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B-Cell Epitopes of Canine Parvovirus: Distribution on the Primary Structure and Exposure on the Viral Surface

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Ten antigenic sites on canine parvovirus (CPV) were mapped with a complete set of overlapping nonapeptides of the capsid proteins VP1 and VP2: five of these sites were recognized by sera from CPV-infected dogs, three were recognized by a rabbit anti-CPV antiserum, and two were recognized by murine monoclonal anti-CPV antibodies. A region covering the first 21 amino-terminal amino acid residues of VP2 was recognized by three sera from infected dogs, one neutralizing rabbit antiserum, and one neutralizing murine monoclonal antibody. Immunoabsorption experiments with full virions indicated that at least 6 of the 10 antigenic sites are located on the surface. Of these six, three sites occur in the amino terminus of VP2. When superimposed on the three-dimensional structure of canine parvovirus (J. Tsao, M. S. Chapman, M. Agbandje, W. Keller, K. Smith, H. Wu, M. Luo, T. J. Smith, M. G. Rossmann, R. W. Compans, and C. R. Parrish, Science 251:1456–1464, 1991), the other three epitopes are located on two loops of VP2 which form the highly exposed "spike" around the threefold-symmetry axis of the virus. Thus, these regions (amino terminus and loops 1 and 3) are of interest as major target sites for induction of neutralizing antibodies.

Canine parvovirus (CPV) belongs to the feline parvovirus subgroup of the genus *Parvovirus* within the family *Parvoviridae* (36). CPV exhibits extensive antigenic and genetic similarities to feline panleukopenia virus (FPLV) and mink enteritis virus (MEV). In animals of more than 6 weeks of age, the clinical symptoms and pathology of CPV, FPLV, and MEV are very similar (20). Nevertheless, the host ranges of these viruses are distinct (1, 22, 25, 38).

CPV infection in dogs is usually followed by enteritis of variable severity and is often associated with a relative lymphopenia (20). Acute myocarditis occurs in puppies up to 16 weeks of age and causes a high mortality rate (20 to 100%). Serum antibodies appear to mediate immunity against parvoviruses (16, 26). Current vaccines are based on live viruses (3, 4, 10). A limitation of the vaccines is that in puppies, maternally derived antibodies block viral replication (27) and thus the development of a protective immunity. This could also hold for recombinant VP2 capsids when they are used as immunogens (12, 33). For such cases, synthetic or subunit vaccines might present a preferable alternative.

Capsids of CPV and other members of the rat virus group within the genus *Parvovirus* exist as icosahedra assembled from 60 structural protein subunits. Of these subunits, fewer than 10 are present as VP1, and the remainder exist as VP2 and VP3 in variable proportions (8, 18, 37, 39). VP1 and VP2 are different transcription products from the same gene. Although there is uncertainty about the exact length of VP1 because of the presence of different possible splicing sites (22, 28, 29), it might consist of up to 748 amino acid residues (28). The primary structure of VP2 corresponds to that of the 584 carboxy-terminal residues of VP1 (22, 28, 29). VP3 is derived from VP2 by proteolytic removal of approximately 20 amino-terminal residues (7, 17, 37). The function of VP3 is unknown, but it seems to be necessary for infectivity, since VP3 is always present in some degree in infective virus preparations (8).

Amino acid sequences derived from the viral genomes of CPV, FPLV, MEV, and raccoon parvovirus are highly homologous. There are only six consistently occurring amino acid differences in capsid protein VP2 of CPV compared with those of FPLV, MEV, and raccoon parvovirus (14, 21, 22, 28, 29). Of these, residues Asn-93 and Ala-103 were found to be critical in epitope recognition for a CPV-specific and -neutralizing monoclonal antibody (MAb) (22). Residues Ala-300 and Thr-301 are critical amino acids for a neutralizing MAb specific for both CPV and FPLV (22, 24). Residues Asn-93, Ala-300, and Thr-301 are located in loops 1 and 3 at the extremities of the threefold spike on the viral surface (39).

In the case of parvoviruses and other nonenveloped viruses, the target for vaccine development is the capsid protein. Epitope-mapping studies of the capsid proteins of CPV have been carried out either with recombinant strains of CPV and FPLV (20, 22, 23) or through more or less random analysis of a number of synthetic peptides and expression products of VP1 (30). A recent study with anti-CPV MAbs and fragments of VP1 as expression products indicated that a major neutralizing epitope was localized in the very amino-terminal domain of VP2 of CPV (13). In that same study, rabbit antisera against the expression products bound to the virus, and some were able to neutralize virus infectivity in vitro. Polyclonal antisera directed against CPV and other members of the feline parvovirus subgroup have not been reported in epitope-mapping studies. Nevertheless, mapping epitopes of antiparvovirus antibodies from target animals would be a logical step in developing a second-generation vaccine.

