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A Cassette Vector for the Construction of Antigen Chimaeras of Poliovirus

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SUMMARY

A cassette vector has been constructed which allows the rapid and extensive modification of one of the neutralizing antigenic sites of the Sabin 1 poliovirus vaccine strain, P1/LSc 2ab. Unique restriction endonuclease sites flanking antigenic site 1 have been engineered into a full-length infectious Sabin 1 cDNA clone with minimal alteration to the coding sequence. This facilitates replacement of this region by oligonucleotides encoding foreign amino acid sequences. Our results indicate that this region is highly flexible in terms of the number and sequence of amino acids which can be accommodated.

The icosahedral poliovirus particle is composed of 60 copies of each of four capsid proteins, VP1 to VP4, which enclose a positive-sense ssRNA genome of approximately 7500 nucleotides (Kitamura *et al.*, 1981). Because of their importance in protective immunity the antigenic sites on the capsid proteins of the three poliovirus serotypes have been studied in detail (Emini *et al.*, 1983; Ferguson *et al.*, 1984, 1985, 1986; Minor *et al.*, 1986; Page *et al.*, 1988; van der Werf *et al.*, 1983). These studies have revealed the existence of at least four independent antigenic sites, which induce the production of neutralizing antibodies. Antigenic site 1 is a continuous epitope, consisting of residues 91 to 102 of capsid protein VP1. Sites 2, 3 and 4 are conformational, being composed of residues from more than one capsid protein. These sites can be readily located on the three-dimensional crystallographic model of the virus where they form part of the surface topography (Hogle *et al.*, 1985). As part of a wider study of poliovirus antigenicity relevant to the development of new and improved poliovirus vaccines, we have reported the construction of a type 1/type 3 poliovirus chimaera (Burke *et al.*, 1988). This virus, which exhibits dual antigenicity, was constructed by the replacement of antigenic site 1 of the Sabin type 1 poliovirus vaccine strain (Sabin & Boulger, 1973) by the corresponding region of a type 3 strain using oligonucleotide-directed mutagenesis (Kramer *et al.*, 1984) on an infectious full-length Sabin 1 cDNA clone (Stanway *et al.*, 1986). The virus induced an immune response against both type 1 and type 3 polioviruses in mice, rabbits and primates. Similar recombinants based on the neurovirulent type 1 poliovirus strain, P1/Mahoney have also been reported (Martin *et al.*, 1988; Murray *et al.*, 1988). The ability to construct such precisely engineered chimaeras has led us to explore the use of Sabin type 1 vaccine strain as a vehicle for the presentation of antigenic domains of other pathogens, for example hepatitis A virus. In this paper we report the construction of a cassette vector which allows rapid and extensive modification of antigenic site 1 of the Sabin strain (P1/LSc 2ab) of poliovirus type 1. Unique restriction sites, flanking antigenic site 1, have been engineered into an infectious full-length cDNA clone of this virus using oligonucleotide-directed mutagenesis allowing replacement of site 1 by complementary oligonucleotides of appropriate sequence, thereby obviating the subcloning and mutagenesis steps previously required in the construction of chimaeras. Our results indicate that antigenic site 1 of poliovirus type 1 exhibits a high degree of flexibility, both in the number and sequence of amino acids that can be accommodated, whilst usually retaining virus viability.

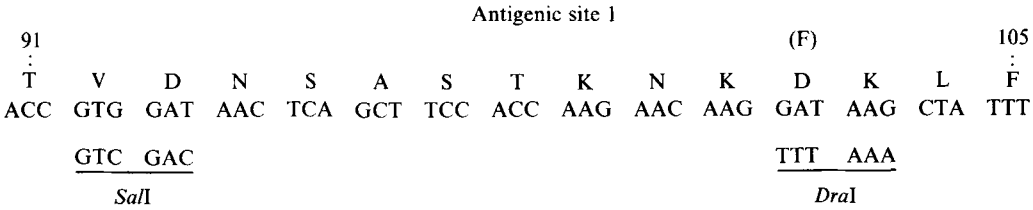

Fig. 1. Nucleotide and amino acid sequence of antigenic site 1 of poliovirus Sabin 1 illustrating changes introduced in the construction of the cassette vector, pCAS1. The cDNA sequence of the genome-sense strand is shown (nucleotides 2750 to 2794), together with the location of the introduced restriction sites. The resulting amino acid change of aspartic acid (102) to phenylalanine is indicated in parentheses.

