Genomic and Antigenic Variations of Porcine Reproductive and Respiratory Syndrome Virus Major Envelope GP_5 Glycoprotein

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ABSTRACT

The objective of the present study was to evaluate the importance of genomic and antigenic variations which may have affected the major envelope glycoprotein GP_5 of porcine reproductive and respiratory syndrome virus (PRRSV) isolates responsible for outbreaks in Quebec and Ontario, in comparison with the modified-live U.S. vaccine strain (MLV) and the European prototype strain from Lelystad (LV). Nucleotide sequence analyses of the open reading frame (ORF)5 genes showed that all of the isolates studied were heterogenous, amino acid (aa) identities varied from 88 to 99% with the MLV strain, and between 51 and 54% with the LV strain. The aa substitutions were randomly scattered across the protein, although one region between residues 26 and 39 was found to correspond to a hypervariable region which involved 0 to 3 potential N-glycosylation sites. The ORF5 encoded products of 5 of these isolates, including the MLV and LV strains, were expressed in *E. coli* as recombinant proteins fused to the glutathione S-transferase (GST) protein and used to raise hyperimmune anti-ORF5 sera in rabbits. The reactivity patterns of strain-specific hyperimmune anti-ORF5 sera and a panel of 4 monoclonal antibodies directed against the ORF5 gene product of the Quebec IAF-Klop strain of PRRSV, indicated that GP_5 of field isolates also underwent antigenic variations. The data suggest that neutralizing epitopes, independent of conformation and glycosylation, are also associated with antigenic variability of the GP_5 of PRRSV.

RÉSUMÉ

L'objectif de ce travail était d'évaluer l'importance des variations génomiques et antigéniques ayant pu affecter la glycoprotéine majeure d'enveloppe GP_5 d'isolats du virus du syndrome reproducteur et respiratoire porcin (SRRP) associés à des épidémies de la maladie survenues dans des élevages de porcs du Québec et de l'Ontario, comparativement à la souche vaccinale atténuée (MLV) d'origine américaine et la souche prototype européenne de Lelystad (LV). Les analyses des séquences en nucléotides ont révélé que les ORFs 5 des isolats cliniques étaient hétérogènes, les niveaux d'identité des séquences en acides aminés (aa) prédites variant de 88 à 99 % comparativement à la souche MLV, et de 51 à 54 % avec la souche LV. Bien que la majorité des substitutions d'aa déduites de l'analyse des gènes étaient distribuées de façon aléatoire, la région délimitée par les résidus 26 à 39 s'avéra très variable impliquant entre autre de 0 à 3 sites potentiels de N-glycosylation. Les produits des ORF5 de cinq de ces isolats, incluant les souches MLV et LV, furent exprimées dans la bactérie *E. coli* sous la forme de protéines recombinantes fusionnées à la glutathione S-transférase (GST) et des sérums hyperimmuns furent préparés chez des lapins. Les profils de réactivité des différents isolats observés avec les sérums hyperimmuns de lapin anti-ORF5, de même que ceux obtenus avec quatre anticorps monoclonaux dirigés contre la GP_5 de la souche québécoise de référence IAF-Klop du virus du SRRP, ont démontré que la GP_5 étaient aussi sujettes à des variations antigéniques. Les résultats obtenus suggèrent que des épitopes neutralisants, ne dépendant ni de la conformation ni de la présence de résidus carbohydrates, sont aussi associés à la variabilité antigénique de la GP_5 du virus du SRRP.

INTRODUCTION

Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of an economically important disease of pigs and is characterized by reproductive failure in sows and gilts and respiratory problems affecting pigs of all ages (1). The PRRSV is a member of a new group of enveloped, positive-strand RNA viruses, presently classified within the family *Arteriviridae*, which also includes equine arteritis virus (EAV), simian haemorrhagic fever virus, and lactate dehydrogenase elevating virus (LDV) (2). Together with members of the family *Coronaviridae*, these viruses have been recently grouped into the order *Nidovirales* (3). The genome of PRRSV is about 15 kb in length and contains 8 open reading frames (ORFs) (2,4). The ORF1a and ORF1b (at the 5′ end) represent nearly 75% of the viral genome and code for proteins with apparent polymerase and replicase activities (2,4). Six putative structural proteins have been identified and assigned to distinct smaller ORFs, namely ORFs 2 to 7, located at the 3′ end of the genome (5,6). The major structural proteins consist of a 25 kDa envelope glycoprotein (GP_5), an 18–19 kDa unglycosylated membrane protein (M), and a 15 kDa nucleocapsid (N) protein, encoded by ORFs 5, 6 and 7, respectively (5–7). Recent

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findings on the characterization of structural proteins of Lelystad virus (LV), the European prototype strain of PRRSV, indicate that expression products of the ORFs 2, 3 and 4 are also incorporated in virus particles as membrane-associated glycoproteins designated as GP_2,GP_3 and GP_4, respectively (8,9).

