Mapping of linear epitopes on the capsid proteins of swine vesicular disease virus using monoclonal antibodies

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The antigenic linear map of swine vesicular disease virus (SVDV) has been studied using a repertoire of monoclonal antibodies (mAbs) raised against a recombinant SVDV polyprotein, P1. Peptide-scanning analyses, cross-reactivity studies with homologous and heterologous viruses and predicted location on a computer-generated three-dimensional model of the capsid proteins have allowed the identification of five main linear sites. Two sites, the N terminus of VP3 and amino acids 51–60 on VP1, correspond to internal areas, conserved not only between SVDV isolates but also in the related enterovirus coxsackievirus B5. In contrast, three other regions, amino acids 142–161 of VP2, 61–70 of VP3 and the C terminus of VP1, are exposed on the external face of the capsid and subjected to antigenic variation, even among different SVDV isolates. Further minor sites that were antigenically conserved were identified on VP4. In contrast with conformational sites described previously, none of the linear epitopes identified in this work is involved in neutralization of virus infectivity and post-infection swine sera did not inhibit the binding of mAbs with the relevant epitopes. Both of these observations suggest that linear epitopes are poorly immunogenic in pigs. The characterization of linear sites has contributed to a better understanding of the antigenic structure of SVDV and mAbs used to this purpose may provide a useful tool for the improvement of diagnostic methods, such as antigen detection systems, and analyses of the antigenic profile of SVDV isolates.

Introduction

Swine vesicular disease (SVD) is a highly contagious pig disease that causes lesions indistinguishable from those caused by foot-and-mouth disease (FMD). The disease appeared first in Italy in 1966 (Nardelli et al., 1968) and subsequent outbreaks have occurred in Europe and Eastern Asia. SVD is caused by the enterovirus SVD virus (SVDV), which is related to the human pathogen coxsackievirus B5 (CV-B5) (Graves, 1973; Brown et al., 1973; King et al., 2000). It has been suggested that SVDV could have arisen by a single transfer of CV-B5 into pigs (Zhang et al., 1999). Although SVDV occurs as a single serotype, epitope mapping by monoclonal antibodies (mAbs) and sequence analyses have enabled researchers to classify isolates into four distinct antigenic/genetic groups (Brocchi et al., 1997).

The SVDV particle consists of a non-enveloped, icosahedral capsid comprising 60 copies each of the four viral proteins, VP1 to VP4. These structural proteins originate from the processing of the precursor polyprotein P1. During morphogenesis, the polyprotein is cleaved by a virus-encoded proteinase, forming the so-called protomer, which comprises VP0 (VP4 + VP2), VP3 and VP1. Five protomers assemble to form a pentamer and 12 pentamers associate with the viral RNA molecule to form the virus particle.

Using mAbs, several antigenic sites involved in neutralization of SVDV have been identified on the capsid surface (Kanno et al., 1995; Nijhar et al., 1999; Rebel et al., 2000; Borrego et al., 2002). Only one of these sites is shared by CV-B5, while the others are all SVDV-specific. All the antigenic sites described are conformation-dependent and, therefore, discontinuous epitopes are considered to be immunodominant in the immune response to SVDV. For other picornaviruses, such as FMD virus, Theiler’s murine encephalomyelitis virus, hepatitis A virus, poliovirus and other enteroviruses, linear
sites have also proven to be involved in the humoral immune response (Roivainen et al., 1991; Inoue et al., 1994; Bosch et al., 1998; reviewed by Usherwood & Nash, 1995; Mateu, 1995). In the case of SVDV, linear sites recognized by post-infection pig sera have been identified by a peptide-scanning technique (Jiménez-Clavero et al., 2000) and by immunoblotting (E. Brocchi, unpublished results) but their role in the immune response is less known. Furthermore, a recombinant, bacterially expressed and denatured SVDV polyprotein, P1, capable of presenting linear antigenic sites, is recognized by sera from infected pigs and is able to induce an SVDV-specific cellular and humoral immune response in pigs (Jiménez-Clavero et al., 1998).