Taking advantage of codon degeneracy, the nucleotide sequence of Sabin 1 cDNA in the region 2740 to 2800 was searched for sequences at which restriction endonuclease sites unique to the cDNA could be introduced with minimal alteration to the amino acid sequence. It was observed that a *Sal*I site at nucleotide 2753 could be created without alteration to the amino acid sequence and that this site would be unique within the virus sequence. Similarly a unique *Dra*I site could be created at position 2783 resulting in the replacement of aspartic acid (VP1 residue 102) by phenylalanine. The synthetic oligonucleotides 5′ GGAAGCTGAGTTGTCGACGGTTATAATGG 3′ and 5′ CACTGTAAATAGTTTAAACTTATTCTTGG 3′ (bases inducing changes underlined) were used to create *Sal*I and *Dra*I restriction sites at positions 2753 and 2783 respectively on a 3·6 kb *Kpn*I partial fragment (nucleotides 66 to 3660) of an infectious Sabin 1 cDNA (Stanway *et al.*, 1986) subcloned in M13mp18, using the gapped-duplex mutagenesis technique (Kramer *et al.*, 1984). The alterations made to the antigenic site were confirmed by dideoxynucleotide chain termination sequencing (Sanger *et al.*, 1977), and are shown in Fig. 1. The mutated fragment was introduced into a full-length cDNA of Sabin type 1 onto which a T7 promoter had previously been engineered at the extreme 5′ end (K. Burke *et al.*, unpublished data). This full-length clone was subsequently transferred into vector pFBI(2) (Pharmacia), which had been modified to remove its three *Dra*I sites at positions 2052, 2071 and 2763, by insertion of an *Eco*RI linker following *Dra*I digestion. An *Eco*RI–*Sal*I fragment carrying this modified full-length poliovirus clone was ligated into *Eco*RI/*Xho*I-digested pFBI(2)-derived vector thereby destroying this *Sal*I site. The resulting plasmid, pCAS1, therefore contained a full-length Sabin 1 cDNA under the control of a T7 promoter in which the introduced *Sal*I and *Dra*I sites were unique. Recovery of infectious virus from *Nae*I-linearized pCAS1 was achieved following transfection of HEp-2c monolayers with transcripts produced *in vitro* by T7 RNA polymerase (Stratagene) as previously described (van der Werf *et al.*, 1986). The genomic sequence of the recovered virus was verified by primer extension sequencing of viral RNA through a region of approximately 350 bases covering antigenic site 1 (Rico-Hesse *et al.*, 1987). The single substitution of aspartic acid for phenylalanine at residue 102 had no apparent affect on virus viability, furthermore the design of the cassette was such that the altered amino acid would be lost upon insertion of replacement sequences.

Short sequences of hydrophilic amino acids from the capsid protein VP1 of hepatitis A virus (Najaran *et al.*, 1985), which might constitute potential antigenic sites, were inserted into the site 1 cassette vector (as shown in Table 1). Appropriate oligonucleotides were annealed by boiling and allowing to cool to room temperature prior to ligation into *Sal*I/*Dra*I-digested pCAS1. T7 transcripts prepared from the resulting recombinant plasmids were used to transfect HEp-2C monolayers (van der Werf *et al.*, 1986). All plasmids containing the hepatitis A virus inserts gave rise to viable virus 24 to 36 h post-transfection. The sequence of each recovered virus was confirmed by sequencing viral RNA (Rico-Hesse *et al.*, 1987). Similarly oligonucleotides corresponding to known or predicted epitopes from human rhinovirus (HRV) serotypes 2 (Skern *et al.*, 1985) and 14 (Stanway *et al.*, 1984) and from the capsid protein VP1 of coxsackie B4 virus (Jenkins *et al.*, 1987) were ligated into the cassette vector. Resulting plasmids were tested for

