LETTER TO THE EDITOR

EFFECT OF THE SERUM ALBUMIN ON REPLICATION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IN A CELL CULTURE

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Porcine reproductive and respiratory syndrome virus (PRRSV) belongs to the family *Arteriviridae*, genus *Arterivirus.* The virus causes a reproductive failure in sows and respiratory distress in piglets (*1*). PRRSV propagation in cell culture is difficult, but Kim *et al.* (*3*) established the cell line MARC-145 from the cell line MA-104 and used it as a suitable vehicle for the propagation of PRRSV. Initially, we adapted the cell line MARC-145 originally maintained in Eagle's minimum essential medium (MEM) in the presence of 5% calf serum (CS) to the cell line MARC-145:NS that was able to grow in a serum-free MEM. The virus used throughout this work was a Japanese isolate EDRD-1 (*4*) propagated in MARC-145 cells. We found that after inoculation of the virus in MARC-145:NS, the virus did not propagate in the cells maintained in the serum-free medium (SFM). However, in the presence of medium containing 5% CS the virus replicated as good as in the parental cell line. This finding drove us to examine the effect of some constituents of CS on the replication of PRRSV. The MARC-145:NS were grown in SFM (MEM supplemented with 10 mmol/l N,N-bis (2-hydroxyethyl)-2-aminoethanesulfonic acid (Wako), 0.295% tryptose phosphate broth, 0.5% bactopepton, 0.045% L-glutamine, and 0.2% $NaHCO_3$). Additional substances to test their effect on PRRSV replication in MARC-145:NS were as follows: bovine serum albumin (BSA, 0.2%), human serum albumin (HSA, 0.2%), rabbit serum albumin (RSA, 0.2%) (Sigma); ovalbumin (OVA, 0.2%), lactalbumin (LA, 0.2%), lactoferrin (LF, 0.2%), plasmin (0.025%), plasminogen (0.025%) (Wako), bovine-γ-globulin (B-γ-g, 0.2%) (Bayer), and trypsin (0.005%) (Difco). In addition to these substances, we used also two monoclonal antibodies (MAbs) anti-BSA signed as MAb1 and MAb2 for neutralization of BSA and HSA. Sixty µl of anti-BSA MAb1 or MAb2 (Connex) containing 12 µg of antibody was mixed with 120 µl of 10% BSA, 10% HSA or SFM, incubated for 1 hr at 37°C, and centrifuged at 20,000 g for 10 mins. The supernatants were diluted 10 times with SFM and used in testing of the PRRSV replication.

MARC-145:NS cells were seeded in the plastic dishes coated with collagen and grown for 1 day. Then, the virus was inoculated into each dish with the MOI = 1. After absorption of the virus, the dishes were washed three times with SFM and further maintained in 2 ml of SFM containing

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Abbreviations: B-γ-g = bovine-γ-globulin as; BSA = bovine serum albumin; CS = calf serum; HSA = human serum albumin; LA = lactalbumin; LF = lactoferrin; MAb = monoclonal antibody; OVA = ovalbumin; p.i. = post inoculation; PRRSV = Porcine reproductive and respiratory syndrome virus; RSA = rabbit serum albumin; SFM = serum-free medium

one of the additional substance mentioned above. The medium and the cells were collected at the day 1, 3, 5, and 7 p.i. and the amount of infectious virus in the samples was determined by virus titration on MARC-145 cells grown in 96-well plates. The indirect immunofluorescence antibody assay (IFA) was used to detect PRRSV antigen in the infected cells treated with each tested substance. MARC-145:NS cells were cultured on cover slips for 1 day, inoculated with PRRSV, and following absorption SFM containing one of the tested substances was added. At day 3 p.i., each cover slip was fixed by acetone, treated by swine antiserum against PRRSV and later with FITC-conjugated anti-porcine IgG.

At the beginning of experimental work, we supposed that after the cells had adapted to the SFM, the sensitivity of MARC-145:NS to viral replication would change, but replication of PRRSV recovered only after the addition of CS to the medium. One of the major components of CS is serum albumin. Consequently, we tested the effects of various albumins on the replication of PRRSV in MARC-145:NS. The addition of serum albumins as well as the addition of CS had a positive effect on the replication of PRRSV. Two of the tested albumins were of animal origin, so we examined the effects of other animal proteins as LA, LF and OVA on the replication of PRRSV. Addition of these proteins to the maintenance medium had no effect on the replication of PRRSV in MARC-145:NS. Surprisingly, another major component of CS B-γ-g had no effect on replication either. Plasmin, plasminogen and trypsin are involved in virus replication (*2, 5*), so we examined their effects on the replication of PRRSV, but they showed no positive effect. In the presence of neutralized BSA the virus did not replicate in cells. HSA in the presence of anti-BSA MAb1 clearly enhanced the replication of PRRSV in MARC-145:NS, because MAb1 did not neutralize HSA. These results confirmed the essential role of BSA in the virus replication process. Similarly, viral antigens were detected by IFA assay in the cytoplasm of MARC-145:NS grown in SFM containing CS, BSA, HSA, or RSA. On the other hand, viral antigens were not detected in MARC-145:NS maintained in SFM containing remaining substances. The obtained results evidently showed the positive effect of serum albumins BSA, HSA and RSA on the replication of PRRSV in MARC-145:NS.

Substance	Virus titer at day 5 p.i.	
	Medium*	Cells
5% CS	5.75	5.75
0.2% BSA	6.25	5.50
0.2% HSA	4.50	5.00
0.2% RSA	4.25	3.50
0.2% OVA	–	–
0.2% LA	–	–
0.2% LF	–	–
0.2% B-γ-g	–	–
0.005% Trypsin	–	–
0.025% Plasmin	–	–
0.025% Plasminogen	–	–
10% BSA	6.00	6.25
10% BSA + SFM	5.50	4.25
10% BSA + MAb1	–	–
10% BSA + MAb2	–	–
10% HAS + MAb1	4.50	4.00
SFM + MAb1	–	–

*$TCID_{50}$/ml; (–) = virus was not detected.

To our knowledge, this is the first report confirming the essential role of serum albumin in the replication of PRRSV.

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