Differential Reactivity of a Monoclonal Antibody Directed to the Membrane Protein of Porcine Reproductive and Respiratory Syndrome Virus

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ABSTRACT

A monoclonal antibody (2C12) against the 19 kDa membrane (M) protein of a Canadian isolate of porcine reproductive and respiratory syndrome (PRRS) virus was produced. By indirect immunofluorescence (IIF) cytoplasmic fluorescence was observed in infected cells, but the pattern of fluorescence was generally different and intensity was weaker than that observed using the nucleocapsid protein-directed monoclonal antibody SDOW17. When tested by IIF towards a total of 26 PRRS virus isolates from Canada, 122 isolates from the US and 13 isolates from Europe the 2C12 MAb reacted with all the North American isolates tested including the VR-2332 isolate and the vaccine (RespPRRS) isolate. However no reactivity was observed towards the European isolates tested including the Lelystad virus. This reactivity pattern suggests that the epitope recognized by this MAb on the M protein of PRRS virus appears highly conserved among North American isolates but absent or weakly expressed on European isolates of PRRS virus.

RÉSUMÉ

Un anticorps monoclonal (2C12) dirigé contre la protéine de la membrane (M) d'un isolat canadien du virus du syndrome reproducteur et respiratoire du porc (SRRP) a été généré. Par immunofluorescence indirecte (IFI), une fluorescence cytoplasmique fut observée dans des cellules infectées mais le patron de fluorescence était différent et l'intensité plus faible avec cet anticorps monoclonal comparativement à l'anticorps monoclonal SDOW17 dirigé contre la nucléocapside du virus SRRP. Lorsqu'un total de 26 isolats de virus SRRP du Canada, 122 isolats des États-Unis et 13 isolats européens furent évalués par IFI avec l'anticorps monoclonal 2C12, une réaction fut observée avec tous les isolats nord-américains, y compris les souches VR-2332 et vaccinale (RespPRRS). Toutefois aucune réactivité ne fut observée avec les isolats européens y compris l'isolat de Lelystad. Ce patron de réactivité suggère que l'épitope reconnu par cet anticorps monoclonal, sur la protéine M du virus SRRP, est apparemment hautement conservé chez les souches nord-américaines et peu exprimé ou absent chez les souches européennes de virus SRRP.

The causal agent of porcine reproductive and respiratory syndrome (PRRS), a recently described disease of swine in North America and Europe, is a new positive-strand RNA virus (1–3). The virus has tentatively been classified in the Arterivirus group, because of its morphologic, physicochemical and genomic characteristics (4,5). The PRRS virus (PRRSV) possesses 3 major structural proteins of 15, 19, and 26 kDa (6,7) and monoclonal antibodies (MAbs) to the nucleocapsid (N) protein (15 kDa) have demonstrated the existence of conserved epitopes among European and US isolates as well as differentiating epitopes absent from European PRRSV isolates of the virus (6). Using MAbs to the 15 kDa N protein, Canadian isolates of PRRSV have been shown to be antigenically similar to US isolates and different from the European Lelystad isolate (8). Recently, MAbs raised to a British isolate were found to lack reactivity with US PRRSV isolates. Four of these MAbs were directed to the N protein, 1 to a 45 kDa protein of purified virus and 1 of undetermined specificity (9). Two MAbs to the membrane (M) protein (19 kDa), raised to a Quebec PRRSV isolate, were recently reported to react by indirect immunofluorescence with 1 Quebec isolate but not with an Ontario isolate, the US VR-2332 isolate or the Lelystad isolate (10). In the present study we report on the reactivity of a hybridoma, secreting MAbs directed to the M protein of PRRSV, towards a number of PRRSV isolates of North America and Europe.

The MARC-145 (11) cell line was used to propagate the PRRSV isolate (LHVA-93-3) used in the production of monoclonal antibodies. This PRRSV isolate was initially isolated on porcine alveolar macrophages (12) and subsequently adapted and cloned in MARC-145 cells. Virus was purified in caesium chloride density gradients and fractions containing maximum infectivity (density 1.18 g/mL) were formalin inactivated prior to inoculating BALB/c mice. Splenocytes of immunized mice were fused with P3X63-Ag8.653 myeloma cells. Screening of hybridoma cells was done using an enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence (IIF) performed in 96 well plates (8). Both screening assays were performed using PRRSV isolate LHVA-93-3 as antigen. Ascites fluid was produced in pristane-primed

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Received February 8, 1996.

