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Pathogenesis and clinical aspects of a respiratory porcine reproductive and respiratory syndrome virus infection

Kristien Van Reeth *

Laboratory of Veterinary Virology, Faculty of Veterinary Medicine, University of Gent, Salisburylaan 133, B-9820 Merelbeke, Belgium

Abstract

Some pathogenetic and clinical aspects of the respiratory tract infection with porcine reproductive and respiratory syndrome virus (PRRSV) are discussed. The acute and persistent stages of PRRSV infection are treated separately. Special attention is given to the author's work on experimental dual infections with PRRSV and other enzootic porcine respiratory viruses. It was concluded that: (1) Studies on the interactions of PRRSV and its target cell, the pulmonary alveolar macrophage, are very scarce. So far, the hypothesis of impaired macrophage function has not been proven. (2) The possibility that PRRSV causes disease in growing pigs in combination with other viral or bacterial infections has been demonstrated experimentally. Variation in disease resulting from such combined PRRSV infections will probably hamper future research into disease mechanisms. (3) There remains a need for more data on the clinicopathological significance of the persistent stage of PRRSV infections. © 1997 Elsevier Science B.V.

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1. Introduction

During the last several years, pathogenesis and clinical signs of porcine reproductive and respiratory syndrome virus (PRRSV) have been studied intensively. As a result, the basic pathogenetic features of PRRSV infection are known by now. Infection most frequently occurs by the respiratory route and virus replication in the respiratory tract is followed by viraemia and dissemination throughout the body. The virus has been

* Tel.: +32-9-2647366; fax: +32-9-2647495; e-mail: maurice.pensaert@rug.ac.be.

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isolated from nasal turbinates, tonsils, lungs, serum, plasma, buffy coat samples, spleen and several lymph nodes of experimentally infected pigs (Duan et al., 1997; Rossow et al., 1995). Other tissues including thymus, brain, heart, trachea and bone marrow are more sporadically virus positive. Immunohistochemistry has been used to show that PRRSV infects macrophage-like cells in the lungs, tonsils, lymph nodes, heart, thymus and spleen (Halbur et al., 1995a). Transplacental infection occurs in late gestation sows, but is beyond the scope of this review. Lymphadenopathy, myocarditis, encephalitis and vasculitis have been reported (Rossow et al., 1995), but interstitial pneumonia is the most consistent lesion. The lungs are probably the portal of entry as well as the target organ and the virus has a predilection for pulmonary alveolar macrophages (PAMs). This paper focuses on the pathogenesis of the respiratory tract infection with PRRSV.

The clinical importance of such PRRSV infection, like that of many other respiratory infections, is difficult to assess. There is the fact that respiratory disease and poor productivity have increased in several countries since the enzootic appearance of PRRSV. Still, experimental PRRSV infections run a largely subclinical course and conditions that may exacerbate the clinical effects are multiple and thus remain difficult to study. Some work on dual infections with PRRSV and other viruses has been performed in the authors' group and will be discussed further.

A particular feature of PRRSV is its capacity to persist in the infected pig for several weeks. PRRSV specific serum antibodies appear within 2 weeks after exposure to the virus, but virus clearance does not occur until 4–6 weeks post-inoculation (PI) (Yoon et al., 1993; Bilodeau et al., 1994). The first 2 weeks of infection are considered as the 'acute stage' during which maximal virus titers are recovered from all susceptible organs throughout the body. The period thereafter is designated as the 'persistent stage' and is characterized by lower levels of virus replication in some organs only. For convenience, the acute and persistent stages of PRRSV infection are treated separately in this review.

2. The acute stage of PRRSV infection

2.1. Pathogenesis of PRRSV in the lungs

PRRSV replicates extensively in the lungs and virus titers are as high as $10^{5.5}$–$10^{5.9}$ $TCID_{50}$/g lung tissue (Van Reeth et al., unpublished). The virus has a very selective tropism for macrophages of the alveolar spaces and alveolar septa (Pol et al., 1991; Halbur et al., 1994). Viral antigen has also been detected in sloughed pneumocyte-like cells (Halbur et al., 1994) and in bronchiolar epithelium (Pol et al., 1991). PAMs, however, make up 80–94% of the infected lung cell population (Duan et al., 1997) and these cells undergo rapid cytopathic effects.

