Epitope mapping of the nucleocapsid protein of European and North American isolates of porcine reproductive and respiratory syndrome virus

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Two major genotypes of porcine reproductive and respiratory syndrome virus (PRRSV) have been described, which correspond to the European and North American isolates. PRRSV nucleocapsid (N) protein has been identified as the most immunodominant viral protein. The N genes from two PRRSV isolates, Olot/91 (European) and Quebec 807/94 (North American), were cloned and expressed in: (i) baculovirus under the control of the polyhedrin promoter and (ii) Escherichia coli using the pET3x system. The N protein from both isolates was expressed much more efficiently in E. coli as a fusion protein than in baculovirus. The antigenicity of the protein was similar in both systems and it was recognized by a collection of 48 PRRSV-positive pig sera. The antigenic structure of the PRRSV N protein was investigated using seven monoclonal antibodies (MAbs) and overlapping fragments of the protein expressed in E. coli. Four MAbs recognized two discontinuous epitopes that were present in the partially folded protein, or at least a large fragment comprising the first 78 residues. The other three MAbs revealed the presence of a common antigenic site localized in the central region of the protein (amino acids 50–66). This region is well conserved among different isolates of European and North American origin and is the most hydrophilic region of the protein. However, this epitope, although recognized by the MAbs and many pig sera, is not useful for diagnostic purposes. Moreover, none of the N protein fragments were able to mimic the antigenicity of the entire protein.

Introduction

The causative agent of porcine reproductive and respiratory syndrome is an arterivirus, isolated first in Europe in 1990 (Wensvoort et al., 1991) and then in the United States (Collins et al., 1992). Porcine reproductive and respiratory syndrome virus (PRRSV) causes an important disease in pigs characterized by reproductive failure in sows and gilts, pneumonia and an increase in perinatal mortality (Terpstra et al., 1991). This virus is now endemic in domestic pigs in the United States and Europe. The North American and European isolates of PRRSV represent two distinct genotypes (Mardassi et al., 1994; Magar et al., 1995; Murtaugh et al., 1995; Meng et al., 1995; Suarez et al., 1996), which have important antigenic differences (Nelson et al., 1993; Wensvoort et al., 1992; Dea et al., 1996). However, even among different isolates of the same genotype there are considerable antigenic, genetic and pathogenic differences (Meng et al., 1996).

PRRSV is an enveloped RNA virus approximately 62 nm in diameter. The virus genome is a single-stranded RNA of positive polarity, approximately 15 kb in size. The genome contains eight overlapping open reading frames (ORF) (Meulenberg et al., 1993). The virion contains six structural proteins encoded by ORFs 2–7 (Meulenberg et al., 1995b; van Nieuwstadt et al., 1996; Meulenberg & Petersen Den Besten, 1996). ORF7 encodes the nucleocapsid (N) protein. Recently, we have shown that the majority of antibodies produced during infection are specific for the N protein of PRRSV (Sanz et al., 1995; Plana-Durán et al., 1997) which is therefore a suitable candidate for the detection of virus-specific antibodies and diagnosis of the disease. Since there are important antigenic differences between both genotypes, the identification of antigenic sites on the N protein could be used in diagnostic assays that discriminate between PRRSV genotypes.

In this study we have cloned and expressed ORF7 from...
two different PRRSV isolates, Olot/91 and Québec 807/94, using insect cells and *Escherichia coli*. We have also constructed an antigenic map of the N protein of the European PRRSV Olot/91 isolate using a panel of monoclonal antibodies (MAbs) and pig antisera in combination with a collection of overlapping fragments. This study has allowed the identification of three major epitopes on the N protein, one of which is well conserved among the different isolates.

**Methods**

- **Cells, viruses and bacterial strains.** *Spodoptera frugiperda* clone 9 (Sf9) cells were grown and maintained in suspension or as monolayer cultures at 27 °C in TNM-FH medium (Summers & Smith, 1987) supplemented with 5% foetal calf serum (Gibco BRL) and gentamicin. Wild-type and recombinant baculoviruses were propagated in Sf9 cells as described by Lopez de Turiso et al. (1992). PRRSV isolates were grown in alveolar macrophages as described previously (Plana-Durán et al., 1997).