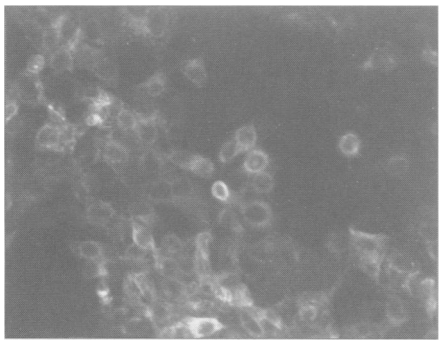

Figure 1. Indirect immunofluorescence on MARC-145 cells infected with the LHVA-93-3 isolate of PRRSV using MAb 2C12.

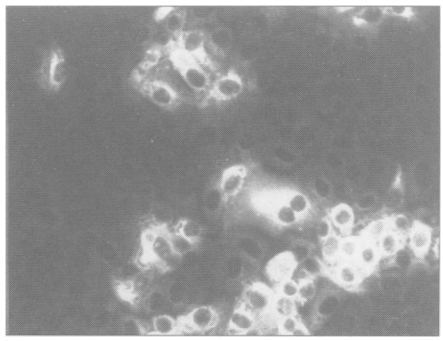

Figure 2. Indirect immunofluorescence on MARC-145 cells infected with the LHVA-93-3 isolate of PRRSV using MAb SDOW17.

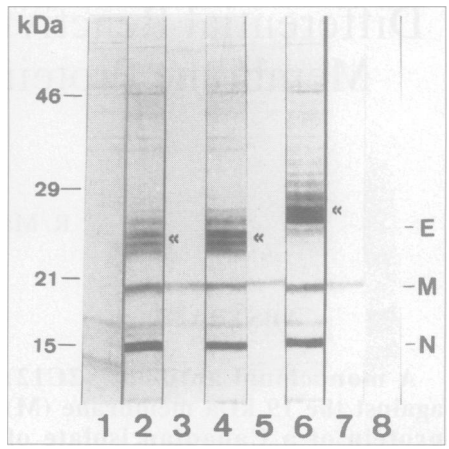

Figure 3. Immunoblotting of MAb 2C12 towards PRRSV isolates. The MAb 2C12 reacted with the 19 kDa protein of Canadian LHVA-93-3 (lane 3), LHVA-93-5 (lane 5) and U.S. NVSL (lane 7) isolates. Nucleocapsid (N), membrane (M) and envelope (E) structural proteins were detected using a porcine anti-PRRSV serum to NVSL isolate: noninfected MARC-145 cells (lane 1), LHVA-93-3 (lane 2), LHVA-93-5 (lane 4) and U.S. NVSL (lane 6). Lane 8; MAb 2C12 and noninfected MARC-145 cells. Heterogeneity in the molecular weight of the E protein of Canadian and US isolates can be seen (arrows).

BALB/c mice and the isotype of MAbs in ascites fluid was determined using a commercially available kit (Bio-Rad Laboratories Canada, Mississauga, Ontario). The protein specificity of the MAbs was determined by immunoblotting using partially purified (through 30% w/w sucrose) PRRSV preparations separated by SDS-PAGE under reducing conditions and transferred to a nitrocellulose membrane. The reactivity of the 2C12 MAb and the reference SDOW17 (6) MAb was tested by IIF on acetone-fixed MARC-145 cells (8) or porcine alveolar macrophages infected with the PRRSV isolates. A total of 26 Canadian, 122 US and 13 European PRRSV isolates were tested. Canadian isolates tested included previously characterized isolates (12,13,14) as well as more recently identified field isolates, from Quebec, Ontario and Alberta. The US isolates tested included the VR-2332 isolate (2,3), the NVSL isolate, the vaccine virus (RespPRRS, Boehringer Ingelheim Animal Health Inc, St. Joseph, Missouri, USA) and field isolates from several states. European PRRSV isolates tested included the Lelystad isolate (1), 10 isolates from France, 1 German isolate and the UK H1 isolate (15).