In this paper, we have followed a different approach to study the linear antigenic map of SVDV, based on the production and characterization of mAbs against linear epitopes. Considering that the precursor polyprotein P1 should present linear epitopes better than the assembled capsid proteins, we have biased the production of mAbs to linear rather than conformational epitopes by immunizing mice with the recombinant, bacterially expressed protein P1. Results obtained with more than 60 mAbs have now enabled us to define the antigenic linear map of SVDV and to analyse the location, variability and immunogenicity of these linear epitopes.

Methods

- **Synthetic antigens.** The recombinant SVDV P1 polyprotein, expressed in *Escherichia coli* as a fusion polypeptide with glutathione S-transferase (GST) and corresponding to the Spanish isolate SPA/1/93, was kindly provided by M. A. Jiménez-Clavero (CISA, Valèdeolmos, Spain). For use in immunoassays, P1 was solubilized after incubation with 8 M urea in 1% SDS. For immunization of mice, the protein was purified by centrifugation through a sucrose gradient, as described previously (Seechurn et al., 1990).

- **Production of mAbs.** One BALB/c mouse was primed and boosted with a 50 µg dose of the purified recombinant polyprotein P1, corresponding to isolate SPA/1/93, which belongs to the most recent antigenic group (Brocchi et al., 1997). Fusion and growth of hybridomas was carried out as described previously (Galfré & Milstein, 1981; Brocchi et al., 1993). Direct ELISA against either P1 or the antigenically homologous, purified SVDV isolate Italy 92 was used for mAbs screening.

- **Neutralization assays.** A number of assays were performed to determine the ability of the mAbs to neutralize virus infectivity. A standard assay in which virus is first incubated with each mAb in a liquid phase was carried out as described previously (Brocchi et al., 1995). A post-adsorption neutralization assay was also performed with some mAbs. In the latter case, virus (100 TCID₅₀) was added to IBRS-2 cell monolayers grown in 96-well microplates and incubated for 30 min at 4 °C. Non-adsorbed virus was then removed and, after washing, mAbs at a high concentration (undiluted supernatant of hybridomas or ascites fluid at a 1:50 dilution) were added and incubated at 4 °C for 30 min. Then, mAbs were removed and, after the addition of fresh medium, plates were incubated at 37 °C. Cytopathic effect was controlled 24–48 h post-infection.

- **Western blot analysis.** Hybridoma supernatants were tested against recombinant P1 and purified virus, resolved by SDS–PAGE in 8 and 10% gels, respectively, and transferred to a nitrocellulose membrane according standard protocols (Harlow & Lane, 1988).

- **ELISA.** For direct ELISA, purified virus (5 µg/ml), recombinant P1 (1 µg/ml) or synthetic peptides for ‘pepscanning’ (10 µg/ml) were adsorbed directly onto NUNC Maxisorp plates (50 µl per well). Then, hybridoma supernatants were added, followed by incubation with both anti-mouse immunoglobulin labelled with horseradish peroxidase (HRP) and substrate solution. For trapping ELISA, a rabbit anti-SVDV immune serum coated to microplates was used to trap the virus; the reaction was then continued as for direct ELISA.

For competitive ELISA, the competition between ten positive and two negative pig sera with anti-P1 mAbs was evaluated. Positive sera included two experimental hyperimmune serum samples, two field serum samples collected in 1997 and submitted to the laboratory for routine diagnosis and six serum samples representing early (collected between 6 and 11 days post-infection) and late (collected between 43 and 56 days post-infection) stages of an experimental infection (Brocchi et al., 1995).

Sequential dilutions of sera and anti-P1 mAbs at a dilution that had given an absorbance reading of 1.5 at 492 nm in a previous titration were simultaneously reacted against recombinant P1 or purified SVDV coated to plates, followed by anti-mouse immunoglobulins conjugated to HRP and substrate solution.

- **Location on the three-dimensional (3D) structure of SVDV capsid.** Location of the identified antigenic areas on the computer-generated 3D model of SVDV, isolate SPA/1/93, was carried out as described previously (Jiménez-Clavero et al., 2000).

