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Review

Immunopathogenesis of porcine reproductive and respiratory syndrome in the respiratory tract of pigs

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) impairs local pulmonary immune responses by damaging the mucociliary transport system, impairing the function of porcine alveolar macrophages and inducing apoptosis of immune cells. An imbalance between pro- and anti-inflammatory cytokines, including tumour necrosis factor-α and interleukin-10, in PRRS may impair the immune response of the lung. Pulmonary macrophage subpopulations have a range of susceptibilities to different PRRSV strains and different capacities to express cytokines. Infection with PRRSV decreases the bactericidal activity of macrophages, which increases susceptibility to secondary bacterial infections. PRRSV infection is associated with an increase in concentrations of haptoglobin, which may interact with the virus receptor (CD163) and induce the synthesis of anti-inflammatory mediators. The balance between pro- and anti-inflammatory cytokines modulates the expression of CD163, which may affect the pathogenicity and replication of the virus in different tissues. With the emergence of highly pathogenic PRRSV, there is a need for more information on the immunopathogenesis of different strains of PRRS, particularly to develop more effective vaccines.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is caused by an arterivirus, PRRS virus (PRRSV) (King et al., 2012). Two genotypes of PRRSV have been identified: (1) European or type 1 genotype (PRRSV-1), the prototype of which is Lelystad virus (LV); and (2) North American or type 2 genotype (PRRSV-2), the prototype of which is the reference strain ATC2332. Significant antigenic and pathogenic differences have been reported between and within genotypes (Stadejek et al., 2006, 2008; Darwich et al., 2011), correlated with a lack of cross-protection by vaccines (Geldhof et al., 2012). Highly pathogenic strains of PRRSV (HP-PRRSV) have been identified within both genotypes (Xiao et al., 2010; Hu et al., 2012).

The respiratory form of PRRS primarily affects growing and finishing pigs, causing interstitial pneumonia, which induces respiratory signs and increases susceptibility to infection with other pathogens (Rossow, 1998). Although infection with PRRSV may be subclinical, clinical disease becomes evident when secondary infections are present (Drew, 2000); PRRSV thus contributes to the porcine respiratory disease complex (PRDC) (Opriessnig et al., 2011).

In 1996, the appearance of disease outbreaks caused by atypical or HP-PRRSV strains was reported in the USA (Halbur and Bush, 1997). These outbreaks were characterised by increased abortions (10–50%), sow mortality (5–10%) and preweaning mortality, primarily due to respiratory disease. HP-PRRSV strains have been isolated in China and South East Asia (Tian et al., 2007; Feng et al., 2008) and Eastern Europe (Karniychuk et al., 2010). Infection with HP-PRRSV strains is associated with severe clinical signs, pulmonary lesions and aberrant host immune responses (Xiao et al., 2010; Amarilla et al., 2012; Hu et al., 2012).

This paper reviews some aspects of the immunopathogenesis of PRRS and demonstrates how PRRSV replication affects the host pulmonary microenvironment, leading to opportunistic secondary infections.

Lung defences in PRRS

PRRSV damages the pseudostratified ciliated epithelium of the respiratory tract, impairing the mucociliary transport system and preventing the removal of microorganisms from the respiratory system (Done and Paton, 1995; Halbur et al., 1995). The primary target cells for replication of PRRSV are porcine alveolar
macrophages (PAMs) (Duan et al., 1997), which are responsible for phagocytosis of microorganisms in the alveoli (Cheung et al., 2000). Replication of PRRSV in PAMs directly impairs their basic functions, including phagocytosis, antigen presentation and production of cytokines (Thanawongnuwech et al., 2001; De Baere et al., 2012). PRRSV induces necrosis or apoptosis in PAMs (Costers et al., 2008) and also induces apoptosis of lymphocytes and macrophages in the lungs and lymphoid organs, impairing the host immune response (Labarque et al., 2003; Gómez-Laguna et al., 2012b).

PRRSV increases the susceptibility of pigs to secondary bacterial infections and other viral infections (Van Reeth et al., 1996; Thacker et al., 1999; Brockmeier et al., 2000; Drew, 2000; Halbur et al., 2000; Wills et al., 2000; Renukaradhy et al., 2010; Diaz et al., 2012). However, under experimental conditions, secondary infections do not always establish in pigs infected previously with PRRSV (Drew, 2000). Concurrent infection with PRRSV and porcine circovirus type 2 (PCV2) is associated with more severe disease and higher piglet mortality (Allan et al., 2000; Harms et al., 2001; Rovira et al., 2002; Opriessnig et al., 2011). Mycoplasma hyopneumoniae potentiates PRRSV-induced disease (Thacker et al., 1999). There is a high frequency of concurrent infection with PRRSV and Streptococcus suis, M. hyopneumoniae or PCV2 in the field (Segalés et al., 2002).