Gross pneumonia with lobular red areas has been reported in some experimental inoculation studies (Pol et al., 1991; Halbur et al., 1993; Halbur et al., 1995b; Van Reeth et al., unpublished). These lesions represent an acute interstitial pneumonia that can be caused by several viruses. Microscopic lung lesions are found consistently and are mainly confined to the alveoli (Pol et al., 1991; Collins et al., 1992; Paton et al., 1992; Halbur et al., 1993; Halbur et al., 1994; Rossow et al., 1995; Halbur et al., 1995b). They

are characterized by marked thickening of alveolar septa containing many macrophages and few lymphocytes and by type 2 pneumocyte proliferation. Alveolar lumina are filled with inflammatory cells and necrotic cell debris. While lung lavage cells from uninfected control pigs consist of > 95% macrophages, lavage fluids from PRRSV infected pigs at 7 days post inoculation (DPI) contain about 50% macrophages, 35% neutrophils and 15% lymphocytes (Zhou et al., 1992).

The tropism of PRRSV for PAMs has led to the central hypothesis that lung defense mechanisms are suppressed following PRRSV infection. It has brought about a tremendous interest in this virus from researchers, industry and practitioners. But at this time, scepticism as to the hypothesis of impaired lung defense should be kept for at least 3 reasons. Firstly, only a small fraction of PAMs are infected. At any time PI, no more than about 1% of lavaged alveolar cells are found PRRSV antigen positive (Mengeling et al., 1995; Duan et al., 1997). But, as suggested by Mengeling et al. (1995) we should take into account that infected AMs may fail to adhere to coverslips or plastic culture plates used in fluorescent antibody tests. As such, the exact number of infected AMs may be quite difficult to score. Secondly, a similar increase in the total number of free bronchoalveolar lavage cells and a proportional reduction of macrophages have been observed with other viral infections of the lungs, such as porcine respiratory coronavirus (PRCV) and swine influenzaviruses. These changes in the cellular composition of lavage fluids largely result from chemotaxis of neutrophils and lymphocytes into the alveolar spaces. Thirdly, there is insufficient evidence that PAM functions are seriously hampered. Also, research into this subject is scanty and inconclusive. In one experiment of Zhou et al. (1992), PAMs from PRRSV infected pigs at 1 week PI were impaired in their ability to synthesise superoxide anions, but expression of interleukin-1β was enhanced.

2.2. *Clinical effects of PRRSV infection*

Researchers from Europe and the U.S. have performed many clinical trials with PRRSV isolates from their respective countries. In these studies, the virus was administered oronasally to 1–14 week old pigs, either gnotobiotic, specific pathogen free or conventional. Considering the variety of experimental conditions, it is not surprising that the clinical outcome differs somewhat. Most experimental infection studies failed to provoke overt disease (Pol et al., 1991; Paton et al., 1992; Plana Duran et al., 1992; Ramos et al., 1992; Yoon et al., 1992; Fichtner et al., 1993; Albina et al., 1994). A transient fever was the most prominent and consistent clinical change. More or less serious respiratory disease has been observed with some U.S. isolates only (Halbur et al., 1993; Rossow et al., 1995; Halbur et al., 1995b). Inoculation with other U.S. isolates remained, however, without any clinical sign (Mengeling et al., 1996). Halbur et al. (1995b) demonstrated that certain U.S. isolates are more pathogenic than other U.S. isolates and than the Lelystad virus.

In our own model of experimental PRRSV infection, 10^5 $TCID_{50}$ of the Lelystad virus strain are introduced into the lungs of 10 week old conventional pigs by aerosol (Van Reeth et al., 1996). A total of 33 pigs were inoculated this way. The inoculation caused fever by the 2nd or 3rd DPI, eventually accompanied by a slight elevation of

respiration rates. In most cases, body temperatures returned to normal by 4 DPI. A significant effect on weight gain during the acute stage of infection was not demonstrated so far. In conclusion, most research groups are of the opinion that the uncomplicated PRRSV infection is mostly inapparent and does not affect productivity.

In the field as well, PRRSV infection may pass without being noticed. In a longitudinal serological study on closed breeding–fattening farms in Belgium, seroconversion of feeder pigs was not associated with disease (Houben et al., 1995). Similar observations have been made in the Netherlands (Wensvoort, personal communication). Also in the U.S., seroconversion to PRRSV has been reported on farms without obvious clinical signs. Researchers in the US suggest that clinical PRRS is probably overdiagnosed by practitioners (Cho et al., 1993).