While the existence of apathogenic or low-pathogenic strains of PRRSV has been demonstrated (1,10), the correlation between genomic variations and virulence variability still remains to be established for this virus. Unlike other porcine viruses, no universally recognized serotyping system is known so far. Compared to the European isolates, the North American strains of PRRSV display a high degree of variability in their ORFs 2,3,5, and 7 coding regions with less than 60% amino acid (aa) identities (5,10–12). Among North American isolates, the ORFs 3, 4 and 5 show the highest degrees of diversity as compared to other coding regions of the viral genome (10,13). Antigenic variability has also been reported by independent investigators using either field convalescent pig sera (14) or monoclonal antibodies (MAbs) directed against the N and/or M proteins (15–18). So far, due to the lack of specific immunological probes, antigenic variability of the GP_5 of PRRSV has never been studied, while such a finding has been well established in the case of the major envelope glycoprotein (G_L) of the EAV (19,20).

We have recently obtained MAbs to the GP_5 of a Quebec reference PRRSV isolate by immunizing mice with the recombinant ORF5 encoded protein expressed in *E. coli*, and established that antigenic determinants of this major envelope glycoprotein are associated with virus neutralization (21). The present report describes the antigenic relationship among Canadian isolates of PRRSV, as established by sequence analysis of their ORF5 genes, through investigation of their reactivity with rabbit hyperimmune sera to *E. coli*- expressed recombinant ORF5 proteins of heterologous strains, and the above mentionned anti-GP_5 MAbs. The U.S. modified-live attenuated vaccine (MLV) strain, as well as LV, were included in these comparative studies.

MATERIALS AND METHODS

CELLS AND VIRUS STRAINS

A total of 9 Canadian field isolates of PRRSV, which were previously found to be different genomically and antigenically in regards to their N and M proteins (15,22), were further analysed in the present studies. The field isolates were recovered from clarified lung homogenates of dyspneic pigs and were initially propagated on porcine alveolar macrophages or MARC-145 (23), a PRRSV permissive cell line (courtesy of J. Kwang, USDA, Clay Center, Nebraska, USA). Subsequently, they were all adapted to MARC-145 cells and cloned by a limited dilution technique. The representative U.S. isolate of PRRSV, MLV strain, was isolated from the commercially available modified-live vaccine (Ingelvac, Boehringer Ingelheim Inc., St. Joseph, Missouri, USA). The vaccine isolate is believed to be derived from the ATCC (American Type Culture Collection, Rockville, Maryland, USA) reference strain VR-2332 (12). The Lelystad (LV) strain was kindly provided to us by G. Wensvoort (Central Veterinary Institute, Virology Department, Lelystad, The Netherlands). For the comparative studies, the majority of PRRSV field isolates were passaged not more than 5 times on MARC-145 cells.

REVERSE TRANSCRIPTION AND AMPLIFICATION OF THE ORF5 GENES

Supernatant fluids of PRRSV-infected cells were clarified and extracellular virions were concentrated by differential ultracentrifugation through a cushion of 30% sucrose (W/V), as previously described (5). Genomic RNA was extracted from the viral pellets by the one-step guanidinium isothiocyanate-acid phenol method (24). The ORF5 coding regions were amplified by RT-PCR as already described (5) using the following oligonucleotide primers: 1005PS (sense: 5′GGATCCATGTTGGGGAAATGCTTGACC3′) and 1005PR (antisense: 5′GGATCCGGCAAAAGTCATCTAGGG3′) for the North American isolates, and the primers ETS5L (sense: 5′GGATCCATGAGATGTTCTCACAAATTGG3′) and ETR5L (antisense: 5′GGATCCCATCTAGGCCTCCCATTG3′) for the LV strain. These primers, containing *Bam*HI restriction sites at their 3′ and 5′ ends, corresponded to the sequence adjacent to the first ATG or the stop codon of the ORF5 coding regions of the Quebec strain IAF-exp91 (EMBL/GenBank accession number L40898) and the European LV strain (EMBL/GenBank accession number M96262) (2,5).

cDNA CLONING AND SEQUENCING ANALYSIS

The RT-PCR amplified products were purified using the GeneClean II nucleic acid purification Kit (BIO 101, La Jolla, Ca), digested with *Bam*HI and ligated into a similarly treated pUC19 plasmid vector (Pharmacia Biotech Inc.). Alternatively, the PCR products with A overhangs were ligated into a TA cloning vector (pCRII vector, Invitrogen Co., San Diego, California, USA), providing single 3′ T overhangs at the insertion sites (22). The genomic region was sequenced on both strands by the dideoxynucleotide chain-termination method (25) using the T7 DNA polymerase (Pharmacia) in an Automated Laser Fluorescent DNA sequencer (Pharmacia LKB). To assess the error rate of the reverse transcriptase and *Taq* polymerase, clones from 3 different RT-PCR events were sequenced. Subsequently, the nucleotide (nt) and aa sequences were computer analyzed with the GeneWorks 2.4 program (IntelliGenetics Inc., Mountain View, California, USA). All comparisons were performed with a k-tupple length of one, and costs to open and to lengthen a gap of 2 and 4 for DNA, and of 5 and 25 for aa, respectively. The degrees of identity in nt and aa of the PRRSV isolates were deduced and compiled from sequencing data. The sequences obtained were analysed and compared with the published sequences of 2 reference European strains LV and PRRSV-10 (EMBL/GenBank accession numbers L04493) (2,4), and those of the U.S. reference strains ATCC VR-2332 (EMBL/GenBank accession number U00153) (12) and ATCC VR-2385 (EMBL/GenBank accession number U03040) (11). The nt sequence accession numbers (EMBL/GenBank/ libraries) of the 9 Canadian PRRSV isolates studied are as follows: U64928 for IAF-Klop; U64929 for IAF-BAJ; U64930 for IAF-DESR; U64931 for IAF 93-653;

