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Pathogenic effects of porcine reproductive and respiratory syndrome virus isolates in swine tracheal ring culture

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Abstract : Pathogenic effects of 29 different porcine reproductive and respiratory syndrome (PRRS) virus isolates were investigated in swine tracheal ring(STR) cultures by examining their effects on the ciliary activity of STR. Inhibition of ciliary movement and destruction of the tracheal epithelium were seen between 72 and 96 hours postinoculation(PI). Virus replication was demonstrated by examining viral infectivity of the supernatants from the STR cultures. PRRS virus antigen in macrophages was detected by a streptavidin-biotin complex(ABC) immunoperoxidase method. Of the 29 PRRS virus isolates, 8 isolates were classified into pathogenic, and the remaining 21 isolates were determined as mildly pathogenic or apathogenic viruses. These results suggest that STR examination may be used as a method for predicting pathogenic variability of PRRS virus isolates.

Key words : PRRSV, pathogenic effects, swine tracheal ring cultures.

Introduction

Porcine reproductive and respiratory syndrome(PRRS) virus has been identified as an important pathogen in swine industry. The etiology and pathogenesis of PRRS virus are now relatively well understood. The virus causes reproductive failure in pregnant sows and chronic respiratory disease in all stages of pigs. The postfarrowing respiratory problems in piglets are characterized by increased severity of pneumonia and delayed growth in nursery through finishing stages of pig production[1,2]. However, the respiratory problems differ in severity from farm to farm. Recent experiments indicated that PRRS virus isolates cause gross and microscopic lesions of varying severity in colostrum-deprived pigs[3,4]. Although respiratory pathogenicity of each PRRS virus isolate could be determined by inoculating them into the host animals, it is a relatively expensive and difficult method. The development

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of an *in vitro* method to predict respiratory virulence of PRRS virus isolates should alleviate this difficulty.

Swine tracheal ring(STR) culture allows the maintenance *in vitro* of living respiratory epithelium. These cultures have been employed to study the respiratory pathogenic effects on the ciliated epithelium of trachea induced by different viruses and bacteria[5,6]. In the present study, replication and pathogenic changes in STR were examined following inoculating of PRRS virus.

Materials and Methods

Virus isolates and cell culture : Twenty-nine PRRS virus isolates including MN-H_L, MN-H_S, MN-W_L[7] and Lelystad virus(kindly supplied by Dr. G. Wensvoort) were used. The MARC-145 cell line[8] was employed for virus propagation and infectivity testing. The MARC-145 cells were maintained in Eagle's minimum essential medium(MEM) supplemented with 3% fetal bovine serum(FBS), 0.15% sodium bicarbonate, and antibiotics.

Tracheal ring cultures : Piglets at 1-day of age were obtained from a PRRS virus negative farm, and the trachea was excised aseptically, sectioned into 26~30 rings at approximately 1mm long. Each ring was placed into 1 well of 24-well tissue culture plate and incubated in a 5% CO_2 Eagle's MEM containing 10% FBS and antibiotics was used in the STR culture. An inverted microscope was used to examine active movement of the cilia in each STR culture after 6 hours of culture.

Virus replication in STR cultures : Initially 4 PRRS virus isolates were randomly selected and tested for virus replication on STR cultures. Each virus(0.1ml $10^{3.0-3.5}TCID_{50}$/0.1ml) was inoculated in quadruplicate into the center of STR and adsorbed at 37℃ for 1 hour. The rings were washed twice with MEM and refilled with 1ml of fresh growth medium. Supernatants(0.1ml per well) were collected from each well and replaced with the same amount of fresh growth medium daily for 7 days. The supernatants in quadruplicate were pooled and kept at -70℃ until virus infectivity was tested.

Examination of pathogenicity for PRRS virus isolates : Following removal of the medium from the 6-hour STR cultures, 0.1ml of each PRRS virus isolate($10^{4.5}TCID_{50}$/ml) was inoculated in duplicate, adsorbed at 37℃ for 1 hour, and 0.9ml of fresh growth medium was added to each well. Ciliary activity of inoculated and uninoculated cultures was observed daily. A scoring system was designed to examine each STR by dividing the ring into 4 partitions. Cessation of ciliary movement in each partition was examined and subjectively scored as 0(no inhibition), 1, 2, 3, and 4(inhibition in all 4 partitions) after 96 hours of incubation. The rings were fixed and sectioned as described previously[6] and examined using a scanning electron microscope(SEM, JOEL JSM-35).

Histopathological or immunohistochemical examination : The STR were fixed in 10% neutral buffered formalin, embedded, sectioned at 4μm, and stained with hematoxylin and eosin(H-E). The sections mounted on aminoalkylsilane-coated glass slides(Sigma Chemical Co., St. Louis, MO) were used for the detection of PRRS virus antigen by a modified streptavidin-biotin complex(ABC) immunoperoxidase method[9]. Briefly, the sections were deparaffinized, dehydrated and endogenous peroxidase was removed. This was followed by two 5-minute baths in 0.05M Tris buffer(TB, pH 7.6). Protease digestion was done in 0.05% protease(Protease XIV) (Sigma Chemical Co., St. Louis, MO) in TB for 5 minutes at 37℃ and followed by two 5-minute cold TB baths. For blocking, a 5% solution of normal goat serum(Sigma Chemical Co, St. Louis, MO) was reacted on the slides for 20 minutes. The tissue sections were flooded with a PRRS virus monoclonal ascites fluid(SDOW 17 supplied by Dr. Eric Nelson, South Dakota State University, Brookings, SD ; IFA titer 〉 1,024) diluted 1 : 1,000 in TRIS phosphate-buffered saline(PBS)(1 part TRIS : 9 parts PBS [0.01M, pH 7.2]) and incubated at 4℃ overnight. After a wash with TRIS, a 5-minute TRIS bath and a 5-minute TRIS bath containing 1% normal goat serum, the sections were flooded with biotinylated goat anti-mouse serum(Kirkegaard & Perry Lab. Inc., Gaithersburg, MD) for 30 minutes. The linking antibody incubation was followed by 3 rinses in TRIS as was done following primary antibody incubation. The sections were then treated with peroxidase-labeled streptavidin(Kirkegaard & Perry Lab. Inc., Gaithersburg, MD) diluted 1 : 500 in TRIS/PBS for 40

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