Poliovirus antigenic hybrids simultaneously expressing antigenic determinants from all three serotypes

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We have constructed six hybrid polioviruses (PVs) modified to express PV type 2 and type 3 antigenic determinants on a PV type 1 (Mahoney) capsid. The hybrids were modified in neutralizing antigenic site (NAg) I and/or NAgII. They were viable, but impaired for growth in comparison to PV1 (Mahoney). Some hybrids modified to express type 2 and type 3 NAgI determinants simultaneously displayed some type 2 but no type 3 antigenicity (in addition to type 1 antigenicity associated with other antigenic sites). Hybrids modified to express a type 2 NAgI determinant and a type 3 NAgII determinant, or vice versa, displayed antigenic characteristics of all three serotypes, although expression of the modified NAgII determinant was weak. We conclude that it is possible to construct a viable hybrid PV simultaneously modified in NAgI and NAgII which expresses antigenic determinants of all three serotypes.

Introduction

There is a growing body of literature reporting the construction of antigenic hybrids of poliovirus (PV) in which various neutralizing antigenic sites (NAgs) have been modified to express heterologous antigenic determinants (Murray et al., 1988a, b; Burke et al., 1988; Martin et al., 1988; Murdin & Wimmer, 1989; Evans et al., 1989; Jenkins et al., 1990; Minor et al., 1990; Kitson et al., 1991; Murdin et al., 1991 a, b). Many of these hybrids are viable, and their antigenicity and some other phenotypic traits have been characterized. Each of these hybrids has been modified at a single antigenic site, and expresses only a single heterologous determinant at such a modified site. There are obvious situations in which it would be useful to express more than one foreign determinant, for instance co-expression of B and T cell epitopes or serotypic variants of an epitope. To determine whether two heterologous determinants can be expressed in a single hybrid PV we attempted to construct trivalent PV hybrids expressing antigenic determinants from all three PV serotypes.

The antigenic structure of PV is well defined and essentially the same for all three serotypes (types 1, 2 and 3). The capsid is composed of 60 copies of each of four capsid proteins, VP1, VP2, VP3 and VP4, of which VP1, -2, and -3 lie on the outer surface of the capsid (Cooper et al., 1978; Melnick, 1985). These three proteins are eight-stranded, anti-parallel \( \beta \)-barrels with a highly conserved structural core and variable loops linking the \( \beta \)-strands (Hogle et al., 1985). The loops form much of the surface of the virion.

There are four NAg sites (NAgI, -II, -IIA and -IIIB) on the virion, to which neutralizing antibodies can bind independently (Jameson et al., 1985; Hogle & Filman, 1987; Minor et al., 1987; Murray & Wimmer, 1988; Wiegars et al., 1989). These sites map almost entirely to the variable surface loops; we have previously presented a summary of them (Murdin & Wimmer, 1989). Of particular relevance here are the loop linking \( \beta \)-strands B and C of VP1 (1BC), a part of NAgI, and the loop linking \( \beta \)-strands E and F of VP2 (2EF), a part of NAgII.

Two strategies were used to construct potentially trivalent PV hybrids. First, hybrids were designed in which 1BC of PV1 (Mahoney) [PV1(M)] was replaced with equivalent sequences from 1BC of both PV2 (Lansing) [PV2(L)] and PV3 (Leon) [PV3(Le)]. Second, 1BC and 2EF of PV1(M) were simultaneously modified so that 1BC was replaced by 1BC from either PV2(L) or PV3(Le), and 2EF was replaced by 2EF from either PV3(Le) or PV2(L), respectively. Here we describe the antigenic properties and growth characteristics of these novel hybrids.

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Methods

Hybrid viruses. The hybrids were constructed in PVI(M) using the mutagenesis strategies described previously (Murray et al., 1988b; Murdin & Wimmer, 1989). Briefly, synthetic oligonucleotides encoding the desired hybrids were introduced into a PVI(M) genomic cDNA clone using mutagenesis cartridges, and RNA transcripts of the mutant cDNA were prepared. The transcripts were infectious when transfected into HeLa cells and the hybrid viruses were recovered from transfected cells. The amino acid sequences of the hybrids are shown in Fig. 1. These were verified by sequencing the virion RNA in the mutated regions.