In this study, epitopes were systematically mapped with all overlapping nonapeptides of the VP1 amino acid sequence (including the whole VP2 sequence) of CPV by using the PEPSCAN procedure (11). This technique is useful in

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delineating epitopes whose short peptide sequences confer enough energy for binding to antibody, but it is not competent to detect epitopes whose residues involved in binding are assembled from distant positions in the sequence (15). Ten antigenic sites were discovered; they are recognized by neutralizing sera from CPV-infected dogs, anti-CPV antibodies from rabbits, or anti-CPV MAbs from mice. Their accessibility to anti-CPV antibodies was studied by absorption with full virions. The locations of the antigenic sites were interpreted with respect to the available structural data on CPV (39). Six of the 10 sites have residues on the viral surface at highly protruding locations, and 3 others also contained surface-exposed residues.

MATERIALS AND METHODS

Cells, viruses, and virus purification. A permanent line of feline kidney cells (CRFK) (ATCC CCL 94) was used for virus production and preparation of replicative-form DNA. Attenuated virus strain CPV-c/780916 (3) was obtained from the American Type Culture Collection (VR-953). FPLV (Rosette strain) was a kind gift from L. Carmichael (Cornell University, Ithaca, N.Y.). Virions used in these experiments were obtained and purified as previously described by López de Turiso et al. (13).

Antibodies. Dog sera from naturally infected dogs were kindly provided by I. Simarro, Complutense University, Madrid, Spain. Production and analysis of the murine anti-CPV MAbs have been previously described (13). For preparing polyclonal antisera, animals were injected with 150 μg of intact full CPV virions per injection. Rabbits were immunized initially in the presence of complete Freund's adjuvant and at days 15 and 30 with incomplete Freund's adjuvant. A guinea pig was immunized twice in a 5-month interval in the presence of incomplete Freund's adjuvant.

Immunoscreening by PEPSCAN. Synthesis of solid-phase peptides on polyethylene rods and immunoscreening with an enzyme-linked immunosorbent assay (ELISA) type of analysis were carried out according to established PEPSCAN procedures (11). A complete set of overlapping nonapeptides was synthesized by using the amino acid sequence data for VP1 of CPV from Reed et al. (28).

Absorption experiments. For absorption treatment, antisera were undiluted and MAbs were 200- or 5,000-fold diluted in phosphate-buffered saline. Absorption was carried out by incubating the antibody sample with intact full CPV virions overnight at 4°C at a concentration of 0.75 mg/ml. Controls consisted of phosphate-buffered saline instead of sample virus. In some cases, inactivated purified foot-and-mouth disease virus type A was used as a negative control. Afterwards, samples were tested by the aforementioned PEPSCAN procedures after appropriate dilution. Following these PEPSCAN analyses, the specimens were recollected, centrifuged to remove viral particles on 20% sucrose for 1 h at 30,000 rpm in a Kontron TST55.5 rotor, diluted 10-fold with tissue culture medium, and then tested for neutralizing activity.

Hemagglutination inhibition and neutralizing activity assays. Hemagglutination inhibition was carried out with pig erythrocytes according to established procedures (2). Neutralizing activity was determined by assaying the CRFK cell monolayer protection ability of an antibody sample as described previously (13).

Analysis of three-dimensional structural data. Coordinates of amino acid residues of capsid subunit VP2 as established by Tsao et al. (39) were kindly provided by M. G. Rossmann and M. S. Chapman, Purdue University, West Lafayette, Ind. Projection of the stereo picture of the VP2 polypeptide backbone and location of antigenic sites therein were elaborated with Sybyl molecular-modelling software (Tripos Associates, Inc., St. Louis, Mo.).

TABLE 1. Antisera and MAbs used in PEPSCAN analysis

Anti-CPV antibody source and code[a]	Neutralizing activity[b]	Hemagglutination inhibition[c]
Dogs, sera 1–6	+	+
Mouse (MAbs[d])		
4B8C6, 3C9, 3C10	+	−
3A6, 4EA8, 5F8A9	+	+
1F11, 2B4, 4AG6, 5BF7	−	−
Rabbits		
Serum 1	+	+
Serum 2	+	+
Guinea pig, serum 1	+	+

[a] Sera from dogs were from naturally infected animals; MAbs and polyclonal anti-CPV sera were from animals immunized with full CPV virions.
[b] Neutralizing titers of polyclonal sera were between 200 and 2,500, while those of MAbs with neutralizing activity also reached these values, except for MAb 3C10, which had a titer of 20.
[c] Hemagglutination inhibition titers were between 1,600 and 6,400
[d] These MAbs were the same as those described by López de Turiso et al. (13).

RESULTS

Antibodies. For the mapping studies, anti-CPV antibody samples from four different animal species were used: naturally infected dogs (six animals), a guinea pig and two rabbits immunized with CPV, and mice immunized with CPV (10 MAbs; Table 1). The polyclonal sera were all able to neutralize virus infectivity in the monolayer protection test and inhibit hemagglutination. Of the MAbs, six had neutralizing ability, and three of these six also inhibited hemagglutination.

