Topographical analysis of canine parvovirus virions and recombinant VP2 capsids

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The distribution of epitopes defined by monoclonal antibodies (MAbs) on the surface of canine parvovirus (CPV) virions and recombinant VP2-capsids was established using immunoelectron microscopy. A correlation appeared to exist between the linear position, neutralizing activity and immunogold staining. Both viral capsids and recombinant capsids gave similar patterns of immunostaining. The neutralizing MAbs that recognized epitopes not previously identified by Pepscan or immunoblotting gave a clear staining. However, MAbs 3C9 and 3C10, identified by Pepscan and immunoblotting as recognizing linear epitopes, did not show any labelling (3C9) or only scattered labelling (3C10). MAb 3C9 recognizes an N-terminal domain of VP2. MAb 4AG6, which recognizes the same linear epitope as 3C10, did not bind to the capsids, indicating a different orientation. An immunofluorescence assay was performed to supplement the B cell epitope characterization. In contrast to other MAbs that gave nuclear and cytoplasmic staining, MAb 3C9 gave a preferential nuclear staining. Based on these results, it is hypothesized that the N terminus of VP2 is barely, or not at all, exposed on the surface of the native virions, but becomes accessible after some virion steric change (e.g. after attachment to the cell receptor).

Canine parvovirus (CPV) belongs to the feline parvovirus subgroup of the genus Parvoviridae (Siegl et al., 1985). CPV capsids have icosahedral symmetry and are composed of 60 copies of structural protein, mostly VP2 with some VP1. VP2 is the N-terminal truncated form of VP1. In DNA-containing particles some VP2 molecules undergo an N-terminal proteolysis, producing VP3 (Cotmore & Tattersall, 1987; Paradiso et al., 1984).

Recently, X-ray diffraction of CPV-2 has been reported (Tsao et al., 1991). Crystallographic analysis of CPV shows features similar to those obtained for the genus picornavirus. Among them is an eight-stranded β-barrel structure that forms a third of the whole protein. This β-barrel structure lies below the capsid surface, which is formed by four different loops. The N-terminal 37 residues of the VP2 molecule have not been well characterized owing to its structural disorder. It has been proposed that this N terminus could extend along the fivefold axis. Between the latter and the great spike of the threefold axis there is a deep depression or canyon which, by analogy with picornaviruses, might contain attachment sites for cellular molecules used as viral receptors.

Epitopes for antibodies have been mapped on the N terminus (López de Turiso et al., 1991; Langeveld et al., 1993), the fivefold cylindrical structure (Rimmelzwaan et al., 1990) and on the great protrusion or spike of the threefold axis (Langeveld et al., 1993; Parrish, 1991). Also, very recently two different groups (López de Turiso et al., 1992; Saliki et al., 1992) have reported the construction of empty CPV capsids derived from the expression of VP2 in insect cells using recombinant baculoviruses. In both cases, these VP2 capsids were used successfully to induce protection in dogs against virulent CPV challenge.

The reactivity pattern obtained by immunoblot staining was identical for the virions and the recombinant capsids. However, no topographical analysis has been made to evaluate the structural differences, if any, between CPV virions and recombinant capsids. It should be remembered that the recombinant capsids contain neither VP1 nor VP3, which are present in the DNA-containing capsids. To obtain information on possible structural or conformational differences we have used immunoelectron microscopy to analyse the distribution of epitopes on the surface of both types of capsids. Also, exploiting the advantage of having monoclonal antibody (MAb) 3C9 which recognizes an N-terminal epitope, we have studied the surface location of the N terminus of the CPV capsid.
Table 1. Immunological characterization of CPV and VP2 capsids with MAbs

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<tr>
<th>MAb</th>
<th>Immunoblotting</th>
<th>Immunofluorescence</th>
<th>Neutralizing activity</th>
<th>Defined epitope*</th>
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<td>VP2 capsids</td>
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* By Pepscan or immunoblotting.

VP2 protein and its involvement in the mechanism of neutralization by MAb 3C9.

