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Differentiation of U.S. and European Isolates of Porcine Reproductive and Respiratory Syndrome Virus by Monoclonal Antibodies†

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Monoclonal antibodies (MAbs) to two U.S. isolates of porcine reproductive and respiratory syndrome (PRRS) virus were prepared. Two MAbs specifically recognized a conserved epitope on the putative 15-kDa nucleocapsid protein of U.S. and European isolates of PRRS virus. Four other MAbs recognized epitopes on the 15-kDa protein of U.S. but not European isolates of PRRS virus. Collectively, this indicates that PRRS viruses contain both conserved and divergent epitopes on the 15-kDa viral protein.

Porcine reproductive and respiratory syndrome (PRRS) was first described in the United States in 1987 (14) and in Europe in 1990 (1). This syndrome has also been called mystery swine disease, swine infertility and respiratory syndrome, and porcine epidemic abortion and respiratory syndrome. Clinically, PRRS is characterized by inappetence and respiratory distress in pigs of all ages; high mortality in neonatal and weaned pigs; poor conception in breeding herds; and increased numbers of aborted, stillborn, and weak pigs in pregnant sows (14, 18, 23). Recently, the etiologic agent of PRRS was isolated by investigators in The Netherlands (Lelystad isolate [23]) and the United States (ATCC VR-2332 [3, 6]). Both the Lelystad and ATCC VR-2332 isolates of PRRS are small, enveloped RNA viruses with morphologic and physicochemical properties similar to those of the non-arthropod-borne togaviruses. Recent evidence on the nucleotide sequence, genomic organization, and replication strategy of the Lelystad isolate and a German isolate of PRRS virus indicates that this virus is similar to members of the family *Arteriviridae*, which consists of lactate dehydrogenase-elevating virus (LDV), equine arteritis virus (EAV), and simian hemorrhagic fever virus (SHFV) (7, 17, 19).

Although the U.S. and European isolates of PRRS virus share similar morphological and physicochemical properties, preliminary results indicate that these isolates are antigenically different. When polyclonal antisera to the Lelystad and ATCC VR-2332 isolates of PRRS virus were used in an immunoperoxidase monolayer assay, results indicated that all isolates shared common antigens. However, the PRRS virus isolates could be divided into two distinct antigenic classes, U.S. and European, with the U.S. isolates being more antigenically diverse than the European isolates (22).

The goals of the present study were to identify the numbers and molecular masses of PRRS viral proteins and to develop monoclonal antibodies (MAbs) which could be used in immunologic assays to determine the heterogeneity of U.S. and European isolates of PRRS virus. Using these MAbs, we demonstrated that a 15-kDa structural viral protein, the putative PRRS virus nucleocapsid protein, contains conserved and variable epitopes on U.S. and European isolates of PRRS virus.

MATERIALS AND METHODS

Cells and viruses. Field isolates of PRRS virus were isolated on either primary procine alveolar macrophages, CL2621 cells (courtesy of Boehringer-Ingelheim Animal Health, St. Joseph, Mo.), or MA-104 cells. One isolate, SD 92-1509 (SD1), was initially isolated on McClurkin swine testis (ST) cells (16). Primary porcine alveolar macrophages were prepared and maintained as described by Wensvoort et al. (23). The CL2621, MA-104, and ST cells were maintained as described previously (2–4).

The ATCC VR-2332 and SD1 isolates of PRRS virus were propagated on MA-104 cells. These virus isolates were harvested and purified on CsCl gradients as described previously (3). Sixty isolates of PRRS virus were obtained from field specimens submitted to the South Dakota Animal Disease Research and Diagnostic Laboratory, and three isolates were obtained from the Department of Veterinary Diagnostic Medicine, University of Minnesota, St. Paul (courtesy of S. Goyal). European isolates were propagated on porcine alveolar macrophage cultures as described previously (22). The SDOW17, VO17, and EP147 MAbs were sent to G. Wensvoort, Central Veterinary Institute, Lelystad, The Netherlands; T. Drew, Central Veterinary Laboratory, Surrey, United Kingdom; A. Brun, Rhône Mérieux, Lyon, France; and A. Bøtner, State Veterinary Institute for Virus Research, Lindholm, Denmark, to test for their reactivities with various European isolates.