PEPSCAN analysis. The mapping of antigenic sites was performed by PEPSCAN with all 740 overlapping nonapeptides that can be derived from the primary structure of VP1, which also covers the 584-residue sequence of VP2. Peptides were considered to represent antigenic sites if peaks occurred in a set and if at least one of the peaks in such a set reproducibly amounted to more than twice the background. Summarized in six scans, 10 sets of nonapeptides could thus be considered sites recognized by anti-CPV antibodies and were designated 1 through 10 (Fig. 1). The amino acid sequences covered by the peptides in these sites and the position numbers of these sequences in VP2 are presented in detail in Table 2 for each antibody specimen found to bind a certain site. Antigenic site 1 was recognized by three of six dog sera tested and was considered the same site, since each of these three dog sera bound peptides 163 through 166 most strongly (dogs 1, 2, and 3, Table 1). The amino acid sequence of this site starts at position 162 or 163 and ends with residue 174 or 175 of VP1 (Table 2). Since VP2 starts at residue 165 of VP1, 10 or 11 amino-terminal residues of VP2, including the (core) sequence SDGAVQ shared by all peptides, must be present in site 1. Sites 2 and 3 are located only seven or eight positions further from the amino terminus of VP2; these sites bound rabbit 1 antibody and murine MAb 3C9, respectively. Antigenic site 4 and sites 5 and 9 all bound antibodies from one dog (dog 4). Exactly the same peptides of site 6 bound both MAb 4AG6 and the weakly neutralizing MAb 3C10. Site 7 directly follows the carboxy-terminal end

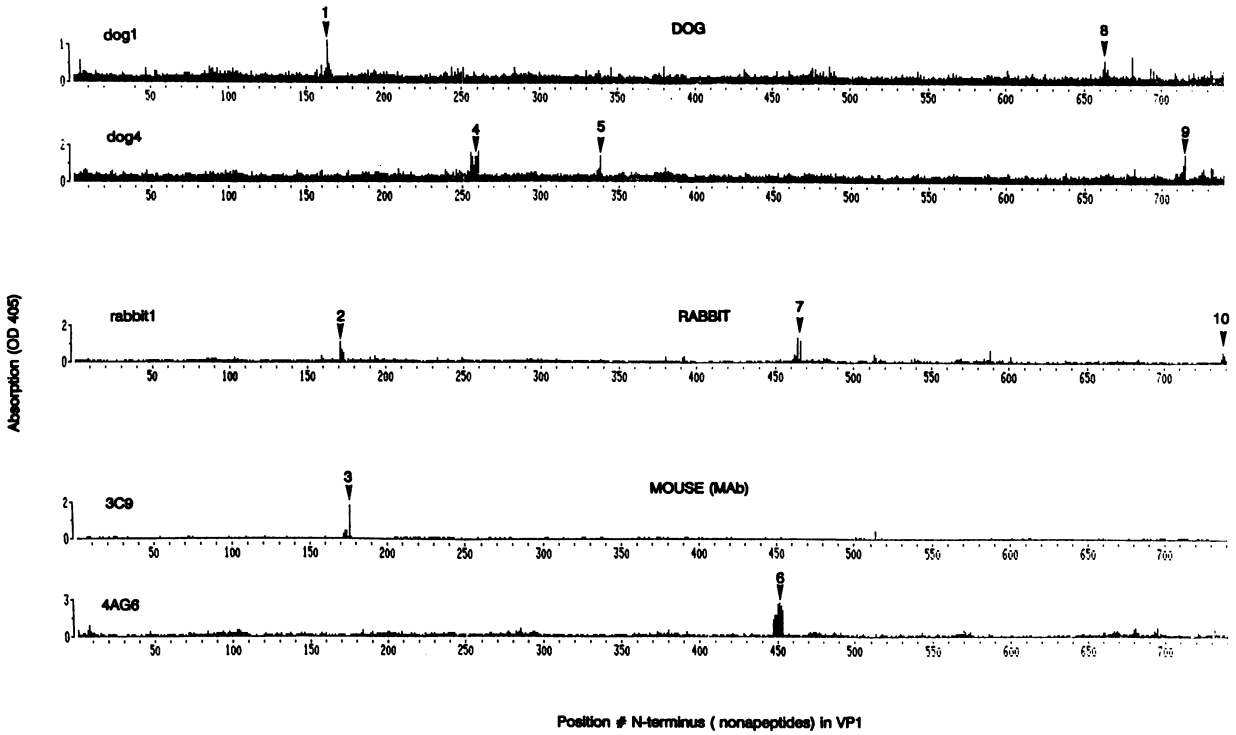

FIG. 1. Mapping of antigenic sites in VP1 of CPV by PEPSCAN analysis. ELISA of all 740 overlapping (immobilized) nonapeptides of VP1 of CPV with selected antibody samples of dog, rabbit, and murine origin. The code for antibody samples at left above each scan follows the nomenclature in Table 1. Arrowheads indicate the locations of peptide sets (antigenic sites) which were reproducibly recognized by antibody and are further described with respect to their sequences in Table 2. Above the arrowheads, antigenic sites are numbered in order of occurrence from amino to carboxy termini along the coat protein and are summarized in Fig. 2A. OD 405, optical density at 405 nm.