Results

Production and preliminary characterization of anti-P1 mAbs

To favour the induction of antibodies directed to linear epitopes, purified P1 subjected to denaturing treatments was purified by centrifugation through a sucrose gradient, as described previously (Seechurn et al., 1990).
chosen as antigen for mice immunization. Recombinant P1 was shown to be a very good immunogen, as almost all hybridomas that originated from one mouse produced antibodies against the recombinant protein. More than 60 mAbs, highly reactive with P1 in ELISA as well as in Western blot assays, were then selected for further characterization.

All except four mAbs strongly recognized purified SVDV coated directly to the solid phase (direct ELISA); furthermore, the same mAbs reacted with individual viral proteins in Western blot assays, suggesting that they were directed to linear epitopes. In particular, only five mAbs reacted to VP1, while 42 and 36% of the mAbs recognized VP3 and VP0, respectively (data not shown).

Comparing results of two ELISAs, in which virus was presented either adsorbed directly to the plate or trapped by an anti-SVDV polyclonal serum, we could distinguish a first group of mAbs able to recognize virus in both presentations from a second group reacting only to directly adsorbed virus (Fig. 1).

In spite of the specific reactivity with SVDV, no mAb was found to neutralize virus infectivity in the standard assay, except mAb 1D5, which showed a very weak neutralizing ability and only when used as undiluted supernatant (data not shown).

Mapping of mAbs on SVDV proteins by pepscanning

In order to determine the precise binding region on the capsid proteins, mAbs were analysed by pepscanning using the collection of 20-mer overlapping synthetic peptides representing the whole P1. Each mAb was tested only against the peptides corresponding to the viral protein recognized in Western blot assays. When two contiguous peptides were recognized, we assumed that the binding area was the common amino acid sequence of the two contiguous peptides.

Reactivity with VP0. More than 20 mAbs were assayed against the 32 peptides of VP0. Aside from the region spanning amino acids 11–30 recognized by one mAb, two contiguous antigenic areas were found on the tract of VP0 corresponding to VP4: amino acids 41–50 and 51–60, each one identified by three different mAbs. Two minor sites were identified on the region corresponding to the N-terminal part of VP2, each one recognized by a single mAb (Figs 1 and 2). The region corresponding to amino acids 142–161 of VP2 (peptide 2151) was the core region of a wider antigenic area extended between positions 112 and 171, which included 12 mAbs showing three overlapping and slightly different patterns of reactivity (Figs 1 and 2). The 12 mAbs reacting with this antigenic area also reacted with the virus in a trapping ELISA, indicating that this area is accessible to the mAbs after immune-capture of the virus (Fig. 1).

Two out of the 12 mAbs further recognized a distant region located near the C terminus of VP2 (peptide 2241, amino acids 232–251). This duplex reactivity was not investigated further.

Reactivity with VP3. Nearly 30 mAbs reacting with VP3 in Western blot analyses were tested against the 23 peptides representing this protein. All antibodies reacted against peptides corresponding to the N-terminal one-third of VP3, defining a substantial antigenic area extending up to amino acid 70 (Figs 1 and 2). Within it, five different epitopes could be distinguished based on the different patterns of reactivity found. Four epitopes spanned positions 11–50 and were partially overlapping. In detail, six mAbs reacted with peptide 3020, thus mapping within the region of amino acids 11–30 (a slight reactivity with the contiguous peptide 3030 was also found in some cases). Two mAbs reacted with peptides 3030 and 3040, having amino acids 31–40 in common, and seven mAbs reacted with peptides 3040 and 3050, thus mapping in
the region spanning amino acids 41–50. Two other mAbs reacted with peptide 3040 alone, corresponding to amino acids 31–50; the target epitope probably maps to its central region corresponding to amino acids 35–45.

The fifth epitope on the N-terminal region of VP3 was identified between amino acids 61 and 70 by ten mAbs reacting with peptides 3060 and 3070, including the unique mAb showing weak neutralizing activity, mAb 1D5.