  E. coli strains DH5 and XL1-blue were used as the host for initial cloning of target DNA into pET vectors (Studier et al., 1990). E. coli strain BL21(DE3)-pLYS(S−ampT−rbcL−m16) (Grodberg & Dunn, 1988) was used as the host for expression.

- **Antisera and MAbs.** A collection of 48 European and 15 North American field pig sera positive for PRRSV and seven MAbs specific for the N protein were used for the antigenic characterization of the N protein and its fragments. The characteristics of these MAbs have been previously described (Sanz et al., 1995). All of them were found to be positive for the native viral protein using indirect immunofluorescence and immunoperoxidase assays.

- **PRRSV Québec 807/94 RNA purification and cDNA cloning.**

  PRRSV Québec 807/94, previously obtained in 1994 from a pig farm in Québec (Canada) with a record of abortions, anorexia, hyperthermia and respiratory problems in piglets, was kindly provided by C. Adlan (Langford Laboratories, Guelph, Ontario). Virus was isolated from the serum of a 7-week-old piglet, grown in pig alveolar macrophages and semi-purified as previously described (Plana-Durán et al., 1992). Briefly, a 21 macrophage culture at an initial density of 3 × 10⁶ cells/ml was infected at an m.o.i. of 10⁻⁶ TCID₅₀ per cell. Between 3 and 4 days post-infection, the culture was centrifuged and the supernatant was concentrated to 4 ml by ultracentrifugation. The concentrated supernatant was loaded onto a discontinuous sucrose gradient (20–50%, w/v) and centrifuged at 32000 r.p.m. in a SW40 rotor (Beckman) for 3 h. An opalescent band in the interphase of the gradient was recovered and diluted with buffer (10 mM Tris–1 mM EDTA). The material was treated with 1% SDS and 500 μg/ml protease K for 1 h at 37 °C to extract RNA. The RNA preparation was then treated three times with phenol and ethanol-precipitated.

  cDNA was synthesized from viral RNA in two steps. The first step was carried out according to standard procedures using reverse transcriptase and oligo(dT), and the product was amplified by PCR, as described below. For PCR amplification, ORF7 primers were derived from the sequence of PRRSV isolate VR-2332 (GenBank accession number U00153). Oligonucleotide CORF7N (TAAATATGCAAAAT-AACACC) was used as sense primer and CORF7C (CCATCATGAGGGTGATCC) as antisense primer. PCR products were cloned in phospatase-treated, Smal-digested pMTL25. Plasmid DNA was sequenced by the dideoxynucleotide chain termination method (Sanger et al., 1977). The nucleotide sequence of Québec 807/94 ORF7 was deposited in the EMBL database, accession number Z82995. Protein sequence analyses were performed with PC/GENE software (Intelligenetics) and the EMBL PredictProtein mail server (Rost, 1996).

- **Construction of AcNPV recombinant transfer vectors and selection of recombinant baculovirus.** A procedure similar to that described previously (Plana-Durán et al., 1997) was followed for the construction of the AcNPV recombinant transfer vector for ORF7 from PRRSV Québec 807/94. Briefly, the ORF7 gene was isolated by BamHI digestion, subcloned into the baculovirus transfer vector pAcYM1 (Matsuura et al., 1987), and transformed into E. coli DH5 cells according to standard procedures. Recombinants were selected by restriction enzyme mapping and by sequencing across the BamHI insertion site.

  Recombinant baculoviruses were obtained from cotransfections involving linearized AcRP23-LacZ⁺ virus DNA (500 ng) and transfer vector DNA (2 μg) using the lipofectin technique (Felgner et al., 1987). The recombinant viruses were selected by their LacZ⁺ phenotypes and plaque-purified before high-titre virus stocks (> 10⁹ p.f.u./ml) were prepared for each recombinant baculovirus (Kitts & Possee, 1993).