of site 6. Antigenic site 7 was recognized by antibody from rabbit 1. This same rabbit serum also bound peptides near the carboxy terminus of VP2 (site 10). Antigenic site 8 was recognized by antibodies from dog 1. In the following, these 10 antigenic sites will consecutively be discussed with respect to features of linear and spatial distribution, cross-reactivity with whole CPV virions (blocking experiments), significance for virus neutralization, and surface distribution and exposure.

Linear and spatial distributions of antigenic sites. The linear distribution of these 10 antigenic sites was related to structural features of the VP2 molecule as established by Tsao et al. (39) (Fig. 2). Since the antigenic sites were found only in the VP2 region of the sequence, position numbering for the VP2 sequence will be applied from now on. Sites 1 through 3 occur within the first 37 amino-terminal residues of VP2, for which no structural information is available (Fig. 2A). Antigenic site 4 is located on loop 1, which extends far from the center of the virus. Site 5 is part of one of the β strands (strand βE) that form the eight-stranded β-barrel structure. Sites 6 and 7 are positioned in the tip of loop 3. Residues 300 through 306 of site 7 are among the most protruding residues of this loop. Site 8, like site 5, forms part of the eight-stranded β-barrel (strand βH). Sites 9 and 10 are present in the carboxy-terminal region of VP2. The spatial occurrence of seven antigenic sites, sites 4 through 10, is shown in a stereo picture of a wire model of the peptide backbone of VP2 (Fig. 2B).

Blocking antibody binding with CPV. The cross-reactivity of full CPV virions with antigenic peptides was investigated to obtain experimental evidence for the surface-exposed nature of the sites on VP2 in CPV. To this end, antibody samples were incubated with whole virions (or buffer as a control) to see whether CPV can block antibody binding to the respective antigenic sites in PEPSCAN. The results (Fig. 3) showed that antibody binding to the three amino-terminal sites of VP2 (sites 1 through 3) can be blocked by CPV virions (Fig. 3A). Other antigenic sites for which a blocking effect was observed were sites 4 (loop 1) (Fig. 3B) and sites 6 and 7 (both loop 3) (Fig. 3C). Remarkably, only the binding of MAb 3C10 but not that of MAb 4AG6 to site 6 was inhibited by CPV, which suggests that these two MAbs bind differently to CPV. This can explain the different neutralizing properties of the two MAbs (Table 1). The binding of rabbit antibodies to site 7 was reduced but not completely abolished by the presence of CPV virions. Binding of antibodies to sites 5, 8, 9, and 10 was unaltered by absorption treatment with CPV (sites 8 and 10 not shown). The blocking effect of CPV was specific, since in the sera that recognize more than one site (e.g., dog 4, Fig. 3B), binding to one site was blocked and binding to the other(s) remained unaltered. The effect of the blocking treatment was further substantiated by testing the absorbed antibody samples in a monolayer protection assay. Dog sera 1, 2, and 4 (Table 1) lost their neutralizing capacities, while three other neutralizing-antibody samples (from rabbit 1 and MAbs 3C9 and 3C10) appeared not to be suitable for such an experiment, since the controls became too dilute to show an effect (data not shown). Thus, from these blocking experiments in PEPSCAN and neutralization assay, it can be concluded that of

TABLE 2. Amino acid sequences of nonapeptide sets with sequences derived from the coat protein of CPV and recognized by anti-CPV antibodies in PEPSCAN of VP2 of canine parvovirus[a]

Antigenic site	Antibody sample		Amino acid sequence of epitope site[b]
1	Dog 1	(-2)	A P M S D G A V Q P D G
	Dog 2	(-3)	L A P M S D G A V Q P D G G
	Dog 3	(-2)	A P M S D G A V Q P D G
2	Rabbit 1	(7)	Q P D G G Q P A V R N
3[c]	MAb 3C9	(8)	P D G G Q P A V R N E R A T
4	Dog 4	(91)	A V N G N M A L D D I H A Q
5	Dog 4	(172)	S L M V A L D S N N T M
6	MAb 4AG6	(283)	R A L G L P P F L N S L P Q S
	MAb 3C10	(283)	R A L G L P P F L N S L P Q S
7	Rabbit 1	(297)	S E G A T N F G D I G V Q Q
8	Dog 1	(498)	L F V K V A P N L T N E
9	Dog 4	(549)	Q Q M S I N V D N Q F
10	Rabbit 1	(573)	Y E K Q L A P R K L Y

[a] Antigenic site numbers and antibody sample codes follow the nomenclature of, respectively, Fig. 1 and Table 1.

