Identification of a New Neutralization Antigenic Site on Poliovirus Coat Protein VP2

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Major neutralization antigenic sites have been previously mapped by us on VP1, the largest capsid protein of poliovirus type 1. Here we report the first identification of the primary sequence of a neutralization antigenic site on capsid protein VP2. Inspection of the amino acid sequence of VP2 led to the selection and synthesis of a peptide (N = 12) that, after linking to a carrier protein, induced an antiviral neutralizing antibody response in rabbits. The response was augmented by a single subsequent inoculation of intact virus; thus, the peptide was also capable of priming the production of neutralizing antibodies. These antibodies were directed only against the site specified by the synthetic peptide. Although the VP2-specific neutralization antigenic site appears not to be strongly immunogenic in the intact virion, it can nevertheless contribute to neutralization of poliovirus. This observation may be important for the development of peptide vaccines.

Poliovirus, a member of the Picornaviridae, occurs in three stable serotypes. Its virion is a naked icosahedron composed of ca. 60 copies each of four structural proteins, VP1, VP2, VP3, and VP4, as well as 1 to 2 copies of VP0, the uncleaved precursor of VP2 and VP4 (15). These proteins encapsidate a single-stranded “messenger-sense” RNA molecule that is 3’ polyadenylated and 5’ linked to a small protein called VPg. The complete nucleotide sequence of the viral RNA of all three serotypes of poliovirus is known (16, 21, 22, 29, 30). Extensive sequence studies of the known poliovirus-encoded polypeptides have allowed us to deduce the amino acid sequences of these proteins and to decode the complete genetic map of the viral genome (7, 8, 17, 25–27).

Various surface-labeling and cross-linking studies have established that VP1 is the predominantly exposed surface protein (2, 18, 23, 33). VP3 and VP2 are also exposed, but to a lesser extent, and VP4 appears to be completely internal, in close association with the viral RNA (34). Recently, considerable progress has been made toward identifying the antigenic sites on the surface of the poliovirion which are responsible for eliciting a neutralizing antibody response. Emini et al. (9) covalently cross-linked F(ab) fragments of monoclonal antibody (N-mcAb) to the virion to show that at least two neutralization epitopes are located on VP1. For our definition of a neutralization antigenic site, N-Ag, and its neutralization epitope(s), N-Ep(s), see references 11 and 35. Briefly, we define an N-Ep as the conformation of a structural element at the surface of a virus to which a monospecific antibody can bind, an interaction leading to the neutralization of the virus. We define an N-Ag as a cluster of N-Eps. Chow et al. (5), by use of synthetic peptides, also demonstrated the presence of potential N-Ags in this protein. Minor et al. (19) and Evans et al. (13) mapped an N-Ag (amino acid residues 93 to 100) in the VP1 of type 3 virus by oligonucleotide and RNA sequence analyses of a series of viral variants which had lost the ability to be neutralized by a given monoclonal antibody. This region is homologous to one of two N-Ags (amino acids 70 through 80 and 93 through 103) of capsid protein VP1 of poliovirus type 1, which we identified by using synthetic peptides and ELISA with N-mcAbs (10, 12). In addition, these peptides and one corre-
FIG. 1. Amino acid sequence of the VP2-specific peptide. Residues from the viral protein sequence are underlined. Additional residues are for spacing and for providing a linkage to carrier protein. Peptide synthesis and carrier linkage reactions were as described by Emini et al. (10).

antibody titers were dramatically increased by a subsequent inoculation of a small amount of intact virus (Table 1). Thus, this peptide was capable of priming a neutralizing antibody response similar to that produced by peptides corresponding to VP1 (10).

The sera produced after the virus inoculation were tested by ELISA for their ability to bind the VP2-specific peptide and various peptides containing sequences from the VP1 protein. These latter peptides represent the three different VP1 neutralizing antigenic sites mentioned before (10). The results (Table 2) indicate that only antibody to the VP2 site was produced by these rabbits. This response is in contrast to that of rabbit antiviral hyperimmune serum prepared against intact virions, in which antibodies were present that bound to most of the VP1-specific peptides but did not bind to the VP2-specific peptide (Table 2). Moreover, it was found that rabbit hyperimmune serum contained antibodies that bind purified VP1. The sera from the peptide-inoculated and vibron-boostered rabbits did not contain this activity (data not shown). It should be added that the serum failed to precipitate denatured VP2, as did sera elicited against VP1-specific peptides that do not recognize VP1 (10). Apparently, the neutralization epitope(s) under study on VP2 is lost upon purification or denaturation of the protein.