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**Fig. 2.** Mapping and characterization of linear epitopes identified by anti-P1 mAbs. Peptides recognized by the mAbs, numbered as explained for Fig. 1, and the amino acid positions in the corresponding viral protein assumed to be the binding area are indicated. When only one peptide reacted with mAbs, the binding area is reported as the whole peptide. When two contiguous peptides were recognized, the binding region is assumed to correspond to the common amino acid positions. When more than two contiguous peptides were reactive, the region spanning the two most distant amino acids is indicated as target area. mAbs have been grouped on the basis of their reactivity to the related viruses Italy 92 (IT92), Italy 72 (IT72), Italy 91 (IT91) and CV-B5. Numbers in each cell show the percentage of reactivity with respect to the homologous virus and it has been clustered as follows: white cells, 0–30% reactivity; grey cells, 31–69% reactivity; black cells, 70–100% reactivity. Antigenic regions and features are as described in the text. Asterisks indicate that a neutralizing conformational (NC) site has been described in that region (Kanno et al., 1995; Nijhar et al., 1999; Rebel et al., 2000; Borrego et al., 2002).

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**Note:** The region spanning amino acids 41–50. Two other mAbs reacted with peptide 3040 alone, corresponding to amino acids 31–50; the target epitope probably maps to its central region corresponding to amino acids 35–45.
Antigenic linear map of SVDV

Fig. 3. Amino acid sequence and location in the 3D model of SVDV of two pairs of peptides distantly located from one another in VP3 and recognized by the same cluster of mAbs. Amino acid sequence (A) and location on the 3D model (C) of peptides spanning amino acids 11–30 (orange) and 201–220 (green) recognized by the same group of six mAbs; amino acid sequence (B) and 3D location (D) of regions spanning amino acids 61–70 (magenta) and 191–210 (red) recognized by another group of ten mAbs. Amino acids common to both pairs of sequences are in bold. The 3D model shows the protomer structure viewed from outside.

As seen in Figs 1 and 2, two peptides located near the C terminus of VP3 were also recognized, but, surprisingly, not independently. Peptide 3200 (amino acids 191–210) was recognized by all the mAbs also reacting with peptides 3060 and 3070, while peptide 3210 (amino acids 201–220) was recognized by all mAbs also reacting with peptide 3020. The high number of antibodies showing the same pattern of reactivity indicates that this was not an artefact but a real reactivity. The amino acid sequence target of mAbs was then compared to determine whether a similitude existed to explain this common reactivity (Fig. 3A, B).

In peptides 3020 and 3210, corresponding to amino acids 11–30 and 201–220 of VP3, respectively, we found the amino acid motif DxQS(6x)F in both regions, suggesting that the observed duplex reactivity could be due to a similar structure of these peptides. However, within regions 61–70 and 191–210, only a common amino acid pair occurred.

Reactivity with VP1. Five mAbs, which reacted with VP1 in Western blot analyses, were assayed against the 28 peptides spanning VP1. They identified two antigenic areas: amino acids 51–60 (two mAbs reacting with peptides 1050 and 1060) and 264–280 on the C terminus (two mAbs reacting with peptides 1270 and 1273) (Figs 1 and 2). The latter two mAbs were reactive in the trapping ELISA.

The fifth mAb against VP1 showed a broad spectrum of reactivity that was interpreted as non-specific reactivity and, thus, was not investigated further.

Location of the main linear antigenic regions on the 3D model of SVDV

Peps scanning analysis enabled us to map five main antigenic linear regions on the viral proteins VP1 to VP3 (called regions 1 to 5), some of them comprising overlapping epitopes and identified by a considerable number of mAbs. These main linear areas were located on the 3D model of SVDV. Epitopes on VP4 were not located, as VP4 is known to be internal.

