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Highly pathogenic porcine reproductive and respiratory syndrome virus infection results in acute lung injury of the infected pigs

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ABSTRACT

Highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) was firstly characterized in 2006 in China. The virus has caused great economic loss to the Chinese swine production during the past years. Herein, we experimentally infected SPF pigs using two strains of PRRSV with different pathogenicity and observed the lung pathological changes looking for new sights on the possible pathogenesis associated with the virulence of HP-PRRSV. The results indicated that the HP-PRRSV-infected pigs died and exhibited severe pathological changes of lungs featuring increased neutrophils, mast cells and mononuclear macrophages, compared with the pigs inoculated with low pathogenic (LP-) PRRSV. Furthermore, the pigs infected with HP-PRRSV showed the higher levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, interleukin (IL)-8 and histamine, leukotriene B4 (LTB4), platelet activation factor (PAF) in sera than those inoculated with LP-PRRSV. Additionally, the fibrosis of lung was observed in the HP-PRRSV-infected pigs. At present, our findings suggest that the aberrant immune responses triggered by HP-PRRSV infection are closely related to acute lung injury (ALI), and especially the pathological changes in lung vascular system are of particular significance. These associated pathological changes of lung are in part responsible for the additional morbidity and mortality observed in HP-PRRSV infection.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is generally considered as a severe viral disease in pigs, causing huge economic loss worldwide each year. The disease was first reported in the late 1980s in the United States (Keffaber, 1989), and similar clinical outbreak occurred in Germany in 1990 and was widespread throughout Europe by 1991 (Goyal, 1993). In the early 1990s, this viral disease was identified in Asia (Murakami et al., 1994; Shimizu et al., 1994). In 1995, a PRRS outbreak was firstly recognized in Beijing of China. Since then, PRRS has been verified in many provinces in China, proving to be one of main swine diseases in China (Gao et al., 2004; Guo et al., 1996). In China in 2006, an unparalleled large-scale, atypical PRRS outbreak caused by the highly pathogenic PRRSV (HP-PRRSV) dealt a heavy blow to the swine industry (Zhou and Yang, 2010; Tian et al., 2007).

Different pathogenic PRRSV strains in the field induced totally different consequences of infected pigs because of genetically extensive variation (Li et al., 2007; Meng, 2000; Nelsen et al., 1999). Our previous studies have shown that the low pathogenic PRRSV (LP-PRRSV) infection resulted in

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no fatality of pigs with light anatomical and histopathological changes in lungs of pigs, whereas the HP-PRRSV-infected pigs presented high mortality accompanying with high fever (40–42 °C), depression, anorexia, cough, asthma, severe dyspnea, disorder in the respiratory tract, lameness, shivering (Zhou et al., 2009). A recent study found that HP-PRRSV could exhibit extensive tissue tropism for pigs (Li et al., 2012). These observations of the pathological changes in tissues of HP-PRRSV-infected pigs suggested that HP-PRRSV infection induced severe pathological changes of lungs.

Various kinds of pathogenic factors can cause acute lung injury (ALI), such as severe infection, shock, thoracic trauma, disseminated intravascular coagulation, gastric acid, smog, and toxic gas aspiration clinically characterized by rapidly progressive dyspnea and intractable hypoxemia (Gattinoni et al., 1998; Atabai and Matthay, 2002). ALI may develop into acute respiratory distress syndrome (ARDS) characterized by damage to the alveolus-capillary interface, usually secondary to an intense inflammatory response of the host lung to infectious or noninfectious invasion (Levy et al., 2005; Bernard, 2005). It has been shown that severe acute respiratory syndrome (SARS) virus and avian influenza A H5N1 virus infection could result in high mortality due to the complication of ARDS. Therefore, infectious pathogens, in which that the majority are viruses, have become one of the most important causes of ARDS (Headley et al., 1997; Chen et al., 2005; Subbarao et al., 1998). Thus, it is intriguing to note that HP-PRRSV infection might be associated with ALI which may have the responsibility for the additional morbidity and mortality of the infected pigs.