Immunizations. Approximately 2 × 10^8 p.f.u. of CsCl-purified virus in 0.1 ml of water was inoculated into the left peripopliteal lymph nodes of New Zealand White rabbits. At the same time, a further 2 × 10^8 p.f.u. in Freund's complete adjuvant was injected subcutaneously. Similar doses in Freund's incomplete adjuvant were administered subcutaneously at 14-day intervals. The animals were bled 42 days after the first inoculation.

Assays. Virus was titrated by plaque assay as described previously (Murdin & Wimmer, 1989). Serial dilutions of serum were titrated by their ability to neutralize 100 TCID₅₀ of PV according to the method of Golding et al. (1976).

Results

Growth characteristics

Viable hybrid viruses were constructed, and their identity was confirmed by sequencing the virion RNA in mutated regions. Amino acid sequences of the hybrids are shown in Fig. 1. Hybrids W1/2/3-1D-23 and -32 have a normal length IBC modified to include partial sequences from IBC of both PV2(L) and PV3(Le). In W1/2/3-1D-23 the left side of the loop carries PV2(L) amino acids, the right side PV3(Le) amino acids, whereas in W1/2/3-1D-32 this is reversed.

Hybrids W1/2/3-1D-23FL and -32FL have an extended IBC modified to include the entire sequences of IBC from both PV2(L) and PV3(Le). In W1/2/3-1D-23FL the left side of the loop carries PV2(L) amino acids, the right side PV3(Le) amino acids, whereas in W1/2/3-1D-32FL this is reversed.

Hybrids W1/2/3-1B/1D-3V1 and -3V2 are modified in both IBC and 2EF. Hybrid W1/2/3-1B/1D-3V1 has IBC replaced with IBC from PV3(Le) and 2EF replaced with 2EF from PV2(L). In hybrid W1/2/3-1B/1D-3V2, IBC is from PV2(L) and 2EF from PV3(Le).

All six hybrids were impaired for growth in comparison to PVI(M) (Fig. 2). The typical yield of these hybrids from a single-step growth cycle was 10- to 100-fold less than that of PVI(M). In addition, all hybrids exhibited a small plaque phenotype in relation to PVI(M) (data not shown).

Antigenicity

The hybrids were tested to determine whether they were susceptible to neutralization by antisera to PV2(L) and PV3(Le), and whether they could induce neutralizing antibodies to PV2(L) and PV3(Le) in rabbits. The results are shown in Table 1.

Hybrids W1/2/3-1D-23 and -32, incorporating only part of IBC from PV2(L) and PV3(Le), displayed some antigenic characteristics associated with the heterologous epitopes. Hybrid W1/2/3-1D-32 could induce high titre PV2(L)-neutralizing antibodies, and in one rabbit (no. 354) induced low titre PV3(Le)-neutralizing anti-
Hybrid W1/2/3-1B/1D-3V1 was able to induce neutralizing antibodies to PV3(Le) and, weakly, to PV2(L), whereas hybrid W1/2/3-1B/1D-3V2 induced neutralizing antibodies to PV2(L) but not to PV3(Le). Once again, the weak neutralizing activity was associated with the determinant expressed in 2EF.

Discussion

We have successfully constructed six novel hybrid PVs in which 1BC and/or 2EF of PV1(M) have been modified to carry amino acid sequences specific for the same loops from PV2(L) and PV3(Le). It is of particular interest that all of these hybrids are viable. In two hybrids, W1/2/3-1D-23 and -32, 1BC had been extended in length from nine to 18 amino acids, further evidence that this site can tolerate substantial changes in its size and shape (see also Murdin et al., 1991b). In two other hybrids, W1/2/3-1B/1D-3V1 and -3V2, both 1BC (NAgI) and 2EF (NAgII) had been simultaneously modified. All hybrids described previously have been modified at one NAg only.