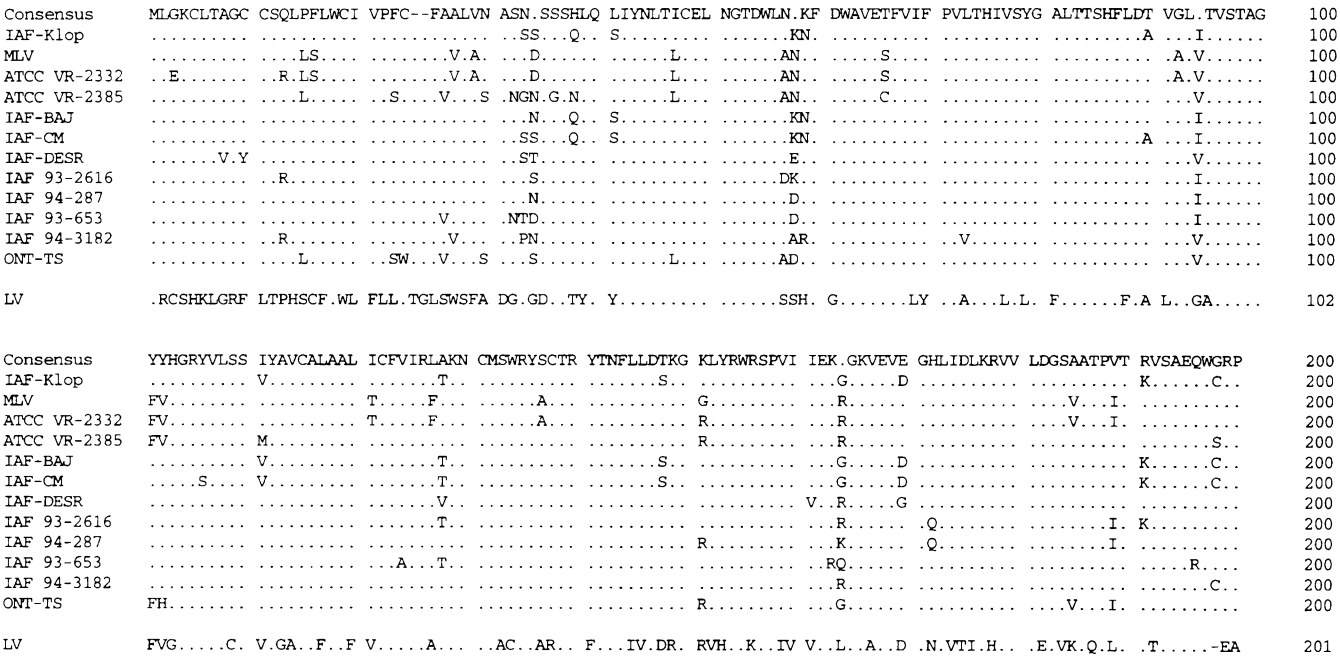

Figure 1. Alignment of the deduced amino acid sequences of the ORF5 encoded proteins of 9 Canadian field isolates of PRRSV, and comparison to that of the U.S. attenuated MLV strain, 2 U.S. reference strains (ATCC VR-2332 and ATCC VR-2385), and 2 European reference strains (LV and PRRSV-10). All sequences are compared with the consensus which is deduced from the ORF5 products of the North American strains; dots indicate the same aa as in the consensus and deletions are indicated by hyphens (-).

U64932 for IAF 93-2616; U64933 for IAF 94-3182; U64934 for IAF 94-287; AF013106 for IAF-CM, and U64935 for ONT-TS.

RECOMBINANT ORF5 FUSION PROTEINS AND IMMUNIZATION

The ORF5 coding regions of the MLV and LV strains, and those of 3 of the Canadian field isolates studied, were subcloned into prokaryotic expression vector pGEX-4T1 (Pharmacia), as previously described (7). Competent *Escherichia coli* cells, strain BL21 (DE3) (Novagen), were transformed by the recombinant plasmid pGEX-ORF5 and grown in 2YT medium containing 2% glucose to reach a density of 1.2–1.5 at 600 nm. Transformed bacteria were induced by addition of 0.1 mM of IPTG to the culture medium and further incubated at 37°C for 6 h with vigorous agitation. The GST-ORF5 fusion proteins, which accumulated within the cells in the form of inclusion bodies, were purified and solubilized in the presence of triton X-100 and 8 M urea, as previously described (26). The solubilized recombinant fusion proteins were refolded by chromatography on G25 sepharose column (Bio-Rad) and purified by affinity chromatography on glutathion-sepharose (Pharmacia) column, according to the manufacturer's directions.

For the preparation of anti-ORF5 hyperimmune sera, New Zealand albino rabbits (1600 g; Charles River Laboratories) were intradermally inoculated with 50–60 μg of recombinant GST-ORF5 fusion protein suspended in Freund's complete adjuvant (Difco), then boosted 3 times by intramuscular injection of 200 μg of GST-ORF5 fusion proteins in Freund's incomplete adjuvant (Difco) at 2-wk intervals. Hyperimmune sera were tested for anti-PRRSV antibodies by indirect immunofluorescence (IIF) using acetone fixed PRRSV-infected MARC-145 cells and fluorescein-conjugated goat's anti-rabbit Ig (Boehringer Mannheim, Laval, Quebec) (16).