Ten CPV-specific MAbs (López de Turiso et al., 1991) were used to characterize CPV virions and recombinant VP2 capsids topographically. CPV and VP2 capsids were purified as previously described (López de Turiso et al., 1991, 1992). To carry out the immunolabeling, samples were adsorbed onto collodion–carbon-coated nickel grids, previously treated with glow-discharge, by floating for 2 to 3 min. They were then washed with PBS and Tris-buffered saline (TBS: 150 mM-NaCl, 50 mM-Tris–HCl pH 8.2) for another 2 min. Samples were then incubated, first with TBG (TBS, 0.1% BSA and 0.1% gelatine pH 8.2) for 15 min, and second, with a 30-fold dilution of the respective MAb in TBS containing 1% BSA, for 50 min. The grids were washed again with TBG for 10 min and incubated with a 15% dilution of 5 nm gold–Protein A or goat anti-mouse IgG/IgM (Janssen) in TBS plus 1% BSA for 30 min. Finally, samples were washed consecutively with TBG (5 min), TBS (5 min) and PBS (2 min), fixed with 2% glutaraldehyde in PBS for 2 min and stained with 0.5% uranyl acetate in water for 5 min. Observations were made with a Jeol 100B electron microscope at 80 kV.

The results obtained are summarized in Table 1 together with other immunological data. Fig. 1 shows immuno electron micrographs of CPV and VP2 recombinant capsids treated with different MAbs. Each of these MAbs represents a different pattern of immunolabeling. Only three MAbs, 4B8, 4EA8 and 5F8, gave a positive pattern of immunogold labeling with both types of capsids. These three MAbs were also good neutralizing antibodies. However, the epitope recognized by the three MAbs had not been previously identified. The type of staining obtained with these MAbs is shown in Fig. 1 for 4EA8. All the viral particles and capsids are homogeneously surrounded by several gold particles. Another MAb, 3C10, which neutralized more weakly than the other three MAbs, gave some labeling in a small percentage of the particles, indicating that accessibility for the immunoglobulins to this epitope was low. MAb 4AG6 showed some labelling but it was only to aggregated proteinaceous material. It is notable that both MAbs 3C10 and 4AG6 recognize the same epitope (Langeveld et al., 1993). This epitope is located on the edge of the canyon, which surrounds the cylindrical structure of the fivefold axis. That these two MAbs show different properties regarding viral neutralization and immunogold labeling (Table 1), indicates that they must recognize this epitope with different orientations. The orientation of 3C10 must partially cover the canyon, because it is supposed, from homology with the structure of picornaviruses (Rossmann & Palmenberg, 1988), that the floor of the canyon is involved in the binding of virus to the cell receptor. Therefore, our hypothesis is that 3C10 neutralizes the virus by preventing the attachment of the virus to the cell receptor. Since the neutralizing ability of this MAb is weaker than that of the other neutralizing MAbs, this inhibition must be only partial.

The rest of the MAbs did not show any gold binding to the capsids or virions. Most of them are not neutralizing. However, it is interesting that the neutralizing MAb 3C9 showed completely negative results. This result was surprising, since the epitope recognized by MAb 3C9 is located on the N terminus (amino acids 11 to 23) in VP2, immediately adjacent to the Gly-rich domain. It has been generally accepted that the N terminus of VP2 is exposed on the capsid surface because it is susceptible to proteolytic cleavage to produce VP3. The N terminus is supposed to extend along the fivefold axis outside the capsid surface. In fact, the reactivity against this epitope was eliminated when the MAb was absorbed with full virions (Langeveld et al., 1993). However, a high dilution (1:5000) of the MAb was necessary. One explanation could be that there are only a few N termini of VP2 outside the virion, one per fivefold axis. Therefore, the available binding sites are relatively low in number when compared to, for example,
Fig. 1. Immunoelectron micrographs of CPV virions and VP2 recombinant capsids labelled with different anti-CPV MAbs. Five nm gold-conjugated goat anti-mouse IgG + IgM was used as the labelling reagent.
those for MAb 5F8. An alternative explanation for this result could be that a domain of the N terminus is positioned within the canyon, leaving it inaccessible to the antibodies. This is possible considering distances and sizes. Once the virus attaches to the cell, the N terminus is extruded from the canyon, becoming accessible to antibodies. A similar situation has been reported for poliovirus; after attachment of the virus to the cell, VP4 and the N terminus of VP1 are extruded (Filman et al., 1989). Also, VP4 contains a myristoyl group, which is essential for assembly of infectious particles (Moscufo et al., 1991). The polyglycine group of paroviral VP2, which is adjacent to the 3C9 epitope, has been ascribed a comparable cell membrane-insertion role, although this remains hypothetical. Also, very recently, the relevance of the RVER domain of the VP2 in cell binding and viral infection has been demonstrated by Tullis et al. (1992) in minute virus of mice.