Factors involved in increased susceptibility of pigs to secondary infections include the pneumovirulence of PRRSV (Martínez-Lobo et al., 2011) and the ability of the virus to persist (>250 days) in lymphoid organs, mainly the tonsils and lymph nodes (Wills et al., 2003). These features are influenced by differences in antigenicity and virulence among PRRSV genotypes, as well as the genetic background of the host. PRRSV-2 exhibits higher pneumovirulence than PRRSV-1, without marked differences in systemic clinical signs, viral load or viral distribution (Martínez-Lobo et al., 2011). However, HP-PRRSV, whether HP-PRRSV-1 or HP-PRRSV-2, is associated with more severe clinical signs, as well as increased severity of lung lesions and viral replication (Amarilla et al., 2012; Hu et al., 2012).

**Role of macrophages in PRRS**

The mononuclear phagocyte system of the lung consists of pulmonary alveolar macrophages (PAMs), pulmonary interstitial macrophages and, in pigs and some other species, pulmonary intravascular macrophages (PIMs) (Longworth, 1997). PAMs are free in the alveolar spaces, where they phagocytise inhaled particles. PIMs are found within the pulmonary capillaries, adherent to endothelial cells; they remove foreign particles from the blood and are able to migrate to sites of injury. PIMs release pro-inflammatory mediators, such as metabolites of arachidonic acid and cytokines that modulate pulmonary microvascular physiology (Bertram et al., 1988; Chitko-Mckown et al., 1991; Carrasco et al., 2002).

PAMs represent the main cellular target for PRRSV replication, although the virus is also able to replicate in other macrophage subpopulations (Duan et al., 1997). In addition, PRRSV replicates in monocyte and bone marrow derived dendritic cells (DCs) in vitro, although such replication has not been demonstrated clearly in vivo (Loving et al., 2007; Wang et al., 2007; Chang et al., 2008; Flores-Mendoza et al., 2008; Gimeno et al., 2011). The main cell surface receptors for PRRSV attachment, internalisation and uncoating are heparan sulphate, sialoadhesin and CD163 (Delputte et al., 2005; Van Gorp et al., 2008).

PRRSV replicates in PAMs and PIMs in vitro, affecting their bactericidal functions (Thanawongnuwech et al., 1997). Since one of the major functions of PIMs is the clearance of bacteria from blood (Winkler and Cheville, 1987; Staub, 1994), viral replication in PAMs and PIMs may affect colonisation of the lungs by secondary viral or bacterial pathogens, as well as haematogenous bacterial dissemination.

PRRSV has a higher tropism for PAMs than for septal macrophages (PIMs and interstitial macrophages) (Gómez-Laguna et al., 2010b; Amariella et al., 2012). Whereas PRRSV replicates primarily in PAMs, expression of pro-inflammatory cytokines has been observed mainly in septal macrophages, pointing to different roles of PAMs and septal macrophages in PRRS. The main function of PAMs is to provide the first line of phagocytic defence against microbial invasion (Cheung et al., 2000; De Baere et al., 2012). Sep tal macrophages, which mostly are activated indirectly by the replication of PRRSV in bystander cells, may modulate local inflammatory and immune responses through secretion of cytokines (Gómez-Laguna et al., 2010b). Sialoadhesin, but not CD163, downregulates phagocytosis in PRRSV-infected PAMs (De Baere et al., 2012).

**Modulation of the immune response by PRRSV and secondary respiratory infections**

PRRSV increases the susceptibility of the host to a wide range of viral and bacterial respiratory pathogens, resulting in increased severity of clinical signs and lung lesions. Concomitant infections with PRRSV and swine influenza virus, porcine respiratory coronavirus (PRCV) or PCV2 are common. More severe clinical signs and growth retardation occur in co-infected pigs than in pigs infected with PRRSV alone (Van Reeth et al., 1996; Harms et al., 2001; Jung et al., 2009; Renukaradhy et al., 2010; Opriessnig et al., 2011). In pigs dually infected with PRRSV and PRCV, exacerbation of clinical signs is associated with an impaired innate immune response in the lungs, specifically a decrease in natural killer (NK) cell-mediated cytotoxicity due to decreased expression of interferon (IFN)- α. The adaptive immune response is also impaired, leading to enhanced apoptosis of PAMs as a consequence of increased concentrations of interleukin (IL)-6 and IL-10 (Jung et al., 2009; Renukaradhy et al., 2010).

Modulation of the innate immune response in PRRSV-infected pigs under field conditions is associated with a decrease in cytotoxicity, but not the percentage, of NK cells (Dwivedi et al., 2012). Functional modulation of NK cells is associated with increased plasma concentrations of IL-4, IL-10 and IL-12, suggesting a role for these cytokines in modulating the host immune response (Dwivedi et al., 2012). Regulatory T cells (Tregs) induced by PRRSV-2, but not PRRSV-1, impair the host immune response (Cece et al., 2012; Silva-Campa et al., 2009, 2010, 2012). Although PRRSV-2 increases the percentage of Tregs, its effect on the immune response in vivo is not yet clear.