[b] Amino acid sequence of peptide set recognized in PEPSCAN (Fig. 1) by the respective antibody sample. Shaded sequences are core residues, i.e. residues which are shared by all peptides of the site; underlined sequences are surface residues deduced from reference 39 and projected onto the surface map in Fig. 4. Numbers in parentheses indicate positions of the amino-terminal amino acids of the sites in VP2; VP2 starts at residue 165 of VP1 in the sequence published by Reed et al. (28). A minus sign before the number means that the amino terminus of the site occurs in the VP1-specific region at the indicated position downstream of the amino terminus of VP2.

[c] This site is comparable to the one (GQPAVRNERATGS) described be López de Turiso et al. (13) for MAb 3C9.

the 10 antigenic sites mapped here, 6 might represent neutralizing epitopes, while sites 5, 8, 9, and 10 do not have any neutralizing property.

Surface residues of antigenic sites. The absorption experiments described above illustrated that 6 of the 10 antigenic sites on CPV were accessible for antibodies that bind to peptides in PEPSCAN. However, close inspection of the reported structural data (39) reveals that except for site 5, each antigenic site contains amino acid residues that are exposed at the viral surface (Table 2). The two-dimensional "road map" in Fig. 4 shows the distribution of the mapped antigenic sites on the surface of the viral capsid within one icosahedral unit. While no structural information is available for the amino-terminal region of VP2 that contains antigenic sites 1 through 3, the reported 8-Å (0.8-nm) space in the cylindrical structure around the fivefold axis should allow exit of one (amino-terminal) chain of VP2 per five icosahedral units. From our results, the external exposure of this amino terminus is evident in view of the fact that these three sites occur in close proximity to each other, antibody binding to them could be blocked by CPV virions, and one of these sites is recognized by a neutralizing MAb (3C9; see above). Which of the constituting amino acids in this region are the accessible residues for antibody binding in the infective virus remains unclear. The surface-exposed residues of antigenic site 4 are loop 1 residues Ala-91, Val-92, Asn-93, and Asn-95, which occur on the tip of a protruding area of the viral capsid, the so-called threefold spike. Six of the carboxy-terminal amino acid residues of antigenic site 6 (Asn-292, Ser-293, Leu-294, Pro-295, Gln-296, and Ser-297) are exposed (Table 2). These residues occur in that region of loop 3 that forms part of the ridge of a deep cleft ("canyon") on the viral surface. Neighboring site 7 occurs with most of its residues surface exposed (Ser-297, Glu-298, Gly-299, Ala-300, Thr-301, Asn-302, Phe-303, Asp-305, Gly-307, Val-308, and Glu-309), forming a big proportion of loop 3, which touches the wall of another depression in the viral surface, indicated in Fig. 4 as "dimple." Thus, 16 amino acid residues of loop 3 appeared to represent the exposed residues of antigenic sites 6 and 7. Antigenic site 8 contains three surface residues (Thr-507, Asn-508, and Glu-509). These residues are part of the ridge of the canyon but on the opposite side of this cleft compared with those of site 6. The surface-exposed residues of antigenic sites 9 (Glu-549, Glu-550, Met-551, Ser-552, Asn-554, Val-555, Asp-556, and Asn-557) and 10 (Tyr-573, Glu-574, and Lys-575) are all located in the dimple area. However, this domain on the intact virion was not able to bind antibodies (see above).

DISCUSSION

Ten antigenic sites in the VP2 subunit of CPV were mapped by using synthetic solid-phase nonapeptides and antibody samples from three different animal species: dog, rabbit, and mouse. Blocking experiments with full virions together with recently acquired knowledge of the capsid structure of CPV (39) have given the basis by which to evaluate which of these sites are of significance for development of a second-generation vaccine.

The amino terminus of VP2 was shown to be an immunogenic domain which is exposed at the viral surface and can elicit neutralizing antibodies. Antibody samples from different animal species, including sera from three naturally infected dogs, recognized this domain. Although the antibodies from each species exhibited different specificities, as exemplified by the core residues of their respective antigenic sites (designated 1 through 3 in this study), the three sites occur in a short stretch of 21 amino acid residues (Fig. 5). With respect to neutralizing MAb 3C9, the present study with synthetic overlapping nonapeptides shows that the core of its epitope occurs from positions 12 through 16 of VP2. This location quite nicely corresponds with the region 11 through 23 reported before by López de Turiso et al. (13), who applied MAb 3C9 to different expression products of VP1. Binding to antigenic sites 1 through 3 by antibody samples could be blocked with whole CPV virions. The latter observation is proof of the external location of at least some of the amino termini of VP2 in full particles. No defined structural information for the 37 amino-terminal residues of VP2 has been obtained from X-ray analyses because of lack of structural order in this region (39). It has been argued by Tsao et al. (39) that there is room for one (amino-terminal) chain to extend through the cylindrical channel at the fivefold-symmetry axis. Yet, the domain might have an enhanced degree of structural organization, since calculations with algorithms for secondary structure revealed that the amino terminus of VP2 possesses a high propensity to form a β turn at positions 7 through 11 (Fig. 5).

