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Peptide Vaccine against Canine Parvovirus: Identification of Two Neutralization Subsites in the N Terminus of VP2 and Optimization of the Amino Acid Sequence

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The N-terminal domain of the major capsid protein VP2 of canine parvovirus was shown to be an excellent target for development of a synthetic peptide vaccine, but detailed information about number of epitopes, optimal length, sequence choice, and site of coupling to the carrier protein was lacking. Therefore, several overlapping peptides based on this N terminus were synthesized to establish conditions for optimal and reproducible induction of neutralizing antibodies in rabbits. The specificity and neutralizing ability of the antibody response for these peptides were determined. Within the N-terminal 23 residues of VP2, two subsites able to induce neutralizing antibodies and which overlapped by only two glycine residues at positions 10 and 11 could be discriminated. The shortest sequence sufficient for neutralization induction was nine residues. Peptides longer than 13 residues consistently induced neutralization, provided that their N termini were located between positions 1 and 11 of VP2. The orientation of the peptides at the carrier protein was also of importance, being more effective when coupled through the N terminus than through the C terminus to keyhole limpet hemocyanin. The results suggest that the presence of amino acid residues 2 to 21 (and probably 3 to 17) of VP2 in a single peptide is preferable for a synthetic peptide vaccine.

Recently, a synthetic vaccine against canine parvovirus (CPV) based on peptides from the N terminus of VP2 has been described (7). After 11 years of unsuccessful attempts, this has been the first example of peptide vaccine giving full protection in the target animal. CPV belongs to the feline parvovirus subgroup of the autonomous parvoviruses; it is a nonenveloped, icosahedral, single-stranded DNA virus. The viral capsid is formed by 60 capsomers composed mainly of VP2 and VP3 and some VP1. VP2 is the amino-terminal truncated form of VP1, and VP3 derives from VP2 by further proteolytical cleavage of about 20 amino acids from the N terminus. In full capsids, some of the N-terminal ends of VP2 protrude outside the capsid (4, 14, 18). Unfortunately, despite the three-dimensional structure for CPV capsids having been solved, no spatial position has been assigned to the N terminus because of its disordered structure (5, 18). It is this N terminal which is used for the synthetic peptide vaccine. Since no three-dimensional structure could be established for this region, its antigenic and functional relevance was not fully considered previously. In fact, the major antigenic regions of CPV had been located around the threefold spike and localized to the cylindrical structure around the fivefold symmetry axis of the capsid's surface (1, 2, 15, 16).

The 23 amino acids of the N terminus of CPV VP2 display the same sequence as the feline panleukopenia virus of cats and the mink enteritis virus. The sequence is also well conserved in the minute virus of mice (MVM) and the H1 parvovirus (hamster). However, the homology with other parvoviruses, including porcine parvovirus, is lower (3). This domain has become important for several reasons: (i) it is involved in the MVM entry to the cell (19), (ii) it contains an epitope recognized by the CPV neutralizing monoclonal antibody 3C9 (8, 13), and (iii) immunization with a mixture of two partially overlapping pentadecapeptides (1L15, 7L15) derived from this region has been able to provide full protection in target animals (dogs and minks) against a challenge with a lethal dose of virulent CPV or mink enteritis virus, respectively (7, 10). A major advantage of using the N-terminal peptides as a vaccine is the lack of reactivity of naturally infected dog sera; only 3 of 16 dogs tested gave a positive reaction against this region. Thus, in dogs, under natural circumstances, the protective immune response is directed toward other domains. This result opens the possibility of circumventing the maternal immunity, the major problem in current CPV vaccines.

The peptides 1L15 and 7L15 have different potencies in inducing virus-neutralizing antibodies, which suggests that for immunization purposes there might well be a better peptide to be selected from the N terminus of VP2. The two peptides overlap each other with the sequence QPDGGQPAV (residues 7 to 15 of VP2). Peptide 7L15 was especially effective in inducing neutralizing activity. Peptide 1L15 contained the antigenic core (SDGAVQ, residues 2 to 7 of VP2) recognized by some CPV-infected dog sera (8). Also, from PEPSCAN analyses with multiple-length solid-phase peptides and antipeptide antisera it was deduced that the main reactivity of the antibodies elicited by these peptides was directed against the amino acid sequence DGGQPAV, residues 9 to 15 (which are part of the overlapping sequence). Therefore, it remained to be seen whether another pentadecapeptide from the VP2 N terminus or an alternative-size peptide is a better candidate for a more straightforward and cheaper vaccine against CPV and its host-range variants.

To study in more detail the immunogenicity of the N terminus, to know whether more than one epitope was present in this domain, and to better define the composition of a peptide

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