Polyclonal antisera. Polyclonal antiserum to U.S. isolate ATCC VR-2332 was prepared in gnotobiotic pigs. Briefly, 3-day-old gnotobiotic pigs were inoculated intranasally with 10^5 50% tissue culture infective doses ($TCID_{50}$s) of ATCC VR-2332 per ml. Pigs were then inoculated subcutaneously with a 2-ml suspension (1 ml of ATCC VR-2332 [titer, 10^6 $TCID_{50}$s/ml] and 1 ml of Freund's incomplete adjuvant) 14 and 28 days later. Serum specimens were collected 70 days

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after the final injection and had a neutralizing titer of 1,024 and an indirect fluorescent-antibody titer of 2,048. Antisera to the Lelystad isolate of PRRS virus was kindly provided by M. Frye, National Veterinary Services Laboratories, Ames, Iowa.

Hybridoma production. Eight- to 10-week-old specific-pathogen-free BALB/c AnN mice were maintained in microisolator plastic cages (Lab Products Inc., Maywood, N.J.). Mice were immunized intraperitoneally with either 10^6 $TCID_{50}$s of ATCC VR-2332 or 10^5 $TCID_{50}$s of SD1 in equal volumes of Freund's complete adjuvant. Identical booster injections were given 5 weeks later. Three weeks after the booster injection and 2 days prior to the harvest of splenocytes, the mice were immunized intravenously with either of the PRRS virus isolates in phosphate-buffered saline (PBS; pH 7.4). Fusion of mouse splenocytes and myeloma cells was done as described by Galfre et al. (9), except that P3/NS-1/1-Ag4-1 myeloma cells and 50% polyethylene glycol (PEG 4000; GIBCO, Grand Island, N.Y.) were used. Culture supernatants from viable hybridomas were screened for antibody against the homologous PRRS virus by a cell culture immunofluorescence (CCIF) assay. Positive hybridomas were subcloned at least twice by limiting dilution. The immunoglobulin isotypes of the MAbs were determined by using hybridoma culture fluids and a commercial immunoglobulin typing kit (ICN Biomedicals, Inc., Costa Mesa, Calif.). Ascitic tumors were produced in pristane-primed BALB/c AnN mice, and ascitic fluid was harvested as described previously (9), filtered (pore size, 0.45 μm; Millipore Corp., Bedford, Mass.), and stored at −20°C.

CCIF assay. The CCIF assay was done with confluent monolayers of MA-104 cells grown in 96-well microtiter plates, which were either inoculated 48 h later with different PRRS virus isolates (multiplicity of infection, 0.1) or left as uninoculated controls. Twenty-four hours after inoculation, cells were fixed in 80% acetone for 10 min at room temperature and air dried. Then, either 50 μl of supernatants from hybridoma cells or ascitic fluid dilutions was added to inoculated and uninoculated cells in duplicate wells and the plates were incubated for 30 min at 37°C. The plates were then washed three times with PBS, 50 μl of fluorescein-conjugated immunoglobulin G (IgG) fraction of goat anti-mouse immunoglobulins (IgA, IgG, and IgM heavy and light chains; Cappel, West Chester, Pa.) was added to each well, and plates were incubated for 30 min at 37°C. Plates were then washed three times with PBS. Finally, 100 μl of PBS was added to each well, and the plates were observed by fluorescence microscopy. Titers of mouse ascitic fluid were expressed as the reciprocal of the highest dilution at which specific fluorescence was observed.

RIP and immunoblotting. A modification of the protein A-Sepharose technique was used for radioimmunoprecipitation (RIP) (10). Briefly, confluent monolayers of MA-104 cells were inoculated with either the ATCC VR-2332 or the Lelystad isolate of PRRS virus (multiplicity of infection, 10) in Eagle's minimal essential media (MEM; GIBCO) containing 2% horse serum. After 1.5 h of incubation at 37°C, the inoculum was replaced with MEM plus 2% horse serum and cells were maintained at 37°C for 4.5 h. At 6 h postinfection, cell monolayers were washed twice with PBS and cysteine- and methionine-free MEM was added. After 1 h, 50 μCi of Tran-^{35}S label (>1,000 Ci/mmol; ICN) per ml was added and the cells were incubated for 10 to 12 h at 37°C. Cells were then rinsed with cold PBS and were resuspended in lysis buffer (10 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate [SDS] containing 1 mM phenylmethylsulfonyl fluoride) for 5 min. Cell lysates from inoculated and uninoculated cells were centrifuged at 10,000 × *g* for 5 min, and 125 μl of each supernatant was incubated overnight at 4°C with 10 μl of polyclonal antiserum or ascitic fluid. After 12 to 18 h, the immune complexes were precipitated with protein A–Sepharose CL-4B beads (Pharmacia, Piscataway, N.J.) for 1 h at room temperature. The precipitates were collected by centrifugation at 10,000 × *g* for 3 min and were then washed three times with lysis buffer and twice with distilled water. Pellets were then resuspended in the sample buffer of Laemmli (15) and heated for 1 min at 100°C prior to analysis by SDS-polyacrylamide gel electrophoresis (PAGE; 12% acrylamide). Gels were dried, and immunoprecipitated virus proteins were visualized by autoradiography.