Why two completely different consequences exist in the pigs infected with various PRRSV strains? To address this question, in this study, SPF pigs were infected with two strains of PRRSV, LP-PRRSV strain HB-1/3.9 and HP-PRRSV strain JXwn06, in an attempt to fully observe the distinct lung pathological changes and to better understand the lung pathogenesis induced by HP-PRRSV.

2. Materials and methods

2.1. Viruses, animals and experimental design

Two PRRSV strains, JXwn06 and HB-1/3.9 with different virulence, were used in the study. The virus JXwn06, a HP-PRRSV strain, was isolated from an intensive pig farm with atypical PRRS outbreak in 2006 (Zhou et al., 2009). The virus HB-1/3.9, a LP-PRRSV strain, was derived from HB-1(sh)/2002 adapted in MARC-145 cells (Gao et al., 2004). These viruses were propagated using highly permissive MARC-145 cells according to the method described previously (Zhou et al., 2009).

Twenty one 28-day-old SPF large white pigs (Beijing Center for SPF Swine Breeding & Management, Beijing, China) were divided randomly into three groups, the JXwn06-infected group ($n = 9$), the HB-1/3.9-infected group ($n = 9$) and control group ($n = 3$). Each group was then housed separately in different isolation rooms, with individual ventilation. Each pig was intranasally inoculated with 2 ml of 10^5 $TCID_{50}$/ml virus (JXwn06 and HB-1/3.9), respectively. The pigs in the control group were mock-inoculated with the same dosages of MARC-145 cells culture supernatant, and then were euthanized and sampled. Clinical signs of virus-inoculated pigs were visually examined and simultaneously the rectal temperatures were measured daily.

Animal use and animal trials in this study have been approved by The Beijing Municipal Committee of Animal Management and The Ethics Committee of China Agricultural University.

2.2. Collection of samples, virus titration and ELISA

On days 3 and 5 post-inoculation (PI), 3 pigs in HB-1/3.9- and JXwn06-infected group were euthanized, and then once death of the rest 3 pigs in JXwn06-infected happened, the 3 pigs were sampled immediately. Meanwhile the rest 3 pigs in HB-1/3.9-infected group were euthanized accordingly. Two portions from each lobe of the left lung were collected immediately and fixed in 4% paraformaldehyde solution. The whole left lung was weighed before and after desiccation at 80 °C drying to constant weight, and then the lung wet:dry weight ratio was determined which was taken as one indicator of lung edema (Lang et al., 2005). Serum samples collected from the infected animals were detected for viral RNA using RT-PCR by amplifying a 312 bp ORF7 fragment of PRRSV. According to the method of Reed-Muench, virus titration in the lungs was determined with some modification. Briefly, three lobes of right lungs were sampled, frozen in liquid nitrogen and then ground into fine powder using a mortar and pestle, weighted for one milligram and homogenized in 1 ml cold phosphate-buffered saline. Clarified homogenates were titrated for viral infectivity in MARC-145 cells cultured in 96 well plates from initial dilutions of 1:10. Viral titers were expressed as mean $TCID_{50}$ per milliliter. In addition, the concentrations of histamine, PAF, LTB4 and IL-8 in serum, and TNF-α in serum and homogenates of lungs were detected using R&D ELISA kit (R&D system, Inc. USA) according to the manufacture's procedure.

2.3. Lung histopathology

The fixed lung samples were dehydrated, embedded in paraffin, and serial sectioned (4 μm). Three sagittal sections from each lung, and six sections per animal, were stained with hematoxylin-eosin and Masson's trichrome. The severities of histopathological changes and pulmonary fibrosis of the lungs were assessed and scored. All the sections were numbered randomly and interpreted by three experimenters blinded to the experimental conditions. Histopathological changes were observed and scored under an Olympus microscope (Olympus Optical Co., Ltd.). Criteria for grading lung histopathological changes were as follows: Grade 0 = no obvious pathological changes; Grades 1–3 = light inflammatory cells infiltration, light hemorrhage, vasculitis or bronchiolitis; Grades 4–5 = inflammatory cells infiltration, hemorrhage, vasculitis or bronchiolitis, cell apoptosis and necrosis, microthrombus; Grades 6–10 = severe inflammatory cells infiltration,