV are mostly targeted to antigenic site 1, whereas a natural type 3 poliovirus infection, either by a wild type or by the attenuated vaccine strain, may result in an antibody response efficiently involving other antigenic sites as well. Our results also indicate that host enzymes acting on poliovirus in vivo can bring about an antigenic change that has relevance in the judging of the efficacy of poliovirus vaccines. In this paper, we present the results of studies of the effects of trypsin and intestinal fluid on the infectivity and the integrity of VP1 of 22 independent type 2 poliovirus isolates and evaluate the effect of trypsin cleavage on the neutralization of poliovirus type 2/MEF-1 and type 2/Sabin strains by sera from persons with different immunization histories.

Cleavage of VP1 of type 2 polioviruses at antigenic site 1 by intestinal fluid and trypsin. Three independent strains of type 2 poliovirus were labeled with [35S]methionine, purified in sucrose gradients, and exposed to specimens of human intestinal fluid and purified trypsin. Polyacrylamide gel electrophoresis revealed that in all cases, the VP1 bands disappeared and a new major band with a molecular mass of approximately 23 kilodaltons appeared (Fig. 1A).

A rabbit antiserum raised against the MEF-1 strain was utilized in a Western blot (immunoblot) analysis of 19 additional independent type 2 poliovirus isolates from different parts of the world. The antibody cross-reacted with blotted VP1 of all tested strains. The band could not be detected in trypsin- or intestinal fluid-treated virus preparations, suggesting cleavage of VP1 (Fig. 1B). Infectivity of the virus preparations was not affected by the treatments.

While this cleavage pattern would indicate a cleavage at about amino acid 95 to 100 of VP1, coinciding with antigenic site 1, we do not know the exact peptide bond cleaved. The amino acid sequence of antigenic site 1 is known for two independent type 2 poliovirus strains, Sabin 2 (13) and Lansing (6). In both strains there is a potential trypsin cleavage site (Arg-100 in Sabin 2 and Lys-99-Arg-100 in Lansing) within the antigenic site.

One or more bands smaller than 23 kilodaltons were usually seen in the trypsin-treated preparations and occasionally in the intestinal fluid-treated preparations. They were not characterized further.

The fact that incubation of the virus preparations with specimens of human intestinal fluid resulted in a cleavage pattern similar to that obtained with purified trypsin suggests that the cleavage also takes place in vivo during natural type 2 poliovirus infections, as we previously reported for type 3 polioviruses (10).

Monoclonal neutralizing antibodies raised against Sabin 2 virus (8) and known to be specific for antigenic site 1 were used for identification of the cleavage site. Five different

* Corresponding author.
monoclonal antibodies neutralized intact Sabin 2 virus, but none of them was able to neutralize trypsin-cleaved Sabin 2 virus (Table 1). These results indicate that the cleavage site of trypsin in the Sabin 2 virus strain is antigenic site 1, similar to that previously reported for Sabin 3.

Antibodies neutralizing protease-treated type 2 poliovirus in human sera. A set of 54 sera collected in Finland in 1977 from different age groups, previously analyzed for type 3 poliovirus antibodies in a similar way (10), was examined for antibodies neutralizing intact or trypsin-treated type 2 poliovirus (Sabin strain) by using the neutralization index determination. The sera were arbitrarily divided into two groups, representing ages below or above 17 years. The younger age group had supposedly developed poliovirus antibodies solely as a response to IPV immunization, while the older age group had in principle had an opportunity to be exposed to natural poliovirus infection before the abrupt stop of indig-

TABLE 1. Neutralization of Sabin 2 virus preparations by antigenic site 1-specific monoclonal antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Neutralization index* for:</th>
<th>Intact virus</th>
<th>Trypsin-cleaved virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>433</td>
<td></td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>434</td>
<td></td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>435</td>
<td></td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>436</td>
<td></td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>269</td>
<td></td>
<td>1.0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Neutralization index is the virus-neutralizing capacity of a serum specimen expressed as a logarithm of the reduction of the plaque titer of a standard virus preparation brought about by a 1-h incubation of the virus with the antibody preparation; a neutralization index > 0.5 is considered significant.

FIG. 1. Cleavage of VP1 of type 2 polioviruses by human intestinal fluid and trypsin. (A) Strains Sabin 2 (lanes 1 and 2), MEF-1 (lanes 3 to 5) and Lennette 77726 (lanes 6 to 8) were grown in Vero cells, metabolically labeled with [35S]methionine, and purified in sucrose gradients (14). Samples of virus preparations were treated for 1 h at 37°C with human intestinal fluid (4 μL/μL of virion protein) (lanes 3 and 6) or with tosylsulfonyl phenylalanyl chloromethyl ketone-treated trypsin (1 μg/μL of virion protein) (lanes 1, 4, and 7) or were untreated controls (lanes 2, 5, and 8). Proteins were separated by 10 to 20% polyacrylamide gradient gel electrophoresis (5), electrically transferred (12) to nitrocellulose sheets, and analyzed by autoradiography. Lane 9, Molecular weight markers. (B) Proteins of crude virus preparations of strains 81-4789/81 and II-299/52 before (lanes 10 and 12) and after treatment with intestinal fluid (1 volume to 3 volumes of virus) (lanes 11 and 13). Proteins were separated by electrophoresis, transferred to nitrocellulose, and analyzed for immunoreactivity with an antisera to the MEF-1 strain. Similar results were obtained with 17 other strains of type 2 poliovirus (II-316/52, II-364/56, II-215/59, 171/2/7/72, 185/2/7/73, II-867/74, 76/78, 92/78, LS 2575/80, 4141/82, 273/82, B 1139/82, 5394/83, P2 Eng/23/84, 282/N/84, 121/84, 967/N/84) as well as after treatment with trypsin (80 μg/ml).