Immunoblotting was done under reduced or nonreduced conditions by using concentrated and fluorocarbon-extracted lysates of MA-104 cells inoculated with either the ATCC VR-2332 or the Lelystad isolate of PRRS virus (10). Briefly, virus proteins were separated on SDS-polyacrylamide (12% acrylamide) gels and were transferred to nitrocellulose paper by electroblotting as described previously (20). Polyclonal antiserum or ascitic fluid was diluted 1:100 in PBS containing 0.05% Tween 20 (PBS-T_{20}), and the mixture was incubated with nitrocellulose strips for 1 h at room temperature. Strips were washed three times in PBS-T_{20} and were then incubated with ^{125}I-labeled protein G (0.1 μCi/ml; ICN) for 1 h at room temperature. Nitrocellulose strips were then washed three times in PBS-T_{20} and once in PBS and were air dried prior to visualization of antibody-protein complexes by autoradiography.

RESULTS

Immunoblotting and RIP were used to determine the number and relative molecular masses of the immunogenic viral proteins expressed in MA-104 cells inoculated with the ATCC VR-2332 and Lelystad isolates of PRRS virus. The SD1 isolate did not grow to a sufficient titer to be used in the immunoblotting and RIP assays. Three proteins with molecular masses of 15, 19, and 26 kDa were identified by the immunoblotting method with polyclonal antisera to ATCC VR-2332 and lysates of MA-104 cells inoculated with ATCC VR-2332 (Fig. 1, lane B). These three proteins were not observed in lysates from uninoculated cells (Fig. 1, lane A). A broad band of 60 to 80 kDa was observed in both inoculated and uninoculated MA-104 cells, indicating that this band was probably cellular proteins. Similar PRRS viral proteins were identified by immunoblotting by using antisera to Lelystad isolates and lysates of MA-104 cells inoculated with the Lelystad isolate (data not shown). Immunoprecipitations were performed with [^{35}S]cysteine–[^{35}S]methionine-labeled PRRS virus (ATCC VR-2332 and Lelystad isolates) by using polyclonal pig antisera, and the same three proteins were identified (data not shown). As in the immunoblotting assay, these proteins were precipitated only in lysates of MA-104 cells inoculated with either the ATCC VR-2332 or the Lelystad isolate of PRRS virus. Collectively, the results of the immunoblotting and RIP assays indicated that there were at least three proteins associated with the PRRS virus.

Cell culture fluids from three primary fusion products contained antibodies that recognized PRRS viral antigens in the cytoplasms of cells infected with the ATCC VR-2332 isolate of virus. Subcloning of these three primary products produced six hybridomas. Hybridomas SDOW12 and

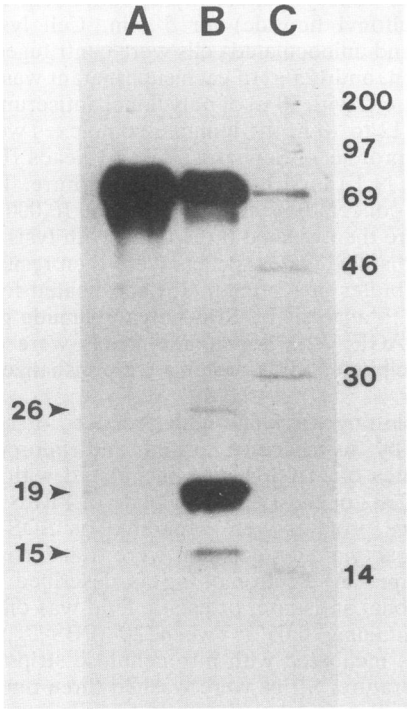

FIG. 1. Immunoblot of lysates from uninoculated MA-104 cells (lane A) and MA-104 cells inoculated with the PRRS virus ATCC VR-2332 (lane B) by using hyperimmune pig antisera. Arrows indicate the positions of PRRS virus proteins. Molecular mass standards are given in lane C (in kilodaltons).