Table 1. *Amino acid sequences inserted into pCAS1 and their origin*

Virus	Amino acid sequence*	Location
Sabin 1	NSASTKNKD	VP1 94–102
Cas 1	NSASTKNKF	VP1 94–102
Sabin 3	NEQPTTRAQ	VP1 92–100
Hepatitis A	(N)EQNPVD(D)	VP1 15–20
	KDLKGKANRGKMD	VP1 29–41
	ELKPGESRHTSD	VP1 70–81
	TFNSNNKEY	VP1 99–118
	NSNNKEYT(D)	VP1 111–118
	(N)ATDVDG(KD)	VP1 150–155
	NTRRTGN(KD)	VP1 191–197
	(N)GLGDKTDS	VP1 217–224
	DPRSEEDKRFE	VP1 290–300
Rhinovirus 2	KLEVTLANY	VP1 81–89
14	KDATGIDNHREA	VP1 85–96
14	(N)MYVPPGAPNP(D)	VP1 151–160
14	(N)KLILAYTPPGARGPQD	VP3 126–141
Coxsackie B4	(N)SAESNNL(D)	VP1 81–87
	IYIKYSSAESNNL†	VP1 75–87

* Residues in parentheses correspond to amino acids which have been retained from the wild-type Sabin 1 sequence.

† A recombinant cDNA containing this sequence did not yield viable virus upon transfection.

viability as described. Sequence analysis of genomic RNA confirmed that recovered virus chimaeras had the expected alteration in the region of their genomes encoding antigenic site 1.

Although all the viruses were neutralized by a polyclonal poliovirus type 1 antiserum and by monoclonal antibodies directed at antigenic sites 2 and 3, they were not recognized by monoclonal antibodies against site 1. Growth characteristics of the virus chimaeras were variable; some grew as well as the parental viruses whereas others took considerably longer to form a c.p.e. in tissue culture (e.g. up to 48 h compared to 16 h for Sabin 1) and showed a reduced yield (up to 100-fold lower). These will be reported in detail along with information on the antigenicity of various chimaeras (M. Ferguson *et al.*, unpublished data). The replication of all the chimaeras in HeLa cells was blocked by a monoclonal antibody specific for the poliovirus receptor (Pipkin *et al.*, 1984) suggesting that they had not acquired additional receptor specificity nor lost their ability to recognize the poliovirus receptor as a result of their new structures at antigenic site 1. In one case a recombinant plasmid containing an insert sequence from coxsackie B4 virus (Table 1) did not produce viable virus upon repeated transfection although the DNA sequence of the engineered site was in frame and otherwise correct. We are currently investigating the reason for the non-viability of this construct.

We present here the Sabin 1 poliovirus vaccine strain as a vehicle for the expression of potentially important epitopes from other pathogens. The established safety record of the Sabin 1 vaccine, coupled with extensive experience of its manufacture and control (Melnick, 1980), make this a particularly attractive vector for such a purpose. Since poliovirus is able to induce a mucosal as well as a systemic immune response the approach may be of considerable value where the pathogen in question infects via the intestinal mucosa where secretory antibodies may play a role in protection from infection (Ogra *et al.*, 1980). The cassette approach to *in vitro* mutagenesis has been reported before for poliovirus (Kuhn *et al.*, 1988), and was then employed in the construction of antigenic site 1 chimaeras based upon the neurovirulent Mahoney strain of poliovirus (Martin *et al.*, 1988; Murray *et al.*, 1988). However, the poliovirus vaccine strain-based cassette reported here has the additional advantage that the introduced restriction sites are unique to the entire pCAS vector allowing replacement of the flanked region in a single step and thus obviating the need for subcloning steps in the construction of recombinant cDNAs. Hepatitis A virus, which like poliovirus is an enterovirus, is an important human pathogen

against which there is no effective vaccine at present (Purcell, 1987). The antigenic and immunogenic characterization of the Sabin 1/hepatitis A chimaeras described here is currently in progress. Such chimaeras may provide us with information on the location of the antigenic sites of hepatitis A virus and may further the production of a safe and effective vaccine. Similarly coxsackie B4/Sabin 1 chimaeras may provide us with information on the antigenicity of this other medically important member of the enterovirus genus. Construction of Sabin 1/HRV chimaeras may not have obvious implications in vaccine development due to the extensive serological diversity of this genus. However, one of the sequences we have chosen to investigate (VP1 151 to 160) corresponds to a region highly conserved between different rhinovirus serotypes and which, as a peptide, induces antibodies which neutralize 60% of rhinovirus serotypes (McCray & Werner, 1987). It will therefore be of interest to determine whether antisera raised against this chimaera will react with rhinoviruses.

Our results indicate that antigenic site 1 of poliovirus is highly flexible and can be exploited to present foreign antigenic determinants. Further studies are in progress to determine whether other antigenic sites of the virus can accommodate similar modifications.

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