Regions 1, 3 and 5 (amino acids 142–161 of VP2, 61–70 of VP3 and the C terminus of VP1, respectively) (Fig. 2) were found to locate on the external surface of the capsid (Fig. 4A, C), while regions 2 and 4 (spanning amino acids 11–50 of VP3 and 51–60 of VP1, respectively) appeared to be exposed on the internal face of the pentamer (Fig. 4B, D). Nevertheless, region 2, which included four different overlapping epitopes, was partially exposed in the protomer (Fig. 4C) but not in the pentamer.

Also, the two pairs of peptides of VP3, which were recognized simultaneously by a same cluster of mAbs, in spite of being distantly located from each other in the primary structure of the protein, were located on the 3D model of the capsid. The two sequences (amino acids 11–30 and 201–220) harbouring the similar amino acid motif DxQS(6x)F were found to be distant from each other in the 3D model of the protomer (Fig. 3C), whereas the two regions (amino acids 61–70 and 191–210), showing no apparent similitude in their amino acid sequence, were found to be close to one another in the 3D model of the protomer (Fig. 3D), suggesting that the two peptides could be part of a single, larger site.

Reactivity with related viruses

The antigenic variability of linear epitopes was investigated by examining the reactivity of mAbs against SVDV isolates representative of three known antigenic groups (Italy 72, Italy 91 and Italy 92). Moreover, considering that internal sites not involved in neutralization are expected to be conserved, an
Fig. 4. Location of the five main linear antigenic sites in the 3D model of SVDV. (A, B) The pentamer structure of SVDV, viewed from both the outside and the inside of the virion, respectively. (C, D) The protomer structure viewed from both the outside and the inside of the virion, respectively. Regions 1, green; 2, red; 3, magenta; 4, blue; 5, orange. For each region, amino acid positions in the corresponding protein are indicated.

additional evaluation of mAb reactivity against the antigenically related CV-B5 was undertaken. This analysis was carried out by direct ELISA against purified viruses coated to the plate, since only in this presentation were all antigenic regions accessible to mAbs (Fig. 2).

As Italy 92 and SPA/1/93 belong to the same antigenic group, the reactivity of each antibody with SVDV Italy 92 was considered as homologous (100% reactivity). Three main patterns were observed: (i) mAbs showing weak or null reactivity with the heterologous SVDV isolates and with CV-B5, thus specific for the homologous antigenic variant; (ii) mAbs reacting with the three SVDV isolates tested but not with CV-B5, thus binding to more conserved epitopes within SVDV but not in CV-B5 (however, within this group, two mAbs, 4G11 and 1A3, were distinguishable as they recognized isolates Italy 72 and Italy 92 but not Italy 91); and (iii) mAbs showing a high percentage of reactivity, not only with the SVDV isolates but also with CV-B5, thus directed to epitopes common to both viruses. These conserved epitopes were consistently detected at more internalized regions as the N-termini of the viral proteins and VP4. In contrast, the former two patterns gave evidence of antigenic variability between SVDV groups in the corresponding epitopes.

Post-adsorption neutralization

As indicated previously, none of the anti-P1 mAbs was able to neutralize virus infectivity when pre-incubated with virus before addition to cells. However, pepscanning results revealed that some mAbs were directed to areas such as VP4 and the N-terminus of VP1, where linear epitopes involved in neutralization, which become exposed and accessible to mAbs only after virus adsorption to cells, have been identified for other enteroviruses and also for SVDV (Li et al., 1994; Jiménez-Clavero et al., 2001). Thus, mAbs directed to VP4 (1D2, 4F9, 4H1, 5G3, 1E10, 2A11 and 5A9) and to the region spanning amino acids 51–60 of VP1 (1C1 and 5H7) were tested in a post-adsorption neutralization assay to determine whether they were able to neutralize virus infectivity under these conditions.
No neutralizing ability was observed for any mAb, even when several attempts, including slight modifications of the method, were performed (data not shown).