The susceptibility of PRRSV-infected pigs to secondary bacterial pathogens has been linked to upregulation of CD14 and lipopolysaccharide (LPS)-binding protein (LBP), which cooperate in the recognition and signalling of LPS. Enhanced expression of these molecules in the lungs increases susceptibility to secondary bacterial infection. Although there is upregulation of CD14 in PRRSV-infected septal macrophages and PAMs, most CD14 enhancement in the lungs of PRRSV-infected pigs is probably due to infiltration of CD14-positive monocytes into the pulmonary interstitium; these may later differentiate into septal macrophages (Van Gucht et al., 2005; Qiao et al., 2011). PRRSV and bacterial endotoxin act synergistically to amplify the inflammatory response of infected macrophages (Qiao et al., 2011). Higher expression of pro-inflammatory cytokines is observed in septal macrophages of PRRSV-infected pigs (Gómez-Laguna et al., 2010b). These findings suggest a role for septal macrophages in the immunopathogenesis of secondary bacterial infections in PRRS through upregulation of CD14 and activation of a cytokine cascade (Fig. 2).
Acute phase proteins in PRRS

Acute phase proteins (APPs) are involved in the acute phase response and are synthesised mainly by hepatocytes in response to pro-inflammatory cytokines (Eckersall, 2000; Petersen et al., 2004). APPs can be classified as ‘positive’ or ‘negative’, depending on whether their serum concentrations are increased or decreased, respectively, in the acute phase response. Haptoglobin (Hp), C-reactive protein (CRP), serum amyloid A (SAA) and ‘pig-major acute protein’ (Pig-MAP) are the main APPs in pigs (Petersen et al., 2004). APPs induce pro-inflammatory reactions and fever, but overexpression can also induce an anti-inflammatory state (Ceciliani et al., 2002; Petersen et al., 2004).

A lack of significant changes in serum concentrations of pro-inflammatory cytokines in PRRS may point to a strategy by which PRRSV is able to escape from the host immune response, thereby preventing activation of hepatocytes to induce an efficient acute phase response. Early expression of Hp and Pig-MAP is observed in PRRS, whereas serum concentrations of CRP and SAA exhibit a delayed and variable enhancement (Gómez-Laguna et al., 2010c). CRP is involved in complement activation and opsonisation, as well as inducing production of cytokines by macrophages (Ceciliani et al., 2002; Petersen et al., 2004). SAA is chemotactic for monocytes, T cells and polymorphonuclear leucocytes (Ceciliani et al., 2002; Petersen et al., 2004). The late increase in CRP and SAA may contribute to the inefficient early immune response in PRRSV-infected pigs.

Hp modulates the immune response through complex interactions with a range of effectors. In experimental infections with PRRSV, there is an increase in serum concentrations of Hp, along with increased expression of IL-6 and tumour necrosis factor (TNF-α) (Asai et al., 1999; Gómez-Laguna et al., 2010c). Hp interacts with CD163, the receptor for PRRSV, increasing expression of the anti-inflammatory cytokine IL-10 (Díaz et al., 2005). CD163 removes haemoglobin–Hp complexes circulating in the blood (Kristiansen et al., 2001), decreasing the amount of haemoglobin available for bacterial pathogens and reducing oxidative stress. Hp may play a role in the pathogenesis of PRRS through modulating the immune response and inducing anti-inflammatory cytokines, such as IL-10.

Samples of saliva and meat juice are alternatives to serum for measuring and monitoring APPs during PRRSV infection in pigs (Gutiérrez et al., 2009; Gómez-Laguna et al., 2010a). Pen-based oral-fluid samples also represent a reliable, cost-effective approach to PRRSV surveillance in pigs (Prickett et al., 2008; Kittawornrat et al., 2010).

Table 1

| Cytokine | Role in PRRS | References |
|----------|--------------|------------|
| IL-1     | Chemotaxis of monocytes and neutrophils Early upregulation in BALF and in the lung parenchyma Poor and delayed enhancement in serum | Thawanongnuwech et al. (2001) Labarque et al. (2003) Van Gucht et al. (2003) Gómez-Laguna et al. (2010b, 2010c) Renukaradhya et al. (2010) García-Nicolás et al. (2011) |
| IL-6     | Induction of acute phase proteins Upregulation in situ in the lung parenchyma | Asai et al. (1999) Gómez-Laguna et al. (2010b) |
| TNF-α    | Inhibition of PRRSV replication Induction of acute phase proteins Downregulated in PRRSV-infected PAMs | López-Fuertes et al. (2000) Gómez-Laguna et al. (2010b) Albina et al. (1998) |
| IFN-α    | Correlation between virus specific IFN-α-SCs and IFN-γ-SCs Interference with PRRSV replication Downregulation in PRRSV-infected PAMs and/or PBMCs | Van Reeth et al. (1999) Royaee et al. (2004) |
| IFN-γ    | Inhibition PRRSV replication Enhancement by PRRSV vaccination with IL-12 or IFN-α Delayed onset of PRRSV specific IFN-γ-SCs Higher expression with highly virulent PRRSV strains | Bautista and Molitor, 1999 Foss et al. (2002) Meier et al. (2003) Thawanongnuwech et al. (2003) Diaz et al. (2005, 2006) |
| IL-10    | Correlation with the expression of PRRSV in the lung Inhibition of the expression of IFN-γ and other cytokines | Diaz et al. (2006) Gómez-Laguna et al. (2010b) |
| TGF-β    | Induction of regulatory T cells after PRRSV-2 infection Correlation with the expression of PRRSV in the lung | Silva-Campa et al. (2009) Gómez-Laguna et al. (2012a) |