- **Analysis of baculovirus-derived recombinant proteins.** Sf9 cells were infected with the recombinant baculoviruses at an m.o.i. of
Antigenic map of PRRSV nucleocapsid protein

Fig. 2. (a) Hydrophilicity profile of the N protein of PRRSV Québec 807/94. The distribution of hydrophilic regions along the predicted amino acid sequence of the protein was determined using the algorithm of Hopp & Woods (1981) and a window setting of six amino acids as recommended by the authors. The sequence of the protein is shown above the profile. (b) Alignment of the predicted amino acid sequences of the PRRSV N protein. Amino acid sequences of the isolates Québec 807/94, IAF-ex91, VR2332, Olot/91 and Lelystad were aligned. The conserved region is boxed.

1 p.f.u. per cell. Cells were incubated at 27 °C for 3 days, collected by centrifugation, washed with PBS and sonicated three times at 23 kHz for 30 s in a Soniprep apparatus (MSE). Cell extracts were analysed by gel electrophoresis on 15% SDS–polyacrylamide gels and were then either stained with Coomassie blue or transferred onto nitrocellulose membranes (Hybond-C, Amersham) for immunoblotting as described by Harlow & Lane (1988). The N protein of PRRSV Olot/91 was found to be more soluble than that of the Québec 807/94 strain, with 60% of the protein recovered in the supernatant of the lysed cells. In either case, enough soluble protein was recovered to allow for its use as a coating antigen in ELISA.

Cloning of PRRSV Olot/91 ORF7 fragments in the pET3x expression vector. The complete ORF7 coding sequence was obtained from the recombinant plasmid pPRRSV-8 (Plana-Durán et al., 1997), as shown in Fig. 1. ORF7 fragments generated were as follows: ORF7 gene (HpaI–AflIII); A (nt 16–237, Hpal–EcoNI); B (nt 54–291, PCR); C (nt 237–387, TaqI–AflIII); D (nt 16–126, Hpal–EcoNI); E (nt 126–237, EcoNI–TaqI); and F (nt 151–201, PCR). All of the fragments were recovered by BamHI-compatible digestion and subcloned into BamHI-digested phosphatase-treated PET3x (a, b and c) (Studier et al., 1990). Vector PET3x was used for this purpose because of the small length of the fragments to be cloned. PET3x yields a fusion protein of 260 amino acids of the gene 10 protein, which when ligated to the small fragments confers more stability to the final protein. The ligation mixtures were used to transform XL1-blue or DH5 competent cells. The resulting colonies were screened by digestion with appropriate restriction enzymes. To check the orientation, the junction sequences of the inserts were sequenced by using the oligonucleotide 5′-CTATCCGCAAC-GTTATGGGC 3′ as primer. After verification of the sequence, strain BL21(DE3)-pLysS competent cells were transformed with each of the recombinant PET3x-derived plasmids. Chloramphenicol and ampicillin agar plates were used to select for the transformed cells.
PCR. PCR was done with a Perkin-Elmer thermal reactor. For fragment B, oligonucleotide a (TGGGGGATCCCGACGCTCAA-TCAA) was used as forward primer and b (GAAAACAGATCTTCC- CGCTGAGT) as reverse primer. For fragment F, oligonucleotide pro1 (CCTGAGAAGCCACATTTTTCTCCTTAGC) was used as forward primer and pro2 (GTGTGGCGATGTCACTTACGACG) as reverse primer.

For all PCR amplifications, 50 ng template DNA was mixed with 800 ng of each primer. The samples were subjected to 25 cycles of amplification under the following reaction conditions: denaturation at 94 °C for 1 min, annealing at 42–60 °C for 1 min, extension at 72 °C for 1 min plus a final extension step at 72 °C for 7 min. The reactions were done with Vent DNA polymerase (New England Biolabs).