Most secreting hybridomas were found to be unstable. One hybridoma (2C12) was positive by ELISA and IIF and was maintained and subcloned. The MAbs of hybridoma supernatants and in ascites fluid were determined to be of the IgG1 isotype. When ascites fluid produced with this hybridoma was tested by IIF, generally moderate cytoplasmic fluorescence often with a perinuclear and reticulate aspect could be observed in infected MARC-145 cells (Fig. 1). Intensity of fluorescence varied between some isolates, stronger in some and weaker in others. In contrast, intensity of SDOW17 directed to a conserved epitope on the 15 kDa N protein of US and European PRRSV isolates (6) was intense and more uniformally spread through the whole cytoplasm (Fig. 2). By immunoblotting, the 2C12 ascites fluid reacted with the 19 kDa protein of 2 Canadian and 1 US PRRSV isolates (Fig. 3), all propagated in MARC-145 cells. Major structural proteins, N, M, and E (envelope) were resolved using an anti-PRRSV serum to the NVSL isolate that was collected from a pig 32 d postinoculation. A marked difference in the electrophoretic mobility of the E protein of the Canadian and the US isolates was observed. The extent and significance of the molecular heterogeneity of this major surface envelope glycoprotein remains to be clarified and is currently being investigated. When tested by IIF towards various other PRRSV isolates, M protein-specific MAb 2C12 reacted with all Canadian isolates and US isolates including VR-2332, NVSL and vaccine strain (RespPRRS). However fluorescence was not observed with the Lelystad or other European PRRSV isolates tested (Table 1).

TABLE 1. Reactivity by indirect immunofluorescence of membrane protein MAb 2C12 and nucleocapsid protein MAb SDOW17 towards North American and European PRRSV isolates

PRRSV isolates	2C12	SDOW17
Canada	+ (26/26)	+ (26/26)
US	+ (122/122)	+ (122/122)
Europe	− (13/13)	+ (13/13)

+ Fluorescence observed
− Absence of fluorescence

The reactivity pattern observed by IIF suggests that the M protein epitope recognized by the 2C12 MAb is highly conserved among North American isolates of PRRSV and absent or very weakly expressed on European isolates, at least for those isolates tested to date. Additional European isolates may have to be tested to further confirm these observations. Previous reports have indicated antigenic differences linked to the N protein of North American and European isolates of PRRSV (6,8,9). Recently, the isolation of a distinct serotype of PRRSV from Ontario, different from North American strains and similar to European PRRSV isolates, was reported (10). This conclusion was essentially based on the nonreactivity of 2 N MAbs, EP147 and VO17, with this isolate. However these MAbs have been shown not to react with some North-American isolates (16). The positive reactivity with the 2C12 MAb, recognizing an apparently highly conserved epitope on North American isolates, suggests that this

Ontario isolate is antigenically more similar to North American isolates than to European isolates. This is supported by the observation of antigenic differences among North American isolates as defined by an extended panel of MAbs to the N protein (16). Although the amino acid sequence identity between North American and European PRRSV isolates is reported to be higher for the M protein (78 to 81%) than for the N protein (57 to 59%) of PRRSV (17), the results obtained in the present work suggest that significant antigenic differences between North American and European isolates of PRRSV may be detected on the M protein of PRRSV as well. The assembly of panels of MAbs to the M protein and to other structural proteins will be of interest for the further study of antigenic variability and the differentiation of PRRSV isolates.

ACKNOWLEDGMENTS

The authors are grateful to K. Eernisse and Dr. J. Pearson (National Veterinary Services Laboratory, USDA, Ames, USA) for supplying MARC-145 cells, NVSL isolate and PRRSV porcine antiserum; to Dr. G. Wensvoort (Institute for Animal Science and Health, Lelystad, The Netherlands) for the Lelystad isolate of PRRSV; to Dr. G. Marsolais (Ministère de l'Agriculture des Pêcheries et de l'Alimentation du Québec, Institut Armand-Frappier, Laval, Quebec), Dr. S. Carman (Veterinary Laboratory Services, Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, Ontario), and Dr. J. Cho (Animal Diseases Research Institute, Agriculture Canada, Lethbridge, Alberta) for supplying additional Canadian isolates of PRRSV; to Dr. T. Drew (Central Veterinary Laboratory, Weybridge, United Kingdom) for the H1 isolate; and to Lyne Delorme, Diane Longtin and Louise Lessard for technical assistance.

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