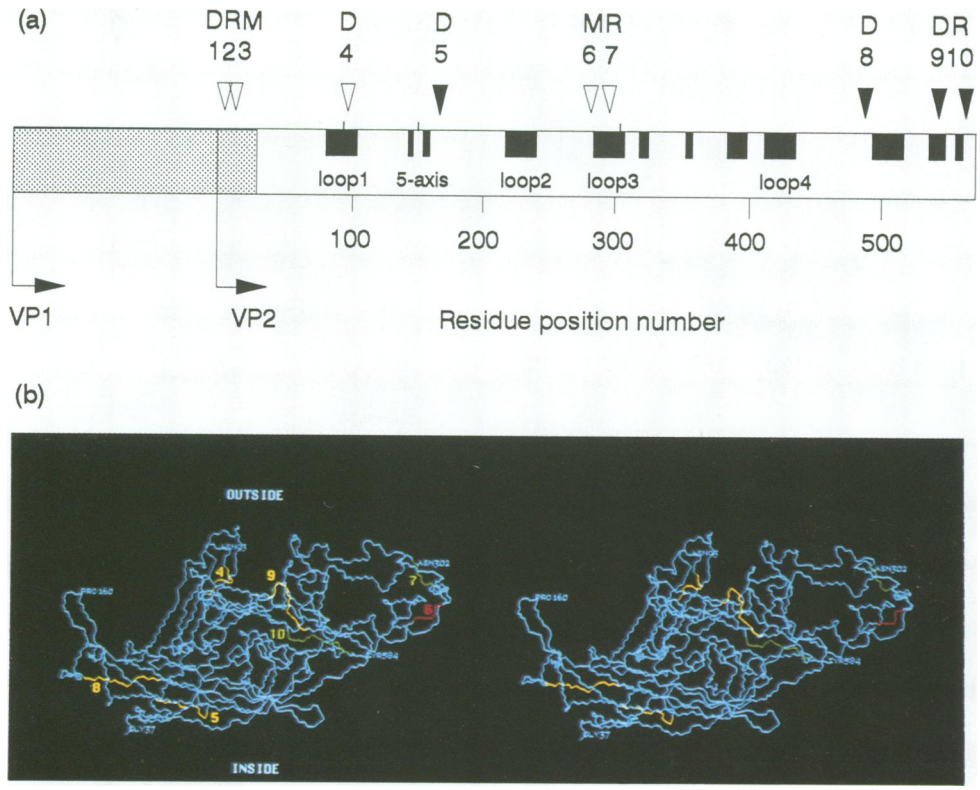

FIG. 2. Representation of the antigenic sites in relation to several structural aspects of VP2. (a) Correlation of linear occurrence to structural features such as loops and to surface. Numbers above bar diagram correspond to positions of antigenic sites of Fig. 1 and Table 2. Black blocks indicate relative locations of surface-exposed residues, including those of loops 1 through 4 and the loop constituting the cylindrical protrusions around the fivefold-symmetry axes of CPV (39). Shaded blocks are the amino termini of VP1 and VP2, for which no structural information was obtained in the X-ray analyses (39). Residue positions follow the numbering from the amino terminus of VP2 (position 1 of VP2 corresponds to residue 165 of VP1). D, R, and M indicate, respectively, dog, rabbit, and murine origins of antibody reactive with the corresponding sites. Open arrowheads, antigenic sites for which antibody binding can be blocked by CPV virions (Fig. 3). (b) Stereo model of the polypeptide backbone of the VP2 subunit, exhibiting spatial locations of the core residues of antigenic sites 4 through 10. The yellow, red, and green fragments represent the core residues of antigenic sites 4 through 10 recognized by the respective dog, mouse, and rabbit antibodies. Numbering of sites corresponds to that in panel a. Five labels are added to indicate the following amino acid residues of VP2: Gly-37 and Tyr-584 as amino- and carboxy-terminal residues of the model, Asn-93 as part of the tip of loop 1, Pro-160 as part of the tip of the loop forming the cylindrical structure around the fivefold axis of CPV, and Asn-302 occurring in loop 3. The orientation of the protein backbone is similar to that of the model in Fig. 2 of Tsao et al. (39). INSIDE and OUTSIDE roughly illustrate the orientation of the polypeptide in the capsid of CPV.

For other parvoviruses, the surface location of the amino terminus of VP2 in full capsids and not in empty particles has been shown by treatment with trypsin of closely related parvoviruses H-1 and minute virus of mice (7, 19, 37). The spatial orientation of the amino terminus of VP2 in recombinant capsids expressed in the baculovirus system (12, 33) is still to be investigated. Also, there is evidence that for VP2 to be infective, about 20 amino acids have to be cleaved away from the amino terminus of VP2, resulting in the capsid protein VP3 (7, 8, 17). The effect of neutralizing antibodies which bind to the amino terminus of VP2 of CPV could very well be hindrance of a proteolytic cleavage that unmasks a viral domain essential for infection.