Growth, induction and analysis of E. coli transformed cells. Single clones of E. coli BL21 (DE3) -pLysS cells containing the recombinant pET3x-derived plasmids were grown and induced with 0.4 mM IPTG (Boehringer Mannheim) as described previously (Martinez-Torrecuadrada & Casal, 1995). After induction at 37 °C for 3 h, cells were centrifuged, washed twice with PBS and resuspended in a final volume of 500 µl PBS for a 50 ml culture. Bacteria were freeze–thawed three times and then lysed by sonication. Loading buffer was added to each sample which was then boiled for 5 min before analysis on 11% SDS–polyacrylamide gels. Detection of protein was carried out by staining with Coomassie blue or by immunoblotting according to standard procedures (Harlow & Lane, 1988).

Purification of the different fragments of the PRRSV N protein was carried out as described previously (Martinez-Torrecuadrada & Casal, 1995).

ELISA. Microtiter 96-well plates (LabSystem) were coated overnight at 4 °C with 100 µl of a 1:100 dilution of the soluble fraction. This was obtained by either sonication (insect cells) or treatment with guanidinium chloride of the N protein (E. coli), or derived fragments, in 0.05 M carbonate buffer (pH 9.6). Standard procedures were then followed for the assay (Harlow & Lane, 1988). The reactivity caused by non-specific binding of sera to Sf9 cells or E. coli extracts was subtracted from that of the recombinant protein to obtain the PRRSV-specific value. The cut-off value was taken as the mean ± 2 SD of the six negative control sera values.

Results

cDNA cloning and sequence analysis of ORF7 from PRRSV Québec 807/94 isolate

A product of 370 bp that contained the complete coding sequence of the PRRSV Quebec 807/94 isolate N protein was obtained after RT–PCR amplification of the viral RNA. This product was cloned in pMTL25 in order to provide BamHI sites compatible with the expression vectors. The resulting plasmid was designated pPRRSC-ORF7. After cDNA sequencing and translation, the predicted protein showed a high amino acid identity (97-9%) (Fig. 2b) with that of the Quebec isolate IAF-exp91 (Mardassi et al., 1994). The only change was Thr9 being replaced by Asn9. With the prototype North American isolate VR2332 (Collins et al., 1992), there was 95-2% identity. All the amino acid changes, apart from one, were located in the first 11 residues. With the Olot/91 isolate, the identity was much lower (61-2%). The changes were scattered throughout the sequence, although there was a central region (residues 47–100) which was more conserved (Fig. 2b). The N protein region located between residues 40 and 60 was very hydrophilic, with a high solvent accessibility (Fig. 2a) and the prediction of secondary structure gave a loop conformation for this region.

Generation of recombinant baculoviruses and analysis of the expressed protein products

The PRRSV Québec 807/94 ORF7 sequence was obtained from pPRRSC-ORF7 and subcloned into the pAcYM1 baculovirus transfer vector. The new vector was designated pAcYM1-ORF7C. In this construct, the nucleotide sequence upstream from the initiation codon was reduced to only 7 nt in order to improve the levels of expression. This recombinant transfer vector was used to generate the recombinant baculovirus AcNPV-ORF7C vector.

AcNPV-ORF7C, together with the previously described AcNPV-PRRSORF7 (Plana-Durán et al., 1997), containing ORF7 from Olot/91, were used to infect Sf9 cells at an m.o.i. of 1 p.f.u. per cell. Infected cells were analysed after SDS-PAGE by Coomassie blue staining and immunoblotting. As shown in Fig. 3, the expressed proteins were of the expected size for the viral N proteins (14-7 kDa for the European and 13-5 kDa for the North American isolate). Western blot analyses using anti-N protein MAb or polyclonal pig antisera identified both N proteins made by the respective recombinant baculoviruses as the correct products. In both cases, an additional band was observed below the major band, which probably indicates an in-frame initiation site occurring at a