SOURCE OF ANTI-PRRSV MONOCLONAL ANTIBODIES

Four MAbs directed against the GP_5 of the Quebec IAF-Klop strain of PRRSV were obtained from BALB/c mice that have been immunized with the *E. coli*-expressed GST-ORF5 recombinant protein of the Quebec reference IAF-Klop strain (21). Three of the anti-GP_5 MAbs are directed against linear neutralizing epitopes of PRRSV (IAF-8A8, IAF-1B8 and IAF-2A5) and the 4th (IAF-3B6) is apparently directed against a non-neutralizing conformational epitope. The MAb IAF-K8, also obtained from a previous study, is directed against a highly conserved epitope of the N protein in both North American and European strains of PRRSV (15). The SDOW17 MAb, also specific for the N protein of PRRSV (18) was a gift from D.A. Benfield (Department of Veterinary Science, South Dakota State University, Brookings, South Dakota, USA).

RESULTS

GENOMIC VARIATIONS BETWEEN THE ORF5 GENES OF CANADIAN, U.S., AND EUROPEAN PRRSV ISOLATES

The aa sequences of the ORF5 of the 9 Canadian PRRSV field isolates were deduced from sequence analyses of the RT-PCR amplified encoding genes. The sequences obtained were compared to that of the MLV strain propagated in MARC-145 cells, and the published sequences of 2 reference U.S. strains (ATCC VR-2332 and ATCC VR-2385) and 2 reference European strains (LV and PRRSV-10). A total of 588 nt substitutions were observed among the 12 North American isolates studied, of which 117 (19.8%) corresponded to purine substitutions (C-T; T-C), occurring mainly at wobble base, thus leading to silent aa mutations. As shown in

TABLE I. Percentage of identity in nucleotides and amino acids between the ORF5 products of different PRRSV isolates

PRRSV Isolate	IAF-Klop	MLV	ATCC VR-2332	ATCC VR-2385	IAF-BAJ	IAF-DESR	IAF 93-2616	IAF 94-287	IAF 93-653	IAF 94-3182	IAF-CM	ONT-TS	LV	PRRSV-10
IAF Klop		89%	89%	88%	99%	94%	94%	94%	94%	93%	99%	90%	63%	63%
MLV	85%		99%	93%	89%	90%	89%	91%	90%	91%	85%	92%	63%	62%
ATCC VR-2332	85%	99%		92%	89%	90%	89%	91%	90%	91%	85%	92%	63%	62%
ATCC VR-2385	87%	90%	90%		88%	89%	88%	90%	89%	90%	87%	92%	63%	63%
IAF-BAJ	99%	86%	86%	88%		94%	94%	94%	94%	94%	99%	90%	63%	63%
IAF-DESR	92%	88%	88%	89%	92%		94%	94%	94%	95%	92%	91%	62%	62%
IAF 93-2616	93%	89%	89%	88%	94%	93%		96%	93%	94%	89%	91%	62%	61%
IAF 94-287	92%	90%	90%	90%	93%	94%	94%		94%	94%	92%	93%	62%	61%
IAF 93-653	91%	87%	87%	89%	92%	93%	94%	94%		94%	91%	91%	62%	61%
IAF 94-3182	92%	89%	89%	90%	93%	94%	93%	94%	92%		92%	91%	62%	62%
IAF-CM	99%	89%	89%	88%	99%	94%	94%	94%	94%	93%		90%	60%	63%
ONT-TS	88%	92%	92%	93%	88%	90%	91%	93%	90%	90%	88%		63%	63%
LV	53%	53%	53%	54%	53%	52%	52%	53%	51%	52%	53%	54%		99%
PRRSV10	54%	53%	53%	54%	54%	54%	53%	55%	52%	54%	53%	54%	98%	

Top right side: % identity in nucleotide; Bottom left side: % identity in amino acids

Fig. 1, the aa sequence analysis of the ORF5 genes of the North American field isolates studied (including the MLV strain) revealed a total of only 147 (6.1%) aa substitutions which appeared to be randomly scattered across the protein, although one region in positions 26–39 appeared to represent a cluster. An overall 90.9% identity was calculated between the Canadian and U.S. isolates (Table I). No insertions or deletions of nt that might have resulted in aa insertion/deletion or frame shifting were found in the ORF5 of the North American isolates studied. In comparison, nt identities between the Canadian field isolates and the European reference strains were only of 62–63% resulting in overall aa identities of 51–54%. When the Canadian isolates were compared to each other, aa identities varied from 88–99%, the ONT-TS isolate being the most variable with 88–93% aa identity. Among the 13 North American isolates studied, the ORF5 product of Quebec field isolates IAF-DESR, IAF 94-287, IAF 93-2616 and IAF 94-3182 appeared the most conserved with less than ten aa substitutions (Fig. 1). In spite of several aa substitutions, the hydropathy profiles of the ORF5 translation products of the North American strains studied were identical to each other, and showed only minor variations with that of LV (data not shown).