IL, interleukin; BALF, bronchoalveolar lavage fluid; TNF, tumour necrosis factor; PRRSV, porcine reproductive and respiratory syndrome virus; PAMs, porcine alveolar macrophages; IFN, interferon; SC, secreting cells; PBMCs, peripheral blood mononuclear cells; TGF, transforming growth factor; PRRSV-2, porcine reproductive and respiratory syndrome virus type 2.
tions of pro-inflammatory cytokines (Baarsch et al., 1995; Conn et al., 1995). Increases in expression of IL-1α, IL-6 and TNF-α in the lungs of pigs infected with PRRSV-1 are correlated with the development of interstitial pneumonia (Gómez-Laguna et al., 2010b). As discussed above, PRRSV impairs the host immune response (Darwich et al., 2010). Expression of PRRSV antigens is correlated with expression of regulatory cytokines, such as IL-10 and transforming growth factor (TGF)-β, in the lungs of pigs (Gómez-Laguna et al., 2010b, 2012a; Table 1). The lack of substantial changes in serum concentrations of pro-inflammatory cytokines may reflect a strategy by which PRRSV evades the host immune response. Therefore, samples of blood and/or serum do not necessarily reflect the events occurring in lungs of PRRSV-infected animals at a given time, although these are very accessible samples.

Differential expression of pro-inflammatory cytokines has been observed in lymphoid organs of PRRSV-infected pigs. Whereas a marked increase in expression of TNF-α and IL-1α was observed in the mediastinal lymph nodes, there was a limited increase in expression of these cytokines in the tonsils and retropharyngeal lymph nodes (Barranco et al., 2012).

Pro- and anti-inflammatory responses in PRRS

PRRSV isolates have been classified on the basis of whether they induce TNF-α, IL-10, both of these cytokines or neither of these cytokines (Gimeno et al., 2011). TNF-α plays an important role in the inflammatory response; this cytokine can act as an antiviral cytokine, protecting cells from infection against viruses or enhancing selective elimination of virus-infected cells through an IFN-independent mechanism. Recombinant porcine TNF-α inhibits PRRSV replication in vitro and PRRSV-infected PAMs have reduced expression of TNF-α (López-Fuertes et al., 2000). Several PRRSV strains are weak inducers of TNF-α and also impair TNF-α production by inhibiting the extracellular signal-regulated kinase (ERK) signalling pathway; this mechanism may contribute to the virulence of HP-PRRS (Hou et al., 2012). Promoter and post-transcriptional downregulation of TNF-α has been associated with PRRSV non-structural proteins (NSPs) 1α, 1β and 2 (Chen et al., 2010; Subramaniam et al., 2010, 2012; Darwich et al., 2011). Specific amino acid residues of NSP1α and NSP1β have been identified that affect TNF-α promoter activity; substitution of specific NSP1β amino acids is a potential tool for the generation of attenuated PRRSV vaccines (Subramaniam et al., 2012). The limited expression of TNF-α in experimental infections with some PRRSV strains may be a mechanism by which these strains impair the host immune response and prevent viral clearance (Table 1; Fig. 1).

IL-10 is a regulatory cytokine that, among other functions, inhibits the synthesis of pro-inflammatory cytokines (Moore et al., 2001). A role for IL-10 in inhibiting or counterbalancing the IFN-γ response and restricting PRRSV replication has been proposed in infections with some PRRSV strains (Bautista and Molitor, 1999; Díaz et al., 2006). Thus, in the same way that IL-10 inhibits the production of IFN-γ, it may also suppress the pro-inflammatory response in PRRSV-infected pigs (Table 1; Fig. 1).

A different behaviour of regulatory cytokines, such as IL-10 or TGF-β, has been observed in PRRSV-infected pigs. There is a significant correlation between PRRSV antigen expression and the expression of regulatory cytokines, such IL-10 and TGF-β, in the lungs, but not in the lymphoid organs, of infected pigs (Barranco et al., 2012; Gómez-Laguna et al., 2010b, 2012a). One hypothesis suggested by these findings is that cells recruited to the pulmonary parenchyma are modulated to secrete IL-10 and/or TGF-β, among other immune mediators. An alternative hypothesis is that the expression of regulatory cytokines plays a key role in the modulation of the inflammatory response within the lung, reducing infiltration and proliferation of inflammatory cells (Spight et al., 2005; Charavaryamath et al., 2006; Backus et al., 2010).

IL-10 is expressed mainly by septal macrophages and, to a lesser extent, PAMs and other cells, whereas TGF-β is expressed mainly in PAMs and secondarily in septal macrophages (Gómez-Laguna et al., 2010b, 2012a). Expression of regulatory cytokines by different subsets of lung macrophages may impair bacterial killing by PAMs and PIMs, as well reduce the efficiency of the local host immune response by septal macrophages. Production of TGF-β occurs in recall responses to some PRRSV-1 strains and is more marked after homologous reinfection, suggesting the presence of TGF-β producing T cell clones dependent on the PRRSV strain (Díaz et al., 2012).