2.3. Dual infections with PRRSV and other infectious agents

The relatively slight pathogenicity of PRRSV by itself and the virus' tropism for PAMs have stimulated research into the combined effects of PRRSV and other infectious agents. Consequently, experimental dual infections have been performed with PRRSV followed by various bacteria such as *Haemophilus parasuis*, *Pasteurella multocida* and *Actinobacillus pleuropneumoniae*. The results of these studies are presented next.

Galina et al. (1994) were the first to demonstrate that PRRSV predisposes 2 week old pigs to infection and disease caused by *Streptococcus suis* (*S. suis*) serotype 2. Earlier, PRRSV infected herds had reported a high incidence of *S. suis* meningitis and septicaemia. A combined experimental infection with PRRSV followed 1 week later by *S. suis* could induce central nervous disorders and suppurative meningitis. Single *S. suis* inoculated pigs, on the other hand, did not show clinical signs and yielded fewer bacteria from their brains and internal organs.

In our group at Gent university, we have focused on dual infections with PRRSV followed by viruses, namely porcine respiratory coronavirus (PRCV) or H1N1-influenzavirus. The selection of these virus species was made on the basis of serological data obtained in intensive fattening herds in Belgium (Van Reeth and Pensaert, 1994b; Houben et al., 1995). These data showed that infections with PRRSV, PRCV and H1N1-influenzavirus occur very frequently during the first 2–3 weeks after multisource feeder pigs enter. Experimental single infections with PRCV or influenzavirus, if administered by aerosol, cause few clinical effects at the age of 10–14 weeks and were therefore considered as good candidates for a dual infection model.

The dual inoculation studies were performed in conventional 10-week-old pigs without antibodies against the respective viruses (Van Reeth et al., 1996). Virus inoculations were by aerosol and a standard 3 day interval between PRRSV and the second virus was applied. The pigs were clinically monitored until at least 14 DPI of the second virus. Under these experimental conditions, dual PRRSV–PRCV and PRRSV–H1N1-infections caused more severe disease and growth retardation than each of the single virus infections. Still, the clinical effects were highly variable in different groups and this is best exemplified as follows. At the time of this writing, 3 separate groups of pigs have been inoculated with PRRSV-influenzavirus. Mean body temperatures in these

groups were elevated ($\geq$ 40°C) during 10 (group 1), 5 (groups 2) and 3 (group 3) days following influenza inoculation and they peaked at 41.4, 40.6 and 40.3°C, respectively. Marked dyspnoea, abdominal breathing and productive coughing were observed in the high fever group (group 1) only, whereas respiratory disease was very mild in the other groups. We calculated that growth retardation in comparison with a single PRRSV inoculated group was 4.6 (group 1), 1.8 (group 2) and 1.9 kg (group 3) at 14 DPI of the 2nd virus. Variation thus occurs and so far undefined factors seemingly influence the clinical response following experimental dual virus infections.

Important to note is that, next to PRRSV, other porcine respiratory viruses are capable of potentiating the effect of a second viral infection. In the author's group, we have previously performed experimental dual infections of feeder pigs with PRCV first and H1N1-influenzavirus 2 or 3 days later (Van Reeth and Pensaert, 1994a). These pigs as well developed higher and longer lasting fever and more pronounced respiratory disease than the respective single virus inoculated pigs. In our opinion, there is insufficient evidence that PRRSV is more important in the etiology of respiratory disease than are a number of other viruses.

The mechanisms of disease of dual PRRSV infections remain to be elucidated. Several findings indicate that systemic or local immunodepression do not play a role in dual infections with viruses. Serum antibody titers against H1N1-influenzavirus or PRCV are equally high in pigs previously inoculated with PRRSV as in single virus inoculated pigs (Van Reeth et al., unpublished). Similarly, it has been shown by others that the humoral immune response against Aujeszky's disease virus is not reduced in PRRSV infected pigs (Molitor et al., 1992; Albina et al., 1995). In the lungs, clearance of influenza virus is not impaired by a prior inoculation with PRRSV (Van Reeth et al., unpublished). Preliminary observations suggest that non-specific immune mechanisms are enhanced rather than suppressed by PRRSV infection. Interleukin-1β mRNA (Zhou et al., 1992) and biologically active interferon-α (Van Reeth et al., unpublished) have been detected in lung lavage cells and fluids of infected pigs. These 'proinflammatory' cytokines can both play a role in antiviral defense and stimulate the pulmonary inflammatory response. They may thus contribute to the pathology of dual virus infections.