Previous investigators have suggested that the first 32 aa of the ORF5 gene product of European PRRSV strains constitute the signal sequence with a putative cleavage site situated at position 32–33 (6). According to the prediction method of von Heijine (27), there is a high probability that

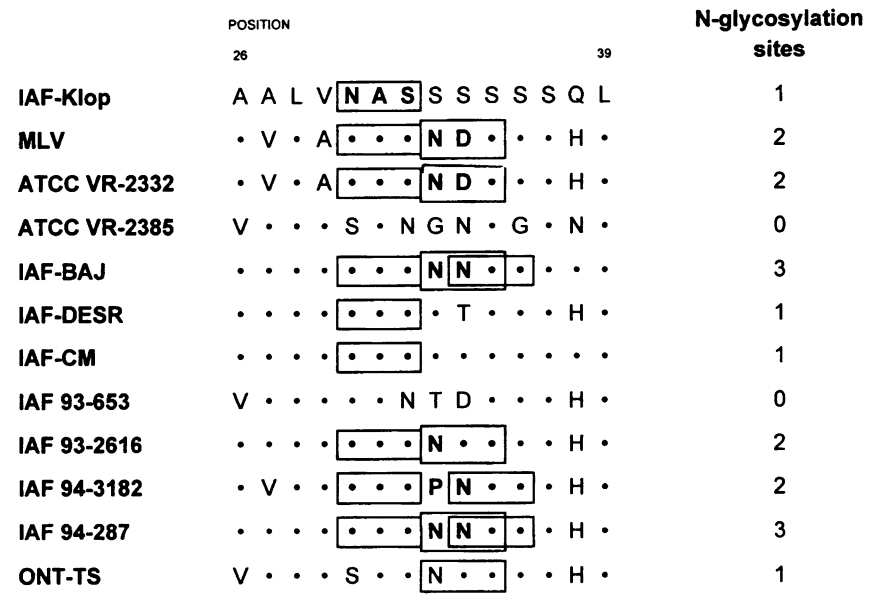

Figure 2. Detailed presentation of the hypervariable region of the GP_5 situated near the N terminal of the ORF5 (position 26 to 39), affecting the number of potential N-glycosylation sites (Boxes).

the putative cleavage site of the Quebec reference IAF-Klop strain be situated at position 25–26. With regards to the high percentage of aa identity (96%) between the N terminal of the Canadian field isolates studied and that of the MLV and the 2 U.S. reference strains (ATCC VR-2332 and ATCC VR-2385), the putative N terminal signal sequence of the North American strains of PRRSV seems to be located at aa 1 to 25. In this region, there was no aa identity between the North American and European strains, and the latters have 2 additional residues inserted at positions 25 (Threonine) and 26 (Glycine) (Fig. 1). This region contains only 4% aa substitutions among the North American strains.

As mentioned above, the region between aa residues 26 and 39 appeared as a hypervariable region with greater than 19% aa substitutions among the North American strains, including the number of potential N-glycosylation sites between aa 30 to 34 (Fig. 2). In this region, ATCC VR-2385 and IAF 93-2616, possess no N-glycosylation site while other isolates have 1–3 potential N-glycosylation sites. The GP_5 possesses 2 other N-glycosylation sites at position 44 and 51 shared by all of the Canadian field isolates studied, the MLV strain, the 2 reference U.S. strains and LV (Fig. 1). The other aa substitutions affected mainly positions 57–59, 94, 101–102, 111, 127–128, 151, 164, 170, 189, and 191. The aa mutations at positions 94 (V for I), 101 (F for Y), 189 (V for I) and 191 (K for R) are conserved among the North