The imbalance between pro- and anti-inflammatory cytokines may modulate the expression of CD163, which is one component of a complex of receptors required for PRRSV entry, including heparan sulphate and sialoadhesin (Delpitte et al., 2005; Van Gorp et al., 2008). Expression of CD163 is upregulated by IL-10 and IL-6, promoting PRRSV entry and replication, but downregulated by TNF-α, TGF-β and IFN-γ (Buechler et al., 2000; Sulahian et al., 2000; Pioli et al., 2004; Weaver et al., 2007; Patton et al., 2009). Other mediators may regulate expression of CD163 (Table 2). There is differential expression of cytokines by different PRRSV isolates (Gimeno et al., 2011) and within different lymphoid organs.
Barranco et al., 2012), indicating that analysis of the expression of pro- and anti-inflammatory cytokines is a useful tool in the study of the immunopathogenesis of different PRRSV strains.

Conclusions

PRRSV impairs local immune responses in the lungs of pigs by a range of mechanisms, including impairment of the mucociliary transport system, decreases in the function and number of PAMs, induction of apoptosis of immune cells and creating an imbalance between pro- and anti-inflammatory cytokines, which may allow the virus to persist in the host. PRRSV decreases the bactericidal activity of macrophages, resulting in increased susceptibility to secondary infections. The variability in cytokine profiles induced by different PRRSV strains, as well as the emergence of HP-PRRSV in Asia and Eastern Europe, highlight the need to evaluate differences in immunobiology among PRRSV strains. PRRSV is able to modulate the immune response by increasing the expression of Hp in the early stages of infection, which may interact with the viral receptor CD163 and induce the synthesis of anti-inflammatory mediators, such as IL-10. There is a need for improved knowledge of the immune response in PRRSV infections in order to improve the efficacy of vaccines to control PRRS.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Table 2

Mediators involved in regulation of CD163 receptor.

| Mediator                  | Upregulation | Downregulation | References               |
|---------------------------|--------------|----------------|--------------------------|
| IL-6                      | +            |                | Buechler et al. (2000)   |
| IL-10                     | +            |                | Buechler et al. (2000)   |
| M-CSF                     | +            |                | Ritter et al. (1999)     |
| Hb–Hp complexes           | +            |                | Ugocsai et al. (2006)    |
| Dexamethasone             | +            |                | Ritter et al. (1999)     |
| TNF-α                     |              | +              | Buechler et al. (2000)   |
| IFN-γ                     |              | +              | Buechler et al. (2000)   |
| TGF-β                     |              | +              | Pioli et al. (2004)      |
| GM-CSF+IL-4               |              | +              | Ritter et al. (1999)     |
| Lipopolysaccharide        |              | +              | Buechler et al. (2000)   |
| 12-O-tetradecanoylphorbol-13-acetate |              | +              | Patton et al. (2009)     |

IL, interleukin; M-CSF, macrophage colony-stimulating factor; HB–Hp, haemoglobin–haptoglobin; TNF, tumour necrosis factor; IFN, interferon; TGF, transforming growth factor; GM-CSF, granulocyte macrophage colony-stimulating factor.
References

Albina, E., Carrat, C., Charley, B., 1998. Interferon-alpha response to swine arterivirus (PoAV), the porcine reproductive and respiratory syndrome virus. Journal of Interferon and Cytokine Research 18, 485–490.

Allan, G.M., McNeilly, F., Ellis, J., Krakowka, S., Rehan, B., McNair, L., Walker, L., Kennedy, S., 2000. Experimental infection of colostrum deprived piglets with porcine circovirus 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) potentiates PCV2 replication. Archives of Virology 145, 2421–2432.

Amarilla, P., Morgan, S., Gómez-Laguna, J., Graham, S., 2004. Induction of CD4+ CD25+ FoxP3+ T cells by Co-infection of porcine dendritic cells with porcine circovirus type 2a (PCV2a) or a combination of both organisms in pigs. American Journal of Veterinary Research 65, 1441–1445.

Carrasco, L., 2012. Enhanced expression of TGF-β in pigs infected with porcine reproductive and respiratory syndrome virus. Veterinary Immunology and Immunopathology 147, 143–148.

Díaz, I., Martin, M., 2012. Different European-type vaccines against porcine reproductive and respiratory syndrome virus have different immunological properties and confer different protection to pigs. Virology 351, 249–259.

Díaz, I., Lar));//2012. Effective responses of pigs after experimental infection with a European strain of porcine reproductive and respiratory syndrome virus. Journal of General Virology 86, 1491–1495.

Díaz, I., Gómez, J., Lar));//2005. Immune responses of pigs after experimental infection with a European strain of porcine reproductive and respiratory syndrome virus. Veterinary Research 36, 32–35.

Drew, T.W., 2000. A review of evidence for immunosuppression due to porcine reproductive and respiratory syndrome virus. Veterinary Research 31, 27–39.

Druet, P., 2002. Autologous and heterologous PRRSV vaccines with different nucleotide sequences have similar safety profiles and identification of cellular targets in the lungs and lymphoid tissues of pigs at different time intervals after inoculation with porcine reproductive and respiratory syndrome virus (PRRSV). Veterinary Microbiology 56, 9–19.

Drew, T.W., McCracken, M., Darwich, L., Mettenleiter, T.C., 1999. Piglet challenge study during early stage of infection under farm conditions. Virology Journal 9, 45.