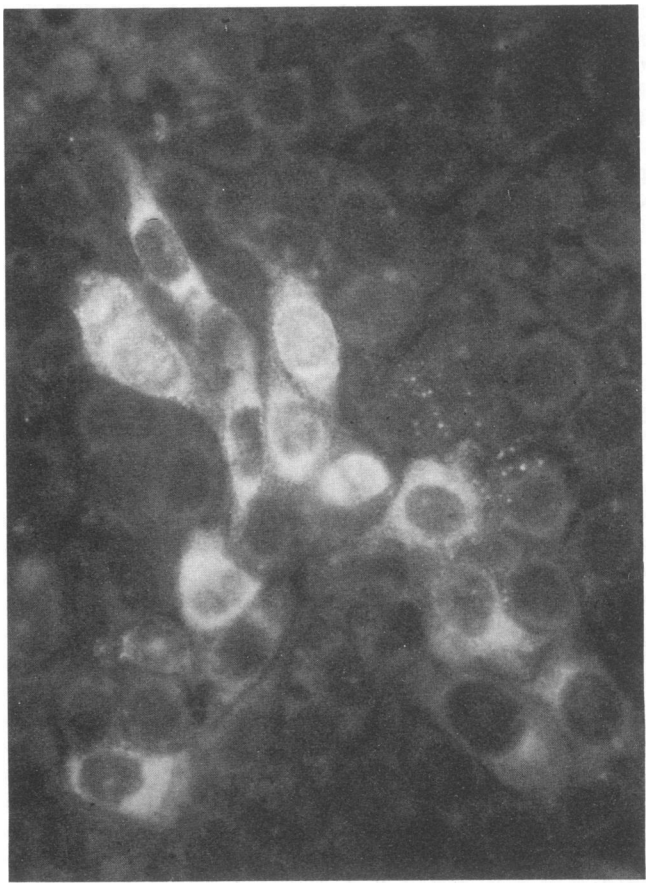

FIG. 2. CCIF staining of PRRS virus-inoculated MA-104 cells with MAb SDOW12. The focus of PRRS virus-inoculated cells shows an intense, often granular cytoplasmic fluorescence observed 3 days postinoculation. Magnification, ×200.

SDOW17 were fusion products from the splenocytes of mice immunized with the ATCC VR-2332 isolate of PRRS virus. The other hybridomas (VO17, VO22, EP147, and EP160) were produced from the splenocytes of mice immunized with the SD1 isolate of PRRS virus. Those hybridomas designated by identical letters originated from the same primary fusion product. Culture and ascitic fluids from each of the six MAbs produced bright, granular fluorescence, as determined by the CCIF assay in MA-104 cells inoculated with the ATCC VR-2332 isolate of PRRS virus (Fig. 2). Similar patterns of fluorescence were also observed in the same cells inoculated with the SD1 or the Lelystad isolate of PRRS virus (data not shown).

Cell culture and ascitic fluids from each of the six hybridomas were also used in RIP and immunoblotting assays. The SDOW, VO, and EP MAbs precipitated by RIP a 15-kDa viral protein in lysates from MA-104 cells inoculated with the ATCC VR-2332 isolate of PRRS virus (Fig. 3, lanes C, F, and I) but not in lysates from uninoculated cells (Fig. 3, lanes B, E, and H). This protein was also precipitated by the SDOW MAbs in lysates containing the Lelystad isolate of PRRS virus (Fig. 3, lane D). In contrast, the VO and EP MAbs precipitated the 15-kDa protein from the ATCC VR-2332 isolate of PRRS virus (Fig. 3, lanes F and I), but not the Lelystad isolate of PRRS virus (Fig. 3, lanes G, J). The SDOW MAbs occasionally precipitated a 74-kDa protein in lysates of MA-104 cells inoculated with either the ATCC VR-2332 or the Lelystad isolate of PRRS virus (Fig. 3, lanes C and D). After separation of viral proteins by SDS-PAGE, none of the six MAbs bound in the immunoblotting assay, suggesting that these antibodies recognized conformationally dependent epitopes on the 15-kDa protein. These results indicate that the SDOW MAbs recognizes a conserved epitope on the 15-kDa protein of the ATCC VR-2332 and Lelystad isolates of PRRS virus. In contrast, the VO and EP MAbs recognized epitopes on a protein with a molecular mass similar to those of the SDOW MAbs, but this epitope was present only on the ATCC VR-2332 isolate and not the Lelystad isolate of PRRS virus.