![Fig. 3. Expression of PRRSV Olot/91 and Quebec 807/94 N protein in baculovirus. Sf9 cells were infected at an m.o.i. of 1 p.f.u. per cell with the two recombinant baculoviruses expressing the respective N proteins. Cells were harvested at 3 days post-infection. Proteins were fractionated on a 15% SDS–polyacrylamide gel, transferred to nitrocellulose membranes for immunoblot analysis and reacted with a mixture of PRRSV N protein MAbs (undiluted supernatant). Bound antibody was detected with a peroxidase-conjugate anti-mouse IgG and 4-chloro-1-naphthol as substrate. Lane 1, Sf9 mock-infected cells; lane 2, Sf9 cells infected with AcNPV-PRRS ORF7 expressing PRRSV Olot/91 N protein; and lane 3, Sf9 cells infected with AcNPV-ORF7C expressing Quebec 807/94 N protein. Molecular mass markers are in kDa on the left-hand side.](https://www.microbiologyresearch.org/figure/3)
methionine codon downstream in the ORF7 sequences (Met17 for Olot/91 and Met25 for Québec 807/94). Similar results have been observed previously for other viral proteins expressed in the baculovirus system (Martinez-Torrecuadrada et al., 1994). The levels of expression were 1–2 µg/10⁶ cells, which are lower than usual for the baculovirus system. The reason for this could be the low solubility of the N proteins. Between 40 and 80% of the protein remained associated with the cell pellet after cellular lysis.

Expression of different PRRSV ORF7 fragments in E. coli

Seven pET3x-derived recombinant plasmids were constructed containing the Olot/91 ORF7 and overlapping fragments of the N protein with various lengths ranging from 51 to 237 nt, as shown in Fig. 1. E. coli BL21 cells were transformed with the respective plasmids and induced for 3 h to express protein. The resultant fusion proteins were analysed by Coomassie blue staining of SDS–polyacrylamide gels (Fig. 4a). In every case, a strong protein band was observed with a size that was in agreement with the expected molecular mass. The fusion proteins were all produced in abundance: from 0·02 mg/ml for fragment C to 0·1 mg/ml for most of the other fragments, as estimated by visual comparison with known quantities of BSA. To confirm the identity of the fusion proteins, fragments were probed with PRRSV-positive pig antisera by immunoblotting (Fig. 4b). Although the binding pattern of the pig sera was variable, most of the sera reacted equally with all the fragments, but not as well as with the complete N protein. However, in some cases the pig sera reacted strongly with fragments A, C and D, weakly with fragment B and failed to react with fragments E and F (data not shown).

The expressed fusion proteins were insoluble, although they could be easily solubilized in 4 M guanidinium hydrochloride. The purity of the fusion proteins obtained after this solubilization step was > 80% (Fig. 4a). Partially purified fusion proteins were used in the antigenicity studies and analysis of the degree of epitope conservation between the European and North American PRRSV isolates.
Table 1. Characterization of PRRSV nucleocapsid MAbs by ELISA

The results, at an absorbance of 405 nm, are recorded as follows: ++, A > 1.0; +, A > 0.5; +/−, A > 0.25; −, A < 0.25.

<table>
<thead>
<tr>
<th>MAb</th>
<th>Antigenic site</th>
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<tbody>
<tr>
<td>1CH5</td>
<td>I</td>
</tr>
<tr>
<td>1DH10</td>
<td></td>
</tr>
<tr>
<td>1BD11</td>
<td>II</td>
</tr>
<tr>
<td>1EB9</td>
<td></td>
</tr>
<tr>
<td>1DA4</td>
<td>III</td>
</tr>
<tr>
<td>1AG11</td>
<td></td>
</tr>
<tr>
<td>1AC7</td>
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Reactivity of PRRSV-specific MAbs with the baculovirus-derived N protein

To investigate the antigenic structure of the PRRSV N protein, each MAb was characterized by its reactivity with each N protein. Only five out of seven specific MAbs reacted strongly with the baculovirus-derived N protein of the European isolate by immunoblotting (Fig. 5a). MAb 1CH5 reacted weakly and 1DH10 did not react with the recombinant N protein. These two MAbs may recognize conformational epitopes that are not properly presented in the recombinant protein. Only MAbs 1AC7, 1AG11 and 1DA4 recognized the N protein from the North American isolate Quebec 807/94 expressed in insect cells (Fig. 5b).