Fig. 2 shows immunoelectron micrographs of CPV virions and VP2 recombinant capsids treated with two
different anti-CPV sera (mouse and rabbit). These positive controls gave the expected results. A roughly homogeneous distribution of gold particles around virions and capsids is obtained with the specific sera. No labelling was obtained in the case of the negative controls (goat anti-mouse and protein A–gold-labelled, respectively).

To gain further insight into the epitope topography of viral particles, we performed an indirect immunofluorescence analysis of CPV-infected CRFK cells. Cells were grown on culture chamber slides (LabTek; Nunc), incubated for 48 h at 37 °C and in 5% CO₂ and then infected with CPV and incubated for a further 48 h. When the first signs of infection were visible, an indirect immunofluorescence assay was performed as previously described (Casal et al., 1984). Briefly, cells were fixed with a 50% (v/v) mixture of methanol–acetone at −20 °C. They were then rehydrated and incubated with the corresponding MAb (undiluted) culture supernatants for 60 min at room temperature, washed with PBS–0.25% BSA and then stained with fluorescein-labelled rabbit anti-mouse antibodies (Sigma) diluted 20-fold in PBS–0.25% BSA for another period of 45 min. Finally, slides were washed with PBS, dried and mounted with 90% glycerol–PBS.

Fig. 3 shows the different types of immunofluorescence obtained with the MAbs. Again, we obtained similar staining with the same groups of MAbs: 4EA8, 4B8 and...
5F8 gave the same pattern. They stained nuclei and, more intensely, cytoplasm. 2B4, a non-neutralizing MAb, gave a similar pattern. However, 3C9 gave an intense nuclear fluorescence. 3C10 and 4AG6 gave a very weak fluorescence, almost negative, diffuse in the cytoplasm and mottled in the nucleus. 3A6, an IgM MAb, gave a periplasmic fluorescence, probably due to its large size. 5BF7 gave negative results.

CPV, an autonomous parvovirus, replicates in the nuclei of infected cells. Also, the assembly of the virion occurs in the nuclei. The fact that 3C9 preferentially recognizes antigens in the nuclei, where the viral capsomers are assembled, confirms the low accessibility of this epitope in intact capsids. However, the other three neutralizing MAbs preferentially recognize assembled capsids. These data may shed some light on the mechanism of virus neutralization by 3C9. It could interact with the process of decapsidation of the virus after attachment to the cell.

There are several conclusions to be drawn from these data. Firstly, and most important, the antigenic similarity between recombinant capsids and full CPV virions was established. This explains the high immunogenicity of these capsids when used to vaccinate dogs against virulent virus challenge (López de Turiso et al., 1992). Secondly, the non-linear epitopes recognized by neutralizing MAbs 4EA8, 5F8 and 4B8 are exposed on the surface of the capsid. Thirdly, the N terminus of VP2 appears not to be fully exposed on the surface of the capsid, at least when the virus is in the natural state, because 3C9, which recognizes an epitope on the N terminus, is not able to label either virions or recombinant capsids. However, some domain of this N terminus must be exposed sometimes outside the virion to allow binding to virus by 3C9 and the proteolytic cleavage of VP2 to VP3. It has been hypothesized that this exposure might occur when the virus attaches to the cell receptor.

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References


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