Ascitic fluid produced by the SDOW hybridomas had high titers of MAb to all three isolates of PRRS virus, as determined by the CCIF assay. Titers of ascitic fluid from the VO and EP hybridomas, however, were 5- to 10-fold lower than the CCIF assay titers obtained with the SDOW MAbs, and these MAbs failed to recognize epitopes on the Lelystad isolate of PRRS virus (Table 1). These results confirmed the RIP assay data that we had derived MAbs that recognized both common and variable epitopes on the U.S. and European PRRS viruses. We then used the CCIF assay and one MAb from each primary fusion product to determine the extent of antigenic variability between field isolates of PRRS virus from the United States and Europe. Since we were unable to import certain European isolates of PRRS virus, we provided the SDOW, VO, and EP MAbs to four laboratories in Europe. The results presented in Table 2 confirmed our preliminary data, obtained by the CCIF assay (Table 1), that the SDOW MAbs recognize a common epitope on all

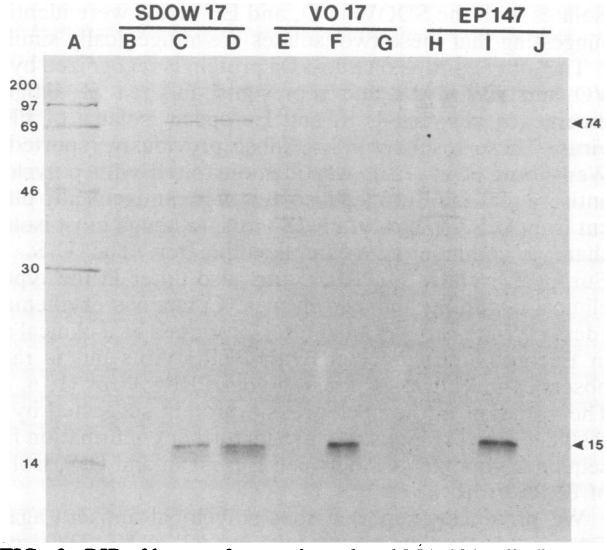

FIG. 3. RIP of lysates from uninoculated MA-104 cells (lanes B, E, and H), cells inoculated with the ATCC VR-2332 isolate of PRRS virus (lanes C, F, and I), and cells inoculated with the Lelystad isolate of PRRS virus (lanes D, G, and J) with SDOW17, VO17, and EP147 MAbs. Arrowheads indicate the positions of PRRS virus proteins. Molecular mass standards are given in lane A (in kilodaltons).

PRRS virus isolates assayed, regardless of the country of origin. In contrast, the VO and EP MAbs identified PRRS viral antigens only on U.S. isolates of PRRS virus. These results indicated that isolates of PRRS virus from the United States and Europe share a common epitope identified by the SDOW MAbs and are also antigenically distinct, as indicated by the VO and EP MAbs.

DISCUSSION

We previously suggested, on the basis of morphologic and physicochemical properties, that the ATCC VR-2332 isolate of PRRS virus is a non-arthropod-borne togavirus similar to LDV, EAV, and SHFV (3). Recently, LDV, EAV, and SHFV were tentatively classified in the family *Arteriviridae* (5, 19). Since our initial report, the genomic organizations of the Lelystad isolate and a German isolate of PRRS virus have also been determined to be similar to those of the arteriviruses (7, 17). In this report, we presented evidence that PRRS virus has viral proteins similar in molecular mass to those described for the arteriviruses. In addition, we derived MAbs to one of the viral proteins and were able to conclusively demonstrate that isolates of PRRS virus from the United States and Europe share common antigenic epitopes, but are also antigenically distinct viruses.