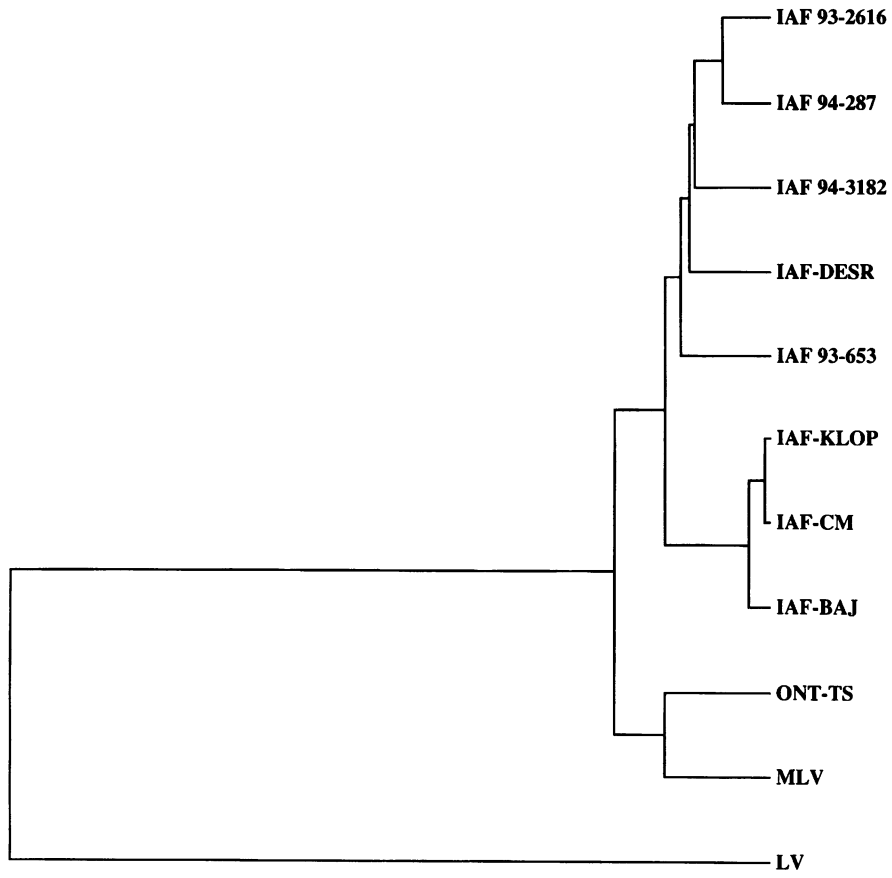

Figure 3. Phylogenetic relationship of 9 Canadian field isolates, the MLV and the prototype European LV strains of PRRSV based on the aa sequence of their ORF5 product. The horizontal lines connecting one sequence to another are proportional to the estimated genetic distance between the sequences.

TABLE II. Reactivity of strain-specific rabbit hyperimmune sera to the recombinant fusion protein GST-ORF5 to 5 different strains of PRRSV, EAV and TGEV

Anti-ORF5 sera to	IIF antibody titers to isolate[a]						
	LV	IAF-Klop	MLV[b]	IAF 93-653	ONT-TS	EAV[c]	TGEV[d]
LV	512	64	32	64	32	< 20	< 20
IAF-Klop	128	1024	128	512	256	< 20	< 20
MLV	256	256	1024	256	256	< 20	< 20
IAF 93-653	64	64	32	512	256	< 20	< 20
ONT-TS	128	128	128	512	1024	< 20	< 20

[a] Antibody titers of rabbit hyperimmune sera expressed as the reciprocal of the highest dilution of serum at which specific cytoplasmic fluorescence was observed
[b] MLV = U.S. attenuated vaccine strain of PRRSV
[c] EAV = Bucyrus strain of equine arteritis virus
[d] TGEV = Purdue strain of swine transmissible gastroenteritis virus

American strains. A single aa deletion was identified at position 198 of the LV and PRRSV-10 strains compared to the North American strains rendering the total number of aa residues of the ORF5 encoded region of the European strains one aa greater than that of the North American isolates (201 versus 200).

The sequencing data of the PRRSV field isolates were used to perform phylogenic analysis by Unweighted Pair Group Method with Arithmetic Mean (GeneWorks version 2.4) and accordingly, the Canadian isolates were grouped in a genotype distinct from that of the reference European LV strain (Fig. 3). However, at least 2 different clades were identified among the Canadian isolates studied, the ONT-TS isolate being classified with the U.S. MLV strain.

ANTIGENIC VARIABILITY OF THE ORF5 ENCODED PRODUCTS

In order to study the antigenic variability of the GP_5 of PRRSV isolates, 5 of the isolates studied belonging to distinct internal branches of the deduced phylogenic tree (Fig. 3) were selected for expression of their ORF5 product in *E. coli*. The GP_5 specificity of each of the antisera that were raised in rabbits against the recombinant GST-ORF5 fusion proteins was confirmed by Western immunoblotting using isopycnic sucrose gradient-purified virus, and by radioimmunoprecipitation using (^{35}S)-methionine-labelled PRRSV-infected cell lysates, as previously described (7,21). Study of the cross-reactivity by IIF of each of these antisera revealed higher antibody titers when tested with its autologous strain (Table II). None of these hyperimmune sera showed reactivity to RK-13 and ST-148 cells infected with the Bucyrus strain of EAV and the Purdue strain of porcine transmissible gastroenteritis virus (TGEV) (IIF antibody titers of < 20), respectively. The antiserum directed against the ORF5 protein of LV weakly recognized (IIF antibody titers of 32 to 64) the 4 North American strains tested, the antibody titers obtained against the homologous strain being 8 to 16 times higher. A difference of 4 to 8 dilutions was also obtained when testing each of the North American anti-ORF5 sera against LV, but the titers obtained were not significantly different from that obtained against heterologous North American isolates, except for the antiserum raised against the ORF5 product of IAF 93-653 isolate which only weakly reacted to the IAF-Klop strain, the MLV and LV strains. None of these 5 anti-ORF5 hyperimmune sera had neutralizing activity (data not shown). Thus, in general, study of the cross-reactivity of anti-ORF5 hyperimmune sera raised against PRRSV isolates of distinct internal branches of the phylogenic tree revealed the occurence of antigenic variability among the ORF5 product of the various PRRSV strains that may involve specific linear non-neutralizing epitopes. The data also suggest that there are linear conserved epitopes among the ORF5 encoded proteins of North American and European PRRSV isolates that are recognized by polyclonal hyperimmune sera.