**Competition with anti-SVDV antibodies**

Ten positive sera, representing early and late stages of infection, and two negative pig serum samples (shown as discontinuous lines) were tested for their ability to inhibit the binding of mAb 3E9 (selected as a representative example) to purified SVDV (A) and P1 (B). Positive serum samples analysed were two hyperimmune serum samples (●), two field serum samples (×), two early experimental serum samples (○) and four late experimental serum samples (●). Results are expressed as percentage of binding inhibition that quantifies the capability of the sera tested to inhibit the mAb binding and was calculated using the formula: [(A492 without serum – A492 in the presence of serum)/A492 without serum] × 100.

![Graph showing inhibition of binding to SVDV antigen](Image)

**Fig. 5.** Analysis of the capability of swine sera to inhibit the binding of the anti-P1 mAb 3E9. Ten positive pig serum samples, representing early and late stages of infection, and two negative pig serum samples were performed (data not shown). Antigenic regions different from those targets of the anti-P1 mAbs or their binding to P1 is not sufficiently high to compete with anti-P1 mAbs. In contrast, the strong humoral response elicited against conformational sites of SVDV during infection may block the accessibility even of linear epitopes in the viral capsid, becoming indirectly responsible for the observed binding inhibition of anti-P1 mAbs.

**Discussion**

The family Picornaviridae includes some important human virus pathogens, such as the agents causing poliomyelitis, the common cold or hepatitis A, and also agents that cause animal diseases of great economical importance, such as FMD. The understanding of their antigenicity and interaction with the immune system is therefore of great interest to improve the control of these diseases. For some of these viruses, a detailed description of antigenic sites has already been reported but attention has been almost exclusively focused on epitopes involved in neutralization. For SVDV, all of the most efficient sites of neutralization are dependent on conformation and have been identified using mAbs. Since the linear antigenic map of SVDV remained almost unexplored, we produced mAbs directed to linear epitopes on SVDV, which allowed characterization of these newly detected sites.

The bacterially expressed polyprotein P1 was an excellent immunogen for mice, as shown previously for swine (Jiménez-Clavero et al., 1998). The positive reactivity shown by all mAbs in Western blot analyses demonstrated that antibodies obtained are directed to continuous epitopes, as expected from the use of a denatured immunogen. However, the strong reactivity with the purified adsorbed SVDV in ELISAs provided evidence that epitopes recognized by mAbs are also present in the viral capsid, being, therefore, of structural significance. In fact, both complete and empty viral capsids represent the main antigenic particles in purified virus preparations and the alteration of the capsid structure during adsorption did not affect mAb binding but, on the contrary, even improved the presentation of internal sites.

Analyses of the repertoire of mAbs produced enabled the identification of five main linear sites (Fig. 2). The region comprising amino acids 142–161 of VP2, designated antigenic region 1, seemed to be the core of a larger antigenic area where three overlapping epitopes could be distinguished. This region was predicted to be an exposed epitope on the 3D model (Fig. 5) and, consistently, accessible to mAbs in the trapping ELISA. In fact, virus trapped by antibodies is assumed to substantially maintain conformation and consequently expose the external surface of the capsid. Furthermore, as expected for external antigenic sites, region 1 was subjected to variability within SVDV variants.

On the N-terminal one-third of VP3, antigenic regions 2 and 3, showing different features, were defined. Region 2, composed of several overlapping epitopes, was conserved...
between the viruses analysed, including the human pathogen CV-B5, and was shown to be internal in the 3D model of the capsid. In contrast, region 3, mapping to amino acids 61–70, was exposed on the outer surface of the capsid and different patterns of reactivity between the viruses examined were shown by the mAbs, according to the differences in the amino acid sequence corresponding to this region (Knowles et al., 2000).

Regions 4 and 5 were mapped at the N- (amino acids 51–60) and C-termini (amino acids 264–280) of VP1, respectively. Region 4 was found to be an internal, conserved site, while region 5, exposed on the surface according to the 3D model, proved accessible to mAbs in the trapping ELISA and subjected to antigenic variability between SVDV isolates. Further contiguous epitopes shared between SVDV isolates and CV-B5 were identified throughout VP4 and the N terminus of VP2. In general, there was a clear correlation between epitope conservation, as reflected by the reactivity of mAbs with the different virus strains tested, and exposition on the external or internal surface, as reflected by the 3D model.