The results obtained by indirect ELISA are shown in Table 1. In this case, the baculovirus-derived N protein reacted well with all the MAbs. However, the antigenicity of the E. coli-expressed recombinant N protein Olot/91 was different from expected. The latter reacted with all the MAbs, including 1DH10 that had reacted very weakly with the baculovirus-derived N protein. Again, the Quebec 807/94 N protein reacted with three MAbs. Since the three MAbs that react with the N protein from both the European and North American isolates are the same, they must be recognizing a common epitope. Epitope mapping was used to identify the conserved epitope.

Characterization of antigenic sites in the PRRSV N protein

Epitope mapping of the PRRSV N protein was accomplished by probing the reactivity of the different fragments of the ORF7 gene expressed in E. coli with a collection of MAbs. After immunoblotting (Fig. 6), three different antigenic sites were found. MAbs 1AC7, 1AG11 and 1DA4 reacted with fragments A, B, E and F, thereby defining antigenic site III. Fragment F, the smallest fragment recognized, comprises the amino acid sequence PEKPHFPLAEDDIRHH (amino acids 50–66). MAbs 1BD11 and 1EB9 reacted with the complete N protein and with the larger fragments A and B, thereby defining site II. They could either recognize a discontinuous epitope located in the first 78 residues or, more likely, an epitope located around the EcoNI restriction site. Finally,
**Antigenic map of PRRSV nucleocapsid protein**

**Table 2. Reactivity of pig sera with PRRSV N protein and fragments**

Percentage of sera, positive or negative, reacting with the PRRSV N protein or derived fragments (A–F). Reactivity was determined as described in Methods.

<table>
<thead>
<tr>
<th>Sera</th>
<th>N protein</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
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<tbody>
<tr>
<td>Positive (%)</td>
<td>100</td>
<td>71.4</td>
<td>65.7</td>
<td>65.7</td>
<td>65.7</td>
<td>62.8</td>
<td>65.7</td>
</tr>
<tr>
<td>Negative (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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Results obtained by ELISA are shown in Table 1. All of the MAbs reacted with the entire N protein. These data confirm that 1CH5 and 1DH10 recognize a discontinuous epitope and the N protein to remain partially folded. The rest of the MAbs displayed the same reactivity as was obtained with the fragments by immunoblotting. The results obtained with fragments C and D suggest the absence of continuous antigenic sites in the N and C termini of the N protein.

**Reactivity of the N protein fragments with PRRSV-specific pig sera**

We investigated the use of these fragments as diagnostic reagents. The reactivity of a collection of 48 field pig sera with each fragment was compared by indirect ELISA. A summary of the results is shown in Table 2. The entire Olot/91 ORF7 gene expressed either in baculovirus or in E. coli reacted with the complete collection of PRRSV-specific pig sera during ELISA and gave the highest readings. Fragment A reacted with 72% of the PRRSV-positive sera. Only two-thirds of the positive pig sera reacted with fragments B, C, D and F. Fragment E, the carboxy-terminal region, showed the lowest reactivity with the pig sera. In summary, none of these fragments compared favourably with the whole N protein. Fragment F, which contains the common antigenic site, did not react with many of the positive sera, suggesting that this epitope is not immunodominant in pigs. The recombinant Québec 807/94 ORF7 protein reacted well with the collection of 15 North American PRRSV-positive sera. In all cases the correlation with the routinely used immunoperoxidase monolayer assay (IPMA) was nearly 100%.

**Discussion**

Two different antigenic groups of viruses have been described for PRRSV, which correspond to the European and North American isolates. Japanese and other Asian isolates are also antigenically related to the North American isolates (Shibata et al., 1996). Newly developed vaccines based on live-
attenuated North American PRRSVs are now being widely introduced in Europe, which may represent a further complication in the serological analysis of infected and/or vaccinated animals. To identify the virus, new diagnostic procedures have to be developed to determine the genotype of the virus either on its own or in mixed populations. These procedures will probably rely on the use of the N protein of PRRSV, given its antigenic properties. N protein has been demonstrated to be the most immunodominant antigen in the pig immune response to PRRSV (Meulenberg et al., 1995a; Sanz et al., 1995; Plana-Durán et al., 1997). Unfortunately, the production and purification of PRRSV antigens from tissue culture is troublesome and costly. To overcome this problem, we have described in this report the expression of the N protein gene in two different systems (baculovirus and E. coli) and the use of these systems in diagnosis, extending the analysis to map antigenic regions and to identify the precise sequence of the conserved epitopes in both isolates.