Eckersall, P.D., 2000. Recent advances and future prospect for the use of acute phase proteins as markers of disease in animals. Revue Médicine Vétérinaire 151, 577–584.

Feng, Y., Zhao, T., Gao, X., Li, Z., Liu, X., 2007. Clinical porcine reproductive and respiratory syndrome virus infection mediated apoptosis in B- and T-cell areas of lymphoid organs of experimentally infected pigs. Transboundary and Emerging Diseases 59, 145–153.

García-Nicolás, O., Quereda, J.J., Gómez-Laguna, J., Barranco, I., Rodríguez-Gómez, I.M., 2006. Viral peptide expression and inflammatory cytokines gene expression and protein levels in tonsil and serum of PRRSV-infected pigs. In: Proceedings of the 6th Symposium on Emerging and Re-emerging Pig Diseases, Barcelona, Spain, 12–15 June 2011, pp. 200.

Gimeno, M., Darwich, L., Pappaterra, G., Pujols, J., Mateu, E., 2006. Different European-type vaccines against homologous and heterologous challenges. BMJ Veterinary Research 8, 281–293.

Gimeno, M., Darwich, L., Diaz, I., de la Torre, E., Pujols, J., Martin, M., Inumaru, S., Cano, E., Domingo, M., Montoya, M., Mateu, E., 2011. Cytokine profiles and phenotype regulation of antigen presenting cells by genotype-I porcine reproductive and respiratory syndrome virus. Veterinary Microbiology 150, 49–62.

Gómez-Laguna, J., Segalés, J., Martín, M., Pujols, J., Mateu, E., 2002. Adjuvant danger signals increase the immune response to porcine reproductive and respiratory virus. Viral Immunology 15, 720–725.

Gómez-Laguna, J., Salguero, F.J., De Marco, M.F., Pallarés, F.J., Bernabé, A., Carrasco, L., Gimeno, M., Darwich, L., Pappaterra, G., Pujols, J., Mateu, E., 2008. Different European-type vaccines against homologous and heterologous adaptiv immune responses in porcine reproductive and respiratory syndrome virus infection. Veterinary Research 43, 30.

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Gómez-Laguna, J., Gutiérrez, A., Pallarés, F.J., Salguero, F.J., Cerón, J.J., Carrasco, L., Gimeno, M., Darwich, L., Pappaterra, G., Pujols, J., Mateu, E., 2012. Characterization of homologous and heterologous immune responses in porcine reproductive and respiratory syndrome virus infection. Veterinary Research 43, 30.

Done, S.H., Paton, D.J., 1995. Porcine reproductive and respiratory syndrome: Clinical disease, pathology and immunosuppression. Veterinary Research 26, 1–14.

Drew, T.W., 2000. A review of evidence for immunosuppression due to porcine reproductive and respiratory syndrome virus. Veterinary Research 31, 27–39.

Druet, P., Costes, S., Nauwynck, H.J., 2005. Analysis of porcine reproductive and respiratory syndrome virus attachment and internalization: Distinctive roles for heparan sulphate and sialoadhesin. Journal of General Virology 86, 1441–1445.

Díaz, I., Lar));//2005. Immune responses of pigs after experimental infection with a European strain of porcine reproductive and respiratory syndrome virus. Journal of General Virology 86, 1491–1495.