3. The persistent stage of PRRSV infection

PRRSV-infected pigs thus act as short-term virus carriers and they can transmit the infection to susceptible contacts for up to 8 weeks PI (Terpstra et al., 1994). Occasional experimental data have shown that stress or immunodepression can induce re-excretion at more than 15 weeks after initial seroconversion (Albina et al., 1994).

There is no consensus on the exact duration and site of PRRSV replication during the persistent stage. The virus has been recovered from serum as well as from lymphoid tissues and lungs for longer periods. In most experiments, viraemia is detected during 2 to 3 weeks PI. Viraemia of up to 6–7 weeks duration has been reported by Yoon et al. (1993) and by Bilodeau et al. (1994). Various factors such as age of the pigs at the time of infection and infection dose probably influence the duration of viraemia (Yoon et al.,

1993). Rossow et al. (1995) suggest virus persistence in lymphoid tissues. At 28 DPI, they could isolate PRRSV exclusively from tonsil, spleen and lymphoid tissues and not from the lungs. Other studies strongly indicate that not lymphoid tissues or blood cells, but lungs are the source of persistent infection (Mengeling et al., 1995; Duan et al., 1997). In the experiments of Duan, PRRSV was isolated from the serum of 6/12, 3/10 and 0/7 pigs at 21, 28 and 35 DPI, respectively. Virus isolation from lung tissue homogenates and/or lavaged PAMs of the same pigs was positive at any of these time points. Circumstantial evidence for virus persistence in alveolar macrophages has been obtained by Mengeling et al. (1995). They have tested serum, lungs, lavaged PAMs and a variety of other tissues for their relative value as samples from which to isolate PRRSV during the persistent stage of infection. PRRSV was isolated most often from medium of 72 h cultured AM. By this method, the virus was isolated from pigs as late as 49 and 70 DPI. The next best procedure for detecting PRRSV was testing lysates of PAMs. The virus was isolated less often from serum, tonsils and lung tissue.

The clinico–pathological significance of PRRSV persistence is still unclear. In some studies, microscopic lung lesions have been found to persist through 21 or 28 DPI (Halbur et al., 1993; Rossow et al., 1995). The general opinion, however, is that lesions are most severe during the acute stage and that they resolve thereafter (Yoon et al., 1992). The duration and evolution of inflammatory changes in the lungs of PRRSV-infected pigs still need more investigation.

Because PRRSV replicates in the lungs during at least 1 month, the potential for dual infections to occur is higher than it is after infection with other enzootic respiratory viruses in which the replication phase is short lasting. But so far, it is an open question whether disease occurs when a secondary infection takes place during the persistent stage of PRRSV. In one experiment of Albina et al. (1995), 6–8-week-old SPF pigs were inoculated with PRRSV and 3 weeks later with *M. hyopneumoniae*. Under these conditions, PRRSV did not enhance the clinical signs of *M. hyopneumoniae* infection. If it should turn out that a relatively short time interval between PRRSV and a second infection is essential for the development of clinical signs, then PRRSV may not be considered of higher significance in causing lung pathology than other enzootic porcine viruses. Because the time interval may determine the clinical outcome of combined infections with PRRSV in the field, further studies on this point are necessary.

4. Concluding remarks

Experimental data have documented the possibility of PRRSV causing disease in growing pigs in combination with other infections. Individual variations in severity of disease and in growth retardation, however, seriously hamper research into the pathogenesis of such combined infections. The role of PRRSV infection in the respiratory disease complex should not be overestimated for several reasons. First, some researchers have failed to demonstrate a synergism between PRRSV and certain viruses/bacteria under experimental conditions. Second, proof is still lacking to show that combined PRRSV infections are the cause of disease problems in the field. Some cases of nursery mortality associated with PRRSV and bacteria (Stevenson et al., 1993) have been

reported, but firm evidence of involvement of multiple PRRSV infections in disease has not yet been given. It should be mentioned that conclusive field studies cannot be based on the results of serology alone. Isolation/demonstration of the agents is necessary as well as information on their association with disease and lesions.

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