The growth characteristics of the hybrids are impaired in comparison to those of their wild-type parent, consistent with previous results obtained with other hybrids modified in 1BC or 2EF (Murdin et al., 1991a, b). Significantly, the two hybrids modified in both 1BC and 2EF are no more impaired than those modified in only one of these loops. Typically the hybrids exhibit a small plaque phenotype and give a final yield in a single-step growth cycle 10- to 100-fold less than PV1(M). We have previously discussed possible reasons for the impaired growth of PV hybrids (Murdin et al., 1991b).

Of the hybrids modified to carry PV2(L)- and PV3(Le)-specific sequences in 1BC (W1/2/3-1D-23, -32, -23FL and -32FL), only W1/2/3-1D-32 expresses strong PV2(L) or PV3(Le) antigenic characteristics. This virus is not neutralized by antisera to PV2, but is able to induce PV2-neutralizing antibodies. We interpret this to mean that the PV2 epitope(s) expressed on the hybrid are distinct from and induce a different set of neutralizing antibodies to the epitopes expressed on PV2(L). We have previously observed a similar effect for another PV2(L) determinant expressed in various ways at 1BC and 2EF on PV1(M) (Murdin et al., 1991b). There was evidence that hybrid W1/2/3-1D-32 expresses a weak PV3(Le) determinant and that hybrid -23 expresses a weak PV2(L) determinant. However, the antigenicity of the heterologous determinants expressed in all four of these hybrids is markedly inferior to that of the same determinants in hybrids constructed to express only a single PV2(L) or PV3(Le) determinant in 1BC. Such hybrids are both neutralized by and induce specific PV2- or PV3-neutralizing antibodies. The results for the

Fig. 2. Single-step growth curves for PV1(M) (●) and hybrid viruses W1/2/3-1D-23, -1D-32 (a, □ and ○), -1D-23FL, -1D-32FL (b, □ and ○), -1B/1D-3V1 and -1B/1D-3V2 (c, ■ and □). Virus was grown and recovered as described previously (Murray et al., 1988b) and then titrated by plaque assay.
Table 1. Neutralizing titres of antisera to the hybrid viruses

<table>
<thead>
<tr>
<th>Serum* (immunizing antigen)</th>
<th>Titre† against</th>
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<tbody>
<tr>
<td></td>
<td>PV1(M)</td>
</tr>
<tr>
<td>Rb659[PVI(M)]</td>
<td>10298</td>
</tr>
<tr>
<td>Rb359[PV2(L)]</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Rb361[PV3(Le)]</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Rb362 (W1/2/3-1D-23)</td>
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</tr>
<tr>
<td>Rb366 (W1/2/3-1D-23)</td>
<td>1024</td>
</tr>
<tr>
<td>Rb364 (W1/2/3-1D-32)</td>
<td>1746</td>
</tr>
<tr>
<td>Rb354 (W1/2/3-1D-32)</td>
<td>2353</td>
</tr>
<tr>
<td>Rb782 (W1/2/3-1D-23FL)</td>
<td>&gt; 2048</td>
</tr>
<tr>
<td>Rb783 (W1/2/3-1D-23FL)</td>
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</tr>
<tr>
<td>Rb784 (W1/2/3-1D-32FL)</td>
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</tr>
<tr>
<td>Rb785 (W1/2/3-1D-32FL)</td>
<td>&gt; 2048</td>
</tr>
<tr>
<td>Rb623 (W1/2/3-1B/1D-3V1)</td>
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</tr>
<tr>
<td>Rb624 (W1/2/3-1B/1D-3V1)</td>
<td>3040</td>
</tr>
<tr>
<td>Rb899 (W1/2/3-1B/1D-3V2)</td>
<td>&gt; 1024</td>
</tr>
<tr>
<td>Rb900 (W1/2/3-1B/1D-3V2)</td>
<td>&gt; 1024</td>
</tr>
</tbody>
</table>

* Sera 659, 359 and 361 were hyperimmune, and 623 PB and 624 PB were pre-bleed sera. Other sera were taken 42 days after the first inoculation; see Methods.