Díaz, I., Gómez, J., Lar));//2005. Immune responses of pigs after experimental infection with a European strain of porcine reproductive and respiratory syndrome virus. Veterinary Research 36, 32–35.
López-Fuertes, L., Campos, E., Doménech, N., Ezquerra, A., Castro, J.M., Domínguez, J., Labarque, G., Van Gucht, S., Nauwynck, H., Van Reeth, K., Pensaert, M., 2003. 9th Report of J. M., Prieto, C., 2011. Comparative pathogenicity of type 1 and type 2 isolates of PRRSV in pigs. Journal of Veterinary Diagnostic Investigation 20, 133–148.
Patterson, J.B., Rowland, R.R., Yoo, D., Chang K.D., 2000. Modulation of CD163 receptor expression and replication of porcine reproductive and respiratory syndrome virus in porcine macrophages. Virus Research 140, 161–171.
Petersen, H.H., Nielsen, J.P., Heegaard, P.M., 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Veterinary Clinical Research 35, 163–187.
Pioli, P.A., Goonan, K.E., Wardwell, K., Guery, P.M., 2004. TGF-β regulation of human macrophage scavenger receptor CD163 is Smad3-dependent. Journal of Leukocyte Biology 76, 500–508.
Prickett, J., lser, C., Chrest, A., Henning, J., Yoon, K.J., Evans, R.B., Zimmerman, J.J., 2008. Detection of porcine reproductive and respiratory syndrome virus infection in porcine oral fluid samples: A longitudinal study under experimental conditions. Journal of Veterinary Diagnostic Investigation 20, 156–163.
Qiao, S., Sun, L., Bao, D., Guo, S., Zhang, S., 2011. Porcine reproductive and respiratory syndrome virus and bacterial endotoxin act in synergy to amplify the inflammatory response of infected macrophages. PLoS Pathogens 7, e1002379.
Renukarady, G.J., Alekke, K., Jung, K., Yang, S., Saif, L.J., 2010. Porcine reproductive and respiratory syndrome virus-induced immunosuppression exacerbates the inflammatory response to porcine respiratory coronavirus in pigs. Journal of Animal Science 88, 3153–3162.
Ritter, M., Buechler, C., Langmann, T., Orso, E., Klucken, J., Schmitz, G., 1999. The scavenger receptor CD163: regulation, promoter structure and genomic organization. Pathobiology 67, 257–261.
Rosow, K.D., 1998. Porcine reproductive and respiratory syndrome. Veterinary Pathology 35, 1–20.
Rovira, A., Balasch, M., Segalés, J., García, L., Planas-Durán, J., Rosell, C., Ellerbrok, H., Mankertz, A., Domingo, M., 2002. Experimental inoculation of conventional pigs with porcine reproductive and respiratory syndrome virus and porcine circovirus 2. Journal of Virology 76, 1322–1329.
Royea, A.R., Husmann, R.J., Dawson, H.D., Calzada-Novas, G., Schnitzlein, W.M., Zuckermann, F.A., Lunney, J.K., 2004. Deciphering the involvement of innate immune factors in the development of the host response to PRRSV vaccination. Veterinary Immunology and Immunopathology 102, 199–216.
Segalés, J., Calsamiglia, M., Rosell, C., Soler, M., Maldonado, J., Martín, M., Domingo, M., 2002. Porcine reproductive and respiratory syndrome virus (PRRSV) infection status in pigs from PRRSV affected herds but not experimentally infected pigs. Veterinary Research 33, 537–536.
Silva-Campa, E., Cordoba, L., Fraile, L., Flores-Mendoza, L., Montoya, M., Hernández, J., 2010. European genotype of porcine reproductive and respiratory syndrome (PRRSV) infects monocyte-derived dendritic cells but does not induce Treg cells. Virology 396, 264–271.
Silva-Campa, E., Mata-Haro, V., Mateu, E., Hernández, J., 2012. Porcine reproductive and respiratory syndrome virus induces CD4+CD8+CD25+Foxp3+ regulatory T cells (Tregs). Virology 430, 73–80.
Silva-Campa, E., Flores-Mendoza, L., Reséndiz, M., Pinelli-Saavedra, A., Mata-Haro, M., Wanga, W., Hernández, J., 2009. Induction of T helper 3 regulatory cells by dendritic cells infected with porcine reproductive and respiratory syndrome virus. Journal of Virology 83, 373–379.
Spith, D., Zhao, B., Haas, M., Wert, S., Denenberg, A., Shanley, T.P., 2005. Immunoregulatory effects of regulated, lung-targeted expression of IL-10 in vivo. American Journal of Physiology. Lung Cellular and Molecular Physiology 288, 1251–1265.
Stadejek, T., Oleksiekiewicz, M.B., Potapchuk, D., Podgoriska, K., 2006. Porcine reproductive and respiratory syndrome virus strain diversity in Europe supports the definition of new genetic subtypes. Journal of General Virology 87, 1835–1841.
Stadejek, T., Oleksiekiewicz, M.B., Scherbakov, A.V., Timina, A.M., Krabbe, J.S., Chabros, K., Potapchuk, D., 2008. Definition of subtypes in the European genotype of porcine reproductive and respiratory syndrome virus: Nucleocapsid characteristics and geographical distribution in Europe. Archives of Virology 153, 1453–1488.
Stadejek, T., Oleksiekiewicz, M.B., Krabbe, J.S., Young, T.F., Thacker, B.J., Thacker, E.L., 2001. Differential expression of proinflammatory cytokines: In vitro PRRSV and influenza A virus infections in dendritic cells. Veterinary Immunology and Immunopathology 81, 199–216.
Thanawongnuwech, R., Young, T.F., Thacker, B.J., Thacker, E.L., 2001. Differential expression of proinflammatory cytokines: In vitro PRRSV and influenza A virus infections in dendritic cells. Veterinary Immunology and Immunopathology 81, 199–216.
Subramaniam, S., Beura, L.K., Kwon, B., Pattainak, A.K., Osorio, F.A., 2012. Amino acid residues in the non-structural protein 1 of porcine reproductive and respiratory syndrome virus involved in down-regulation of TNF-α expression in vitro and attenuation in vivo. Virology 432, 241–249.
Subramaniam, S., Kwon, B., Beura, L.K., Kusyynski, C.A., Pattainak, A.K., Osorio, F.A., 2010. Porcine reproductive and respiratory syndrome virus non-structural protein 1 suppresses tumor necrosis factor-alpha promoter activation by inhibiting NF-κB and NF-κB activation. PLoS ONE 6, 676. 9. 
Su, N.C., 1994. Pulmonary intravascular macrophages. Annual Review of Physiology 56, 47–67.
Sulahian, T.H., Hogger, P., Wahner, A.E., Wardwell, K., Goulding, N.J., Sorg, C., Drost, A., Stehling, M., Wallace, P.K., Morganelli, P.M., Guery, P.M., 2000. Human monocytes express CD163, which is upregulated by IL-10 and identical to p155. Cytokine 12, 1312–1321.
Thacker, E.L., Halbur, P.G., Ross, R.F., Thanawongnuwech, R., Thacker, B.J., 1999. Mycoplasma hyopneumoniae potentiation of porcine reproductive and respiratory syndrome virus-induced pneumonia. Journal of Clinical Microbiology 37, 620–626.
Thanawongnuwech, R., Rungsipipat, A., Disneyan, S., Saisayombat, R., Napakornaporn, S., Halbar, P.G., 2003. Immunohistochemical staining of IFN-γ positive cells in porcine reproductive and respiratory syndrome virus-infected lungs. Veterinary Immunology and Immunopathology 91, 73–77.
Thanawongnuwech, R., Young, T.F., Thacker, B.J., Thacker, E.L., 2001. Differential production of proinflammatory cytokines: In vitro PRRSV and Mycoplasma hyopneumoniae co-infection model. Veterinary Immunology and Immunopathology 79, 115–127.
Thanawongnuwech, R., Thacker, E.L., Halbar, P.G., 1997. Effect of porcine reproductive and respiratory syndrome virus (PRRSV) (isolate ATCC VR-2385) infection on bacterial activity of porcine pulmonary intravascular macrophages (PIMs): In vitro comparisons with pulmonary alveolar macrophages.
macrophages (PAMs). Veterinary Immunology and Immunopathology 59, 323–335.