MABS DIRECTED AGAINST THE GP_5 SELECTIVELY REACT WITH HETEROLOGOUS STRAINS OF PRRSV

To further investigate the antigenic variability of the major envelope glycoprotein GP_5 of PRRSV, the reactivities of 4 anti-GP_5 MAbs were tested by IIF to the 9 Canadian PRRSV field isolates, the MLV and the LV strains. The serological identification of the various PRRSV isolates studied was confirmed by testing their reactivities to MAbs SDOW17 and IAF-K8, 2 anti-PRRSV MAbs directed against common antigenic determinants of the N protein of the North American and European strains (15,18). As summarized in Fig. 4, all of the PRRSV isolates tested, including the LV strain, reacted with high titers (> 1:51 200) to both anti-N MAbs. The 4 anti-GP_5 MAbs reacted with approximately same titers (1:1600 to 1:6400) to the MLV strain, but failed to react with MARC-145 cells that have been infected with LV. None of the 6 anti-PRRSV MAbs tested showed reactivity to EAV- or TGEV- infected cells (IIF antibody titers of < 20). On the other hand, the 4 anti-GP_5 MAbs showed various reactivity profiles to the Canadian isolates studied and accordingly, the latters could be subdivided into 3 subgroups. The isolates classified in subgroup 1 comprised the Quebec reference strain IAF-Klop and 5 other Canadian field isolates (IAF 93-2616, IAF-DESR, IAF-CM, IAF 94-3182, and ONT-TS), as well as the MLV strain. The 2nd antigenic subgroup comprised 2 other Quebec isolates (IAF 93-653 and IAF-BAJ) which were selectively recognized by MAbs IAF-8A8, IAF-1B8 and IAF-3B6, but not by IAF-2A5. The remaining Quebec isolate IAF 94-287 was assigned to the subgroup 3, as it reacted with the MAbs IAF-8A8 and IAF-1B8 but neither with IAF-2A5 nor with IAF-3B6. It should be noted that in some cases, the IIF titers of MAbs were lower when they were tested with heterologous strains as compared to the homologous IAF-Klop strain. The reactivity of MAbs was considered as positive when a specific cytoplasmic fluorescence with PRRSV-infected cells was obtained at a minimum reciprocal dilution of 1:400.

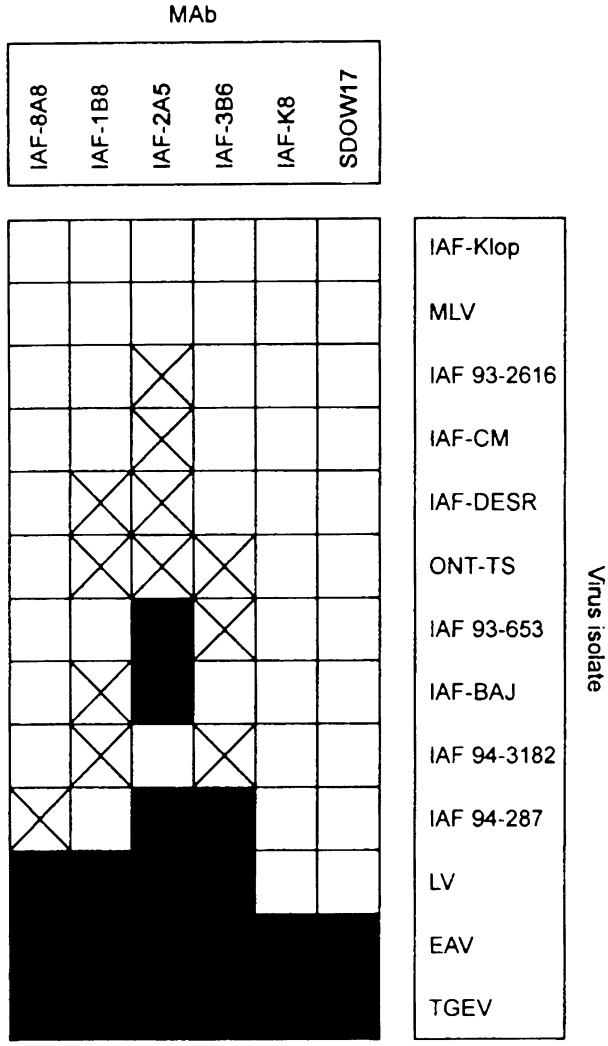

Figure 4. Cross-reactivity of monoclonal antibodies to the IAF-Klop strain of PRRSV with other Canadian field isolates, the MLV and LV strains. The cross-reactivity between the various isolates was tested by indirect immunofluorescence with 4 anti-GP_5 MAbs (IAF-8A8, IAF-1B8, IAF-2A5 and IAF-3B6), and 2 anti-N MAbs (IAF-K8 and SDOW17). Reactivity to the heterologous virus is expressed as the reciprocal of the highest dilution of ascitic fluid giving a positive cytoplasmic fluorescence with PRRSV-infected MARC-145 cells. No reactivity was observed toward the Bucyrus strain of EAV and the Purdue strain of TGEV. □ IIF titers of 1600 to 12 800; ⊠ IIF titers of 400 and 800; ■ IIF titers of < 100.