These data further support the conclusion that the animals that were inoculated with the VP2 sequence-specific peptide and subsequently subjected to a small dose of intact virus apparently only produced antibody capable of recognizing the peptide-specific site on the virion. Binding of antibody to this site led to virus neutralization. The data also support the conclusion that, at least in rabbits, this VP2 site on the virion is not highly immunogenic. Rabbits inoculated several times with virus failed to make antibody capable of recognizing this VP2 region as expressed on the peptide. The hyperimmune sera did, instead, contain antibodies to antigenic sites on the VP1 structural protein. Hence, it appears that a synthetic peptide can induce an immune system to produce antibody to a normally immunorecessive antigenic site on an intact virion. This phenomenon has been noted previously in nonviral systems (see reference 28 and references therein).

The implications of the phenomenon are interesting and may become important for the development of synthetic vaccines. The following points should be considered. First, the extent of antibody induction by a peptide is not only dependent upon the sequence of the peptide but is also governed by its chain length, which influences its folding into different conformations. No rules have yet been found that allow us to predict the properties of peptide-induced antibodies (10, 20). For example, we have found that a decapeptide is not recognized by all the groups of N-mcAbs that recognize the identical peptide shortened by four amino acids (10). Moreover, the major neutralization epitopes of polyomavirus type 1 mutate to neutralization resistance at a high rate, a phenomenon observed also for other viruses (9, 10, 11, and references therein). Hence, variants not neutralizable by antipeptide antibodies probably exist ubiquitously in every population of virus in nature and will appear rapidly upon antibody selection. The limitations contemplated for a peptide vaccine have been seen in studies with foot-and-mouth disease virus, an aphthovirus (24). It may therefore be crucial to search for several different neutralization antigenic sites at the surface of a virus and to use several peptides corresponding to these sites for immunization. As shown here, a peptide used for immunization does not necessarily correspond to an immunodominant neutralization antigenic site. In fact, it appears that the peptide by itself need not be capable of inducing neutralizing antibodies as long as the peptide can prime for such a response (10).

Secondly, it appears that the immune system recognizes only a limited number of dominant neutralization antigenic sites on the surface of a virion, although other potential sites may exist. This restricted response to dominant sites is highly advantageous for a virus if the virus can tolerate mutations (without loss of viability) which phenotypically alter these sites. As emphasized above, those mutations can lead to the rapid appearance of nonneutralizable variants. In this way the virus can evade the immune system of the host while masking other potential N-Ags whose variability may be restricted because they play a role in virion stability or virus uptake. If so, the immune system, once alerted to those minor N-Ags by peptides (either directly or after priming), could produce antibodies that may be able to neutralize the virus.

<table>
<thead>
<tr>
<th>TABLE 2. Recognition of synthetic peptides by rabbit antiviral sera</th>
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<tr>
<th>Serum</th>
<th>Recognition of the following peptide</th>
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<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Hyperimmune</td>
<td>+</td>
</tr>
<tr>
<td>Rabbit A</td>
<td>-</td>
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<td>Rabbit B</td>
<td>-</td>
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<td>Rabbit C</td>
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* Hyperimmune rabbit serum was prepared by several inoculations, at 3-week intervals, of 7.0 log_{10} PFU of virus into each of two rabbits. The animals were bled 2 weeks after the final inoculation, and both sera were pooled. Sera from rabbits A, B, and C were prepared after the final virus inoculation as described in footnote b of this table.

* Peptides 1 through 5 contain the following amino acid sequences from the VP1 structural protein (10): 70 through 75, 97 through 103, 93 through 103, 70 through 81, and 11 through 17. VP2 is the VP2 sequence-specific peptide described in this study. Symbols: +, positive response by ELISA with a 1:10 dilution of rabbit sera; -, ELISA reaction not significantly above background. ELISA procedure was carried out exactly as described by Emini et al. (10).
entire virus population, possibly even across serotype boundaries, and provide long-term protection.

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LITERATURE CITED

ERRATA

Intracellular Location and Kinetics of Complex Formation Between Simian Virus 40 T Antigen and Cellular Protein p53

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Volume 52, no. 2, p. 350–355: In Table 4, page 354, the main boxhead, “% in complex,” should read “% in nucleus.”

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Volume 52, no. 2, p. 719–721: On page 719, column 2, 9 lines from the bottom, “. . . 192 through 203 . . .” should read “. . . 162 through 173 . . .”