Tian, K., Yu, X., Zhao, T., Feng, Y., Cao, Z., Wang, C., Hu, Y., Chen, X., Hu, D., Tian, X., Liu, D., Zhang, S., Deng, X., Ding, Y., Yang, L., Zhang, Y., Xiao, H., Qiao, M., Wang, B., Hou, L., Wang, X., Yang, X., Kang, L., Sun, M., Jin, P., Wang, S., Kitamura, Y., Yan, J., Gao, G.F., 2007. Emergence of fatal PRRSV variants: Unparalleled outbreaks of atypical PRRS in China and molecular dissection of the unique hallmark. PLoS ONE 2, e526.

Ugocsai, P., Barlage, S., Dada, A., Schmitz, G., 2006. Regulation of surface CD163 expression and cellular effects of receptor mediated hemoglobin-haptoglobin uptake on human monocytes and macrophages. Cytometry A 69, 203–205.

Van Gorp, H., Van Breedam, W., Delputte, P.L., Nauwynck, H.J., 2008. Sialoadhesin and CD163 join forces during entry of the porcine reproductive and respiratory syndrome virus. Journal of General Virology 89, 2943–2953.

Van Gucht, S., Van Reeth, K., Pensaert, M., 2003. Interaction between porcine reproductive-respiratory syndrome virus and bacterial endotoxin in the lungs of pigs: Potentiation of cytokine production and respiratory disease. Journal of Clinical Microbiology 41, 960–966.

Van Gucht, S., Van Reeth, K., Nauwynck, H., Pensaert, M., 2005. Porcine reproductive and respiratory syndrome virus infection increases CD14 expression and lipopolysaccharide-binding protein in the lungs of pigs. Viral Immunology 18, 116–126.

Van Reeth, K., Labarque, G., Nauwynck, H., Pensaert, M., 1999. Differential production of proinflammatory cytokines in the pig lung during different respiratory virus infections: Correlations with pathogenicity. Research in Veterinary Science 67, 47–52.

Van Reeth, K., Nauwynck, H., Pensaert, M., 1996. Dual infections of feeder pigs with porcine reproductive and respiratory syndrome virus followed by porcine respiratory coronavirus or swine influenza virus: A clinical and virological study. Veterinary Microbiology 48, 325–335.

Wang, X., Eaton, M., Mayer, M., Li, H., He, D., Nelson, E., Christopher-Hennings, J., 2007. Porcine reproductive and respiratory syndrome virus productively infects monocyte-derived dendritic cells and compromises their antigen-presenting ability. Archives of Virology 152, 289–303.

Weaver, L.K., Pioli, P.A., Wardwell, K., Vogel, S.N., Guyre, P.M., 2007. Up-regulation of human monocyte CD163 upon activation of cell-surface Toll-like receptors. Journal of Leukocyte Biology 81, 663–671.

Wills, R.W., Doster, A.R., Galeota, J.A., Sur, J.H., Osorio, F.A., 2003. Duration of infection and proportion of pigs persistently infected with porcine reproductive and respiratory syndrome virus. Journal of Clinical Microbiology 41, 58–62.

Wills, R.W., Gray, J.T., Fedorka-Cray, P.J., Yoon, K.J., Ladely, S., Zimmerman, J.J., 2000. Synergism between porcine reproductive and respiratory syndrome virus (PRRSV) and Salmonella choleraesuis in swine. Veterinary Microbiology 71, 177–192.

Winkler, G.C., Cheville, N.F., 1987. Postnatal colonization of porcine lung capillaries by intravascular macrophages: An ultrastructural, morphometric analysis. Microvascular Research 33, 224–232.

Xiao, S., Mo, D., Wang, Q., Jia, J., Qin, L., Yu, X., Niu, Y., Zhao, X., Liu, X., Chen, Y., 2010. Aberrant host immune response induced by highly virulent PRRSV identified by digital gene expression tag profiling. BMC Genomics 11, 544.