FIG. 2. Neutralizing antibodies to intact and trypsin-cleaved type 2/Sabin poliovirus in human sera collected in 1977 from different age groups. ●, <17 years; X, >17 years. The neutralizing capacities of sera are expressed by neutralization indices (see Table 1). Symbols in the shaded area represent sera with insignificant neutralizing capacities.

neutralizing poliovirus circulation in Finland in the early 1960s (7). About one-third of the sera from both groups were found to have neutralizing capacity to neither the intact nor the cleaved Sabin 2 virus (Fig. 2). The remaining positive sera had a significantly better capacity to neutralize the intact versus the cleaved virus (P < 0.001, Wilcoxon signed-rank test), but the two age groups were similar in this respect (Mann-Whitney test).

A nationwide campaign with OPV was organized in Finland in 1985 to control an outbreak due to wild-type 3 poliovirus discovered in late 1984 (3). Before this campaign, OPV had never been used in Finland in general immunizations. The effect of one dose of trivalent OPV on the antibody patterns was studied in a group of 17 6-year-old children previously immunized with four or five doses of the regular IPV. Neutralization indices of sera drawn before and 1 month after the OPV were measured against intact and trypsin-cleaved type 2/Sabin and type 2/MEF-1 strains. Standard microneutralization titers of these sera against the two strains have been reported before (11) and were found to be relatively high (geometric mean titers were 348 for Sabin 2 and 1,093 for MEF-1 in the pre-OPV sera). When the sera were used undiluted in the neutralization index test, no remaining infectious virus of any of the four preparations could be detected in most cases. To reveal possible differences in the relative neutralization capacity, the sera were used in further experiments at a 1:10 dilution. All but one of the pre-OPV sera were able to neutralize trypsin-cleaved type 2 poliovirus of either strain tested. For both virus strains, the intact virus was more readily neutralized than the cleaved one by both pre- and post-OPV sera (Fig. 3). The difference was slightly greater after the OPV, which is in contrast to the situation we previously reported for type 3 poliovirus (not shown).

Correlation of documented Sabin 2 virus excretion (reported previously for these vaccinees (11)) to pre-OPV antibody level and to subsequent response to intact and trypsin-cleaved viruses did not reveal major differences between the two virus preparations either (Fig. 4). Virus excretion was usually but not exclusively associated with low pre-OPV antibody levels, and a good antibody response...
of most if not all type 2 polioviruses. The cleavage site of trypsin in the Sabin 2 virus strain was shown to be antigenic site 1, similar to that previously reported for Sabin 3. Likewise, sera from persons immunized solely with IPV have a weaker capacity to neutralize the cleaved type 2 poliovirus than the intact virus. Unlike the situation with type 3 poliovirus, however, a dose of trivalent OPV to persons previously immunized with IPV did not greatly increase the apparent proportion of antibodies binding to antigenic sites other than site 1.

These results indicate that the antigenic site 1 of type 2 poliovirus is immunogenic in humans immunized with the trivalent IPV. However, the prominence of this antigenic site over the others, as yet unidentified for type 2 polioviruses, was not as obvious as it was for type 3 polioviruses (10). Hence, IPV-immunized persons with moderate or high levels of antibodies to the intact type 2 polioviruses usually have significant levels of antibodies against the VP1-cleaved virus as well. Until these results, antigenic site 1 had been the only site documented to be involved in the induction of neutralizing antibodies to type 2 polioviruses (8). A dose of OPV that resulted in excretion of attenuated type 2 viruses in a large proportion of the previously IPV-immunized vaccinees did not decrease the apparent moderate prominence of antigenic site 1-specific antibodies. Even after the OPV, the levels of neutralizing antibodies to the intact and to the cleaved type 2 viruses were in good correlation. This is different from the situation that we previously found for type 3 poliovirus antibodies in the same children. Antibodies in both pre- and post-OPV sera neutralizing trypsin-cleaved type 2/Sabin virus thus appear to be targeted to the same sites as those neutralizing the intact virus.

In conclusion, the observations reported in this paper suggest that while the antigenic site 1 of type 2 poliovirus undoubtedly is immunogenic in human recipients of IPV, involvement of the other as yet unidentified antigenic sites is also important. Consequently, cleavage of the antigenic site 1 by intestinal trypsin does not convert type 2 polioviruses resistant to antibodies resulting from regular IPV immunizations. Hence, the putative improvement of mucosal immunity obtained by the proposed addition of trypsin-cleaved virus into the IPV preparation (10) would be less remarkable for type 2 than for type 3 polioviruses. Attempts to examine the possible benefits of an IPV preparation modified in this way are in progress.

Skillful technical assistance by Mervi Eskelinen is gratefully acknowledged. We thank David Magrath for providing the set of virus isolates, Morag Ferguson for the site 1-specific monoclonal antibodies, and Erkki Savilahti for the specimens of human intestinal fluid.

This work was supported by grants from the Finnish Cultural Foundation, the Finnish Academy, and the Sigrid Juselius Foundation.

LITERATURE CITED
NOTES