DISCUSSION

In the present study, genomic and antigenic variations have been found to be associated with the major envelope GP_5 protein of PRRSV. The North American strains could also be grouped in a genotype distinct from that of the European strains with regards to their ORF5 coding sequences, in agreement with previous findings by others (11–13,15). However, the antigenic diversity of the GP_5 of the North American field isolates was not associated with an extensive degree of aa sequence heterogeneity. It is noteworthy that no correlation could be established between the phylogenetic relationships of the various PRRSV isolates analysed and antigenic subgroups as defined by their reactivities to monospecific polyclonal anti-ORF5 sera and a set of 4 neutralizing MAbs directed against the *E. coli*-expressed ORF5 product of the Quebec reference IAF-Klop strain. The data further suggest that linear neutralizing epitopes, independent of conformation and glycosylation, are also associated with antigenic variability of the GP_5 of PRRSV.

Like most RNA viruses, notably the EAV (20,28,29), PRRSV is also genomically heterogenous. The aa identity of the ORF5 gene product of

7 U.S. isolates of PRRSV was previously reported to vary from 88% to 97% (10). Similarly, aa identities of the ORF5 encoded G_L protein of EAV field isolates ranged between 85.7% and 99.7%, highest degrees of identity existing among the North American isolates (28). These results have been confirmed by a recent report indicating that the North American isolates of EAV are included in a clade apart from that of the European prototype strain (29). The antigenic variability of the G_L protein of EAV has also been established by the selective reactivity of different field isolates with anti-G_L MAbs (20). The genomic variability may be attributed to the lack of proof reading function of viral RNA dependent RNA polymerase. Concurrence of variable regions with the hydrophilic domains of GP_5 can be the result of host's selective humoral immune response directed against the exposed domains of this envelope glycoprotein which in turn favours antigenic drifts. The genomic variations contribute to the emergence of antigenic variants, which is an effective mechanism for evading the host's immune surveillance.

As in the case of EAV (19,20), the ORF5 encoded envelope glycoprotein of PRRSV has been found to be associated with virus neutralization (21) and therefore, contains immunologically important domains. Clinical manifestations of PRRS are diverse and complex and they depend in part on the immune status of the host. Whether mutations within the antigenic domains of the GP_5 may contribute to the establishment of a chronic form of the disease and eventually a persistent infection remains to be demonstrated.

It has been also demonstrated that while anti-ORF5 sera produced in rabbits cross-reacted with the heterologous strains, they gave highest titers when tested in IIF with the autologous strain of PRRSV. This can be due to the concomitant association of common and strain-specific epitopes with the GP_5 of the 5 strains of PRRSV used in our experiments. The shared epitopes are implicated in the cross-reactivity of the strain-specific antisera while strain-specific epitopes resulted in higher titers when these antisera were tested with the autologous strains of the virus. Further experiments such as competitive ELISA with monospecific antisera are needed to elucidate this assumption. In mice, the reactivity patterns of a panel of 4 MAbs with different strains of PRRSV suggested the possibility of at least 3 distinct epitopes associated with the GP_5. It can be anticipated that the 2 MAbs IAF-8A8 and IAF-1B8 are directed against the same or two closely located neutralizing epitopes since all North American PRRSV strains tested were simultaneously recognized by both MAbs (Fig. 4). The MAb IAF-2A5 is apparently directed against a distinct neutralizing epitope since it failed to react or reacted only weakly with half of the strains tested, whereas the MAb IAF-3B6 recognized a non-neutralizing conformational epitope (21). It is noteworthy that high degrees of nt and aa identity between strains do not necessarily result in similar reactivity patterns with MAbs and, only one point mutation in the epitope recognizable by a MAb may change the reactivity of the strain with that MAb. For example, while the 2 Quebec isolates IAF-Klop and IAF-BAJ have 99% identity in nt and aa sequences for their ORF5 gene product, they showed different reactivity patterns with the MAb IAF-2A5 and inversely, the ONT-TS and IAF-DESR with only 90% identity shared identical reactivity patterns with the 4 anti-GP_5 MAbs.

The location of the epitopes associated with the GP_5 of PRRSV is still to be determined. On the other hand, it has been clearly demonstrated that the ectodomain of the G_L glycoprotein of EAV (approximately 95 aa in length) which is located at its N-terminal half, contains a highly immunogenic region consisting of not more than 44 aa residues (19,30). A linear immunodominant epitope maps to this region which induces neutralizing antibody in horses (30). Moreover, characterization of different neutralization-resistant escape mutant viruses with a panel of six anti-EAV neutralizing MAbs, as well as competitive binding assays, indicated that this linear immunodominant region of G_L encompasses three overlapping or closely adjacent neutralizing epitopes (19). In agreement, recent aa sequence analysis of the G_L protein of Canadian, American and European EAV isolates revealed 2 major variable regions encompassing residue positions 61 to 104, predicted to be located in the ectodomain of the protein (29). It remains to be demonstrated if highly variable regions (aa positions 26 to 39, and 57 to 59) identified in the present study are the counterparts of these highly variable regions of EAV bearing major neutralizing epitopes. The region corresponding to aa positions 26 to 39 of the PRRSV ORF5 protein also displayed difference in glycosylation patterns between strains but its significance remains to be established. Interestingly, the facts that all anti-ORF5 MAbs tested were found to be directed to linear neutralizing determinants not affected by the absence of carbohydrate residues (21), and no correlation could be demonstrated between patterns of MAb recognition and aa sequence differences in the N-terminal region of GP_5 among the different PRRSV isolates tested, suggest that the latter MAbs are probably directed to epitopes located in other regions of the GP_5 envelope glycoprotein.

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