When mapping mAbs by pepscanning, two groups of mAbs against VP3 were found to cross-react with peptides corresponding to sequences distantly located from one another on the primary structure. In one case, the two regions (amino acids 11–30 and 201–220) distantly located from one another in the 3D model but containing similar amino acid motifs in both peptides could explain this reactivity. We assume that the antigenic region on SVDV probably corresponds to the N-terminal sequence, being that this region was also recognized by mAbs from SVDV-infected pigs (Jiménez-Clavero et al., 2000). In the other case, the two regions (amino acids 61–70 and 191–210) were found to be close to one another on the 3D model of the capsid, thus suggesting the possibility that both linear regions could be discrete parts of a unique conformational epitope still recognized by antibodies when presented alone in synthetic peptides (van Regenmortel, 1988). In fact, these two regions have been detected as antigenic by pepscan analysis of anti-SVDV sera from experimentally and naturally infected pigs (Jiménez-Clavero et al., 2000). mAbs to these regions could have been induced by the corresponding conformational epitope, partially maintained in P1.

In spite of using a different immunogen and animal host, the linear antigenic map defined by our antibodies shares some features with the one resulting from SVDV-infected pig sera (Jiménez-Clavero et al., 2000) and also with the one described for CV-A9 (Pulli et al., 1998). In particular, amino acid positions corresponding to the N terminus (region 2) and the so-called knob of VP3 (region 3), together with those corresponding to the puff βE-βF of VP2 (region 1), the C-terminal end of VP1 (region 5) and VP4, are linear antigenic areas recognized by both mAbs and post-infection sera.

In contrast, other antigenic sites described previously were not detected in our system. For instance, it was surprising to obtain no mAbs against either the extreme N terminus (amino acids 1–20) or the conserved amino acid motif PALTAEGT (amino acids 29–37) of VP1, despite both antigenic regions being recognized in SVDV and other enteroviruses (Jiménez-Clavero et al., 2000, 2001; Roivainen et al., 1991; Hovi & Roivainen, 1993). However, the antigenicity of the N terminus of VP1 seems to depend on its dynamic structure (Li et al., 1994; Roivainen et al., 1993). In the polyprotein P1 used as immunogen, the N terminus of VP1 is no longer a terminus and is probably subjected to structural constraints that prevent its mobility. Curiously, we have detected a contiguous antigenic area not described previously: region 4 (corresponding to peptides 1050 and 1060), which probably remains buried in the viral capsid (Ketterlinus & Wiegers, 1994).

Linear regions 1, 3 and 5 locate quite close to or even include amino acid positions involved in neutralizing conformational sites of SVDV (Kanno et al., 1995; Nijhar et al., 1999; Rebel et al., 2000; Borrego et al., 2002). However, none of the mAbs directed to linear epitopes was able to neutralize virus infectivity even when tested in post-adsorption neutralization assays aimed at detecting internal epitopes possibly involved in neutralization and becoming exposed after virus adsorption. Evidence of such epitopes in VP4 and the N terminus of VP1 for other enteroviruses, including SVDV, was reported previously (Li et al., 1994; Jiménez-Clavero et al., 2001). Moreover, the weak or null competition between antibodies elicited by SVDV infection and anti-P1 mAbs indicates that linear epitopes identified by these mAbs are low or not immunogenic in pigs. This suggests that, although the immune response in the natural host also includes antibodies to linear epitopes, conformational sites are definitely more immunogenic.

Results presented here have led to a better understanding of the antigenic structure of SVDV and picornaviruses in general. They could also allow an improvement of diagnostic systems based on mAbs. In fact, linear epitopes should be more resistant to damage than conformation-dependent epitopes and, consequently, selected mAbs to linear epitopes conserved between different virus strains could be the tools best suited for an antigen detection system. On the other hand, those mAbs directed to exposed sites that are subject to antigenic variation could be useful for isolate subtyping. Experiments to evaluate the use of mAbs directed to linear epitopes in diagnostic assays are currently under way.

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