† Reciprocal dilution of serum giving 50% endpoint in a neutralization assay against 100 TCID₅₀ of virus.

PV2(L) determinant on hybrid W1/2/3-1B/1D-3V2 and the PV3(Le) determinant on hybrid 3V1 illustrate this point (see also Murray et al., 1988 a, b; Burke et al., 1988).

It is noteworthy that hybrids W1/2/3-1D-23 and -32 are more antigenic than -23FL and -32FL, even though the former hybrids express only part of 1BC from PV2(L) and PV3(Le). NAGI includes nearly the whole of 1BC, and it might be predicted that the whole sequence of 1BC would be a better antigen than a partial sequence. However, it appears that a partial sequence in an approximately correct loop configuration (hybrids W1/2/3-1D-23 and -32) is more antigenic than the entire sequence in an incorrect extended configuration (hybrids W1/2/3-1D-23FL and -32FL).

The hybrids constructed by simultaneously modifying 1BC and 2EF (W1/2/3-1B/1D-3V1 and -3V2) were more successful constructs. Both are neutralized by antisera to all three PV serotypes, although the neutralizing activity of antisera to the determinant expressed in 2EF was weak for both viruses. Hybrid W1/2/3-1B/1D-3V1 induces neutralizing antibodies to all three PV serotypes, although the neutralizing activity induced by the PV2(L)

determinant in 2EF is weak (pre-bleed titres are shown to make it clear that this response is real). Hybrid W1/2/3-1B/1D-3V2 induces neutralizing antibodies against PV1(M) and PV2(L) only. The PV3(Le) determinant in 2EF is unable to induce detectable neutralizing antibodies.

We have previously described a hybrid of PVI(M) (W1/2-1B-NII2/2/2) which is modified in 2EF to express the same PV2(L) determinant as hybrid W1/2/3-1B/1D-3V1, but not modified in 1BC (Murdin & Wimmer, 1989). This hybrid induces somewhat higher absolute titres of neutralizing antibody directed to the 2EF determinant than W1/2/3-1B/1D-3V1, but also induces higher neutralizing titres against itself and against PV1(M). The relative strength of the 2EF determinant appears to be similar in the two hybrids. An analogous hybrid modified in 2EF to express the PV3(Le) determinant expressed in W1/2/3-1B/1D-3V2 is neutralized by antisera to PV3(Le) to the same extent as -3V2 (H.-H. Lu, unpublished results). We conclude that modification of 1BC does not affect the expression of the 2EF determinant.
Thus, 1BC and 2EF can be simultaneously modified to express independent heterologous antigenic determinants. The PV antigenic determinant associated with 1BC induces a good neutralizing antibody response. The determinant associated with 2EF is very weak, at least in the cases of PV2(L) and PV3(Le), but it can be expressed on PV1(M). The response to this determinant may be improved by manipulating more of NAgI than 2EF. Minor et al. (1990) have shown that the response to NAgI of PV3 (Sabin) expressed on PV1 (Sabin) can be improved by manipulating other parts of the site in addition to 1BC, and it is reasonable to expect that NAgI will behave similarly.

The 1BC loop is known to accept a wide range of sequence replacements, but the 2EF loop appears to be less tolerant [for instance we have so far failed to express the 2EF loop from human rhinovirus 14 in 2EF of PV1-(M)]. We have recently found that sequences inserted into the 1BC loop (i.e. PV1 amino acids retained and heterologous amino acids added) are better tolerated than the same sequences replacing the 1BC loop [i.e. PV1 amino acids deleted and replaced by heterologous amino acids; Murdin et al. (1991b) and unpublished data]. Therefore it may also be possible to express a heterologous determinant as an insertion into 2EF, rather than as a replacement.

Expression in 2EF needs to be improved, but nevertheless we have demonstrated that it is possible to construct a PV hybrid expressing two heterologous epitopes.

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References


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