The expression levels obtained in baculovirus for the recombinant N protein were lower than usual. Several hypotheses may explain this. Previous reports of viral N protein expression in insect cells have shown a tendency for this type of protein to aggregate and form inclusion bodies (Rota et al., 1990; Vapalahti et al., 1996). Such aggregation would account for the low solubility and low expression of PRRSV N protein in insect cells. In fact, much of the expressed protein aggregates spontaneously and remains insoluble. The expression levels of the N protein, when expressed as a large fusion protein of 46 kDa, were much higher in E. coli than in baculovirus. The increased levels of expression may explain its better reactivity with the MAbs. After solubilization in 4 M guanidinium hydrochloride the E. coli-derived protein could be used directly in diagnostic assays by simple dilution in PBS.

To map the antigenic sites of the protein, the N gene was truncated from both ends and the fragments expressed were tested for reactivity with MAbs. From the results obtained, the MAbs could be divided into three groups, recognizing at least three different epitopes on the PRRSV N protein. MAbs 1CH5 and 1DH10 recognized a discontinuous epitope (site I) on the N protein. Further testing of the MAbs by Western blot analysis under non-reducing conditions gave identical results (data not shown), suggesting that the presence of disulfide-linked dimers does not play a major role in the formation of this epitope. 1CH5 and 1DH10 competed partially for the same site and were the only MAbs that competed efficiently with all the positive pig sera in a competition ELISA assay (data not shown). The MAbs for PRRSV described to date behave differently. Drew et al. (1995) did not find common recognition sites, but Nelson et al. (1993), Wieczorek-Krohmer et al. (1996) and Dea et al. (1996) reported common antigenic sites among different isolates, although no attempt to define their amino acid composition was made. Our findings demonstrate the existence of common antigenic regions among European and American PRRSV isolates; three out of seven N protein-specific MAbs recognized the epitope PEKPHPLAEADDRHH (amino acids 50–66) in the middle of the protein, which suggests good immunogenicity for this region. Amino acid sequence comparisons of several PRRSV isolates show that the central region of the protein is well conserved. This region (amino acids 50–90) contains the epitope found with group III MAbs and is the most hydrophilic domain of the N protein. The prediction of secondary structure and solvent accessibility indicates that the epitope 50–66 has a high probability of being part of a random structure with a good solvent accessibility, suggesting that this epitope is exposed on the surface of the N protein.

The reactivity of a collection of PRRSV-specific sera with the truncated fragments by indirect ELISA was weak in general, and about one-third of the pig sera did not react with any of the fragments. Therefore, although fragment F contains a common epitope it does not seem a suitable reagent for diagnostic purposes. The lack of immunodominance of the conserved linear epitopes would account for the absence of cross-reactivity for the N antigen among positive pig sera of different origin. This explanation is supported by the finding that MAbs 1CH5 and 1DH10, which compete efficiently with all the European-positive sera, react only with the Olot/91 N protein, but not with Québec 807/94. Therefore, the most immunodominant epitope, which reacts with all the pig sera and the MAbs 1CH5 and 1DH10, is a variable and conformation-dependent epitope. In summary, only the entire N protein, expressed in E. coli, seems to be a suitable reagent for diagnostic purposes. It is expressed at very high levels and is easy to purify. Our results contrast with those for the prototype arterivirus (equine arteritis virus) where the N protein expressed in E. coli was not appropriate for diagnosis and the N-terminal fragment (amino acids 1–69) was the most...
immunoreactive region of the viral N protein (Chirnside et al., 1995). The approach described here should provide a useful test for use in diagnosis of PRRSV infection.

This work was partially funded by grant 351/96 of the Spanish Ministry of Industry and Energy.

References


Received 7 January 1997; Accepted 24 April 1997