In the blocking experiments, two other domains of VP2 were shown to be accessible for antibodies from neutralizing polyclonal and MAb samples. For both loop 1 (containing antigenic site 4) and loop 3 (with antigenic sites 6 and 7), the binding of antibodies could be competed for by full CPV virions. These loops have their tips in the most protruding regions on the capsid surface, the so-called spike around the threefold-symmetry axis (39). Antigenic site 4 was recognized by a canine serum with neutralizing capacity (dog 4, Table 1). Upon incubation of this serum with CPV virions, not only was antibody binding to the site 4 peptides blocked, but also its neutralizing activity was lost. One of the surface residues in this site, Asn-93, had previously been shown to be possibly involved in binding of a neutralizing murine MAb directed against CPV (MAb 14 in reference 22). Loop 1 might therefore represent another domain for virus neutralization. Antigenic site 6 was recognized by two MAbs, of which one (3C10) exhibits a weak neutralizing activity, while the other (4AG6) does not. The two MAbs have different orientations on and/or affinities for the site on CPV in such a way that in PEPSCAN, only 3C10 antibodies are blocked by the virus. The former option might be the correct one, since the reactivities of these two MAbs are apparently equal as judged from immunoglobulin G titers in ELISA (13) combined with similar absorbancy values obtained in PEPSCAN with both MAbs at high dilutions and equal immunoglobulin G concentrations (not shown). Antigenic sites 6 and 7 both form part of loop 3 of VP2 and occur at the edge of two depressions on the viral capsid: canyon and dimple. The

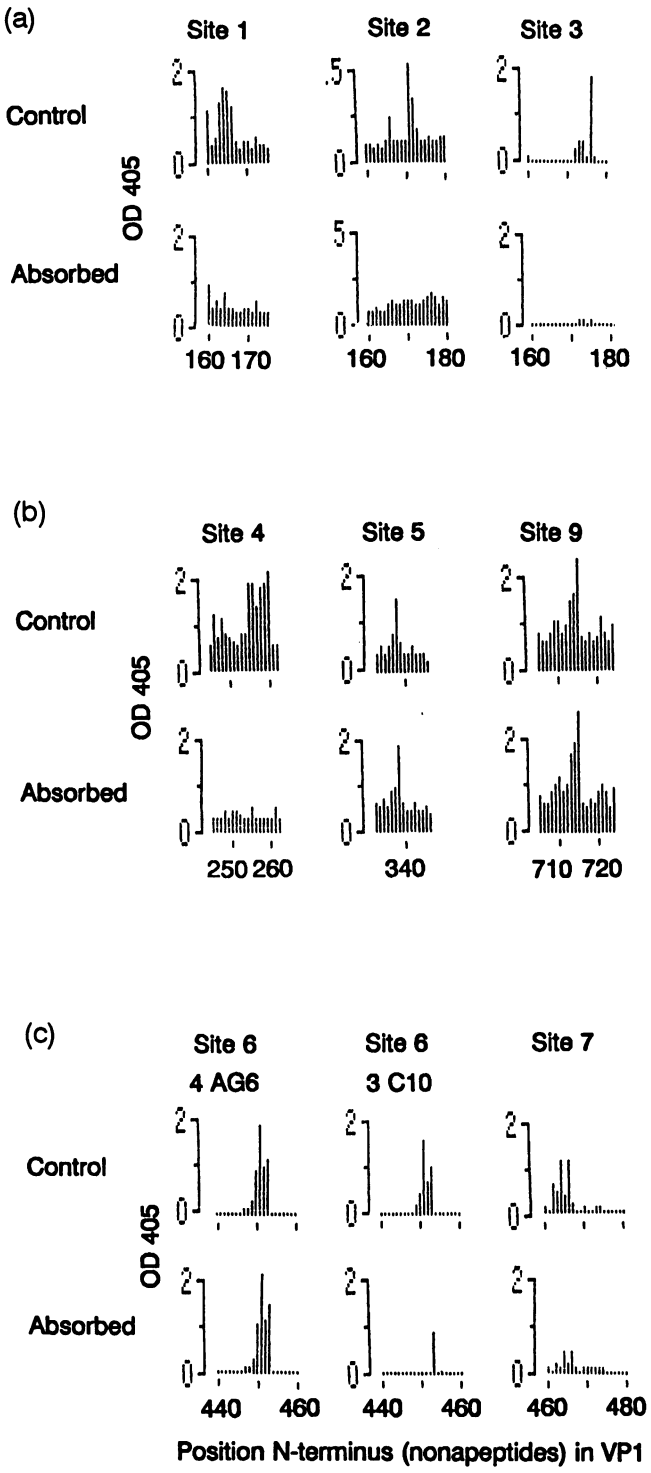

FIG. 3. Blocking of antibody binding by CPV virions to nonapeptides in PEPSCAN analyses. The nonapeptide sets around the antigenic sites depicted in Fig. 1 were incubated with the antibody samples binding to these selected peptides. For each antigenic site, the optical density pattern is shown for nonapeptides incubated with antibody treated with phosphate-buffered saline (control) or virions (absorbed). (a) The three sites of the amino-terminal domain of VP2 (for site 1, serum from dog 2 was used); (b) the three sites recognized by serum from dog 4; (c) sites 6 and 7 from the loop 3 region. OD 405, optical density at 405 nm.