Three viral proteins with molecular masses of 15, 19, and 26 kDa were identified in lysates of cells inoculated with PRRS virus by both immunoblotting and RIP. These three proteins were considered to be PRRS viral proteins, because their presence was observed only in lysates of cells inoculated with either the ATCC VR-2332 or the Lelystad isolate of PRRS virus. We suspect that these proteins are virion structural proteins, because a comparison of our immunoblotting and RIP assay results for the structural proteins of PRRS virus reveals that they have a striking similarity to the virion structural proteins of the arteriviruses LDV, EAV, and SHFV. The viral particles of LDV and EAV have been reported to contain a nucleocapsid protein of 12 to 15 kDa, a nonglycosylated membrane protein of 16 to 19 kDa, and a single envelope glycosylated protein of 24 to 44 kDa for LDV, whereas EAV has two glycosylated envelope proteins of 25 and 30 to 42 kDa (7, 13, 19, 24). The structural proteins of SHFV also have molecular masses similar to those of our PRRS virus isolates (21). Our results, in conjunction with those reported for LDV, EAV, and SHFV, indicate that the 15-, 19-, and 26-kDa proteins are likely the nucleocapsid, nonglycosylated membrane, and glycosylated envelope proteins, respectively, of PRRS virions. However, additional studies with gradient-purified PRRS virions and monospecific polyclonal antisera in immunoblots or RIP assays will be necessary to verify that these three proteins are virion structural proteins. Also, the RIP and immunoblotting techniques identify only those viral proteins to which the host produces antibodies. There are other viral proteins that may be nonimmunogenic or poorly immunogenic and that were therefore not detected by the assays used in our study. Studies on the Lelystad isolate and a German isolate of PRRS virus suggest that the genome has coding capacity for at least six to seven proteins (7, 17), and only three were identified in our study. Nevertheless, the molecular masses of the three major proteins of PRRS virus in combination

TABLE 1. Characteristics of MAbs to PRRS virus

MAb[a]	Titer of ascitic fluid to PRRS virus isolates as determined by CCIF assay[b]:		
	ATCC VR-2332	SD1	Lelystad
SDOW12	1:5,000	1:5,000	1:5,000
SDOW17	1:5,000	1:5,000	1:5,000
VO17	1:1,000	1:1,000	<1:4
VO22	1:500	1:500	<1:4
EP147	1:1,000	1:1,000	<1:4
EP160	1:500	1:500	<1:4

[a] SDOW hybridomas were produced by using splenocytes from mice immunized with the ATCC VR-2332 isolate of PRRS virus; VO and EP hybridomas were produced with splenocytes from mice immunized with SD1. All MAbs were of the IgG1 isotype.

[b] Titer is expressed as the highest dilution of ascitic fluid which produced distinct cytoplasmic fluorescence.

TABLE 2. Reactivities of MAbs with various U.S. and European isolates of PRRS virus determined by CCIF assay

Isolate source	No. of PRRS isolates positive/no. of PRRS isolates tested		
	SDOW17	VO17	EP147
United States	63/63	63/63	63/63
United Kingdom	32/32	0/32	0/32
Spain[a]	10/10	0/10	0/10
The Netherlands[a]	8/8	0/8	0/8
Germany[a]	2/2	0/2	0/2
Luxemburg[a]	1/1	0/1	0/1
Italy[a]	1/1	0/1	0/1
France[a,b]	2/2	0/2	0/2
Denmark[c]	1/1	ND[d]	0/1

[a] Isolates tested by G. Wensvoort by an immunoperoxidase monolayer assay (17).

[b] One isolate tested by A. Brun, Rhône Mérieux, Lyon, France. Reactivity determined by an immunoperoxidase monolayer assay.

[c] Reactivities were determined by an immunoperoxidase monolayer assay and enzyme-linked immunosorbent assay (4a).

[d] ND, not determined.

with the virus morphology, physicochemical properties (3, 18, 23), and genomic organization (7, 17) substantiate the fact that PRRS virus is an arterivirus.