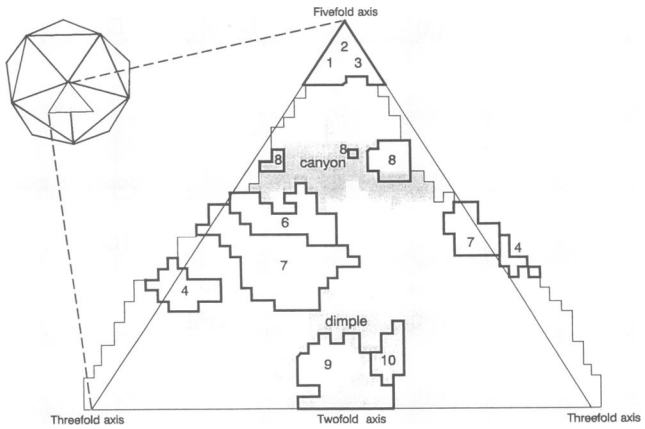

FIG. 4. Distribution of antigenic sites in CPV capsids with respect to capsid surface profile. The exposed portions (underlined residues in Table 2) of the sites are depicted in the surface road map adapted with permission from Tsao et al. (39). Numbers inside heavily outlined boxes indicate antigenic site numbers as used in Fig. 1; numbers for sites 1 through 3 at the fivefold axis are placed where the amino termini of VP2 subunits are supposed to extend from the capsid. The area of the surface map is contributed to by more than one VP2 subunit, which is illustrated by the splitting of some antigenic sites (e.g., sites 7 and 4). The approximate locations of canyon and dimple are shaded. The blowup is intended to illustrate the orientation of the road map on the icosahedral surface.

floor of the canyon has been proposed to be a site for receptor binding at host cell surfaces (32). Though the canyon probably is too narrow for antibodies to reach the receptor attachment site, antibody binding to a site(s) at the canyon edge has been shown to interfere with viral infectivity in picornaviruses like human rhinovirus (31, 34, 35). For site 7, at least one of two residues of VP2 (Ala-300 and Thr-301) was important for binding of a neutralizing MAb specific for both CPV and FPLV (MAb 8 in reference 24). Thus, loops 1 and 3 of VP2 appear to have several properties important for antiviral antibodies: they are immunogenic, have surface-exposed residues, and are recognized in antibody samples with neutralizing properties.

Binding of antibodies to several synthetic antigenic sites of VP2 (sites 5, 8, 9, and 10 and site 6 for MAb 4AG6) could not be blocked by full CPV virions (Fig. 2A and 3). Since the antibodies had developed by immunization with whole CPV virions either during viral infection in the natural host (dog)

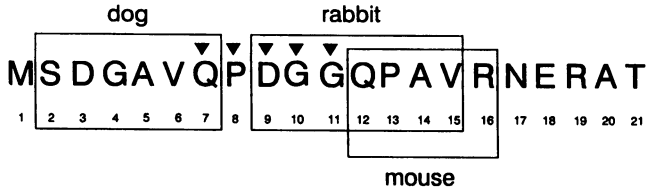

FIG. 5. Outline of residues in the amino-terminal region of VP2 of CPV with respect to location of antigenic sites 1 through 3 of Fig. 1. The core of the site for each species source of the binding antibody is boxed. For the tetrapeptide sequences QPDG and PDGG (indicated by ▼), a high propensity to form a β turn was calculated according to the algorithm of Chou and Fasman (5), with probability values of bend occurrence [$P(t)$] of 6.06×10^{-4} and 3.24×10^{-4}, respectively. Position numbers are indicated for the sequence of VP2.

or by injection with whole CPV virions in experimental animals (mouse and rabbit), these sites must have been generated during denaturation and/or processing of the virus in the animal rather than by capsids in the native state. Like site 5, such antigenic sites can even be located inside the capsid, as was shown, e.g., by immunizing experimental animals with synthetic peptides deduced from VP1 of poliovirus (6, 9, 31).

In conclusion, three different immunogenic neutralization sites appear to exist in CPV. The amino terminus of VP2 might well be a region involved in an infective process. Loops 1 and 3, on the other hand, are possible sites for attachment of neutralizing antibodies, which can interfere with binding between virus and cell surface receptor. Since the amino terminus of VP2 was recognized in sera from several dogs (as well as by a rabbit anti-CPV antiserum and a murine anti-CPV MAb), this region is of special interest for inclusion in a new-generation vaccine.

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