Each of the six MAbs produced to two different PRRS virus isolates was directed to the 15-kDa viral protein, which we believe to be the nucleocapsid protein. Although much effort was expended, we could not obtain MAbs which recognized epitopes on the 19- or 26-kDa viral proteins. We also cannot explain why the 15-kDa protein is immunodominant in the mouse. This was surprising to us, because the 19-kDa protein band was usually the most intense on the immunoblots. However, others have reported difficulty in producing MAbs to arterivirus proteins, such as the neutralizing epitopes on the envelope membrane of LDV (11). While inactivation of LDV with either glutaraldehyde or formalin improved the chances of obtaining MAbs to the envelope glycoprotein of LDV (11), we were unsuccessful in producing MAbs to other proteins using formalin-inactivated PRRS virus. Further attempts to produce MAbs to the 19- and 26-kDa proteins of PRRS virus may require the use of cDNA expression products as antigens to immunize mice. We note that the MAbs bound to one other protein of 74 kDa. However, this was not a consistent finding and may represent nonspecific binding of the MAb. There are no proteins of this molecular mass reported for other arteriviruses (8, 19), and the reported sequences of the Lelystad isolate (17) and a German isolate (7) of PRRS virus lack an open reading frame for a protein of this size.

The ability of the SDOW12 and SDOW17 MAbs to identify all U.S. and a limited number of European PRRS virus isolates indicates that most, if not all, isolates of PRRS virus have at least one conserved epitope on the nucleocapsid protein. We did not anticipate the lack of cross-reactivity of the VO and EP MAbs with the European isolates. It was expected that epitopes on the 15-kDa nucleocapsid protein would be highly conserved, because this internal viral protein would most likely escape immune pressures toward genetic variation. The results suggest that there are both conserved and variable epitopes on the nucleocapsid protein. An alternative explanation is that the SDOW, VO, and EP MAbs recognize different epitopes on different viral proteins of approximately the same molecular mass which cannot be distinguished by the immunoblotting or RIP assay. For example, the 14-kDa nucleocapsid and the 16-kDa nonglycosylated membrane proteins of EAV are very similar in molecular mass (8), and aberrant migration of the marker or viral proteins could influence the determination of the molecular mass of the protein. This was a possibility in our SDS-PAGE system as well, but there was a greater differential in molecular mass between the putative nucleocapsid (15-kDa) and nonglycosylated membrane (19-kDa) proteins of PRRS virus compared with those between the two proteins of EAV virions. Therefore, we favor the explanation that the SDOW and the VO and EP MAbs did recognize different epitopes on the same viral protein.

The epitope identified by the SDOW MAbs is highly conserved among all PRRS virus isolates. Both U.S. isolates (ATCC VR-2332, SD1) used to produce the SDOW, VO, and EP MAbs had similar reaction patterns in the CCIF assay. Although the VO and EP MAbs were produced with the SD1 isolate of PRRS virus, this isolate could not be grown to sufficient quantity on MA-104 or ST cells to be used in RIP or immunoblotting assays. While it would be of interest to compare the SD1 and VR-2332 isolates by RIP and immunoblotting, this was not technically possible. However, the CCIF assay reactions of the ATCC VR-2332 and SD1 isolates with the SDOW, VO, and EP MAbs were identical, suggesting that these two isolates are antigenically similar.

The other epitope on a 15-kDa protein is recognized by the VO and EP MAbs and represents an area of antigenic divergence between U.S. and European isolates of PRRS virus. These results confirm those previously reported by Wensvoort et al. (22), who demonstrated with polyclonal antisera that the European isolates were antigenically different from U.S. isolates of PRRS virus, although most isolates share a common antigenic denominator. The U.S. and European isolates of PRRS virus also differ in the type of clinical syndrome induced in pigs. Cutaneous erythema or edema ("blue ear") is most often described as a clinical sign in European pigs infected with PRRS virus but is rarely observed in U.S. pigs infected with PRRS virus (1, 6, 23). The variation in the nucleocapsid protein suggested by the results of the present study awaits further confirmation from sequence comparisons between European and U.S. isolates of PRRS virus.

We previously reported that polyclonal antisera against EAV and LDV did not react with the ATCC VR-2332 isolate of PRRS virus. Also, antisera against PRRS virus did not cross-react with either EAV or LDV by indirect immunofluorescence (3). Thus, it appears that the *Arteriviridae* may be subdivided into at least three antigenic groups (group I, EAV; group II, LDV; and group III, PRRS virus) on the basis of results of immunofluorescence assays. This division may be similar to that of the *Coronaviridae* (12), a family which the family *Arteriviridae* resembles in genomic organization, replication, and transcription (7, 17, 19). Our results also indicate that the PRRS viruses can be divided into two subgroups, with subgroup A representing the European prototype (Lelystad isolate) described by Wensvoort et al. (22) and subgroup B representing the U.S. prototype isolate (ATCC VR-2332) described by Benfield et al. (3).

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