Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Veterinary Immunology and Immunopathology 167 (2015) 1–14

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Review Paper

Innate and adaptive immunity against Porcine Reproductive and Respiratory Syndrome Virus

Crystal L. Loving[a,*], Fernando A. Osorio[b], Michael P. Murtaugh[c], Federico A. Zuckermann[d]

[a] USDA-ARS-National Animal Disease Center, Ames, IA, United States
[b] Nebraska Center for Virology and School of Veterinary & Biomedical Sciences, University of Nebraska-Lincoln, United States
[c] Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, MN, United States
[d] Department of Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana-Champaign, IL, United States

ARTICLE INFO

Article history:
Received 19 March 2015
Received in revised form 29 June 2015
Accepted 1 July 2015

Keywords:
PRRSV
Immunology
Antibody
Cell-mediated immunity
Innate immunity

ABSTRACT

Many highly effective vaccines have been produced against viruses whose virulent infection elicits strong and durable protective immunity. In these cases, characterization of immune effector mechanisms and identification of protective epitopes/immunogens has been informative for the development of successful vaccine programs. Diseases in which the immune system does not rapidly clear the acute infection and/or convalescent immunity does not provide highly effective protection against secondary challenge pose a major hurdle for clinicians and scientists. Porcine reproductive and respiratory syndrome virus (PRRSV) falls primarily into this category, though not entirely. PRRSV causes a prolonged infection, though the host eventually clears the virus. Neutralizing antibodies can provide passive protection when present prior to challenge, though infection can be controlled in the absence of detectable neutralizing antibodies. In addition, primed pigs (through natural exposure or vaccination with a modified-live vaccine) show some protection against secondary challenge. While peripheral PRRSV-specific T cell responses have been examined, their direct contribution to antibody-mediated immunity and viral clearance have not been fully elucidated. The innate immune response following PRRSV infection, particularly the antiviral type I interferon response, is meager, but when provided exogenously, IFN-α enhances PRRSV immunity and viral control. Overall, the quality of immunity induced by natural PRRSV infection is not ideal for informing vaccine development programs.

The epitopes necessary for protection may be identified through natural exposure or modified-live vaccines and subsequently applied to vaccine delivery platforms to accelerate induction of protective immunity following vaccination. Collectively, further work to identify protective B and T cell epitopes and mechanisms by which PRRSV eludes innate immunity will enhance our ability to develop more effective methods to control and eliminate PRRS disease.

Published by Elsevier B.V.

Contents

* Corresponding author.
E-mail address: crystal.loving@ars.usda.gov (C.L. Loving).

http://dx.doi.org/10.1016/j.vetimm.2015.07.003
0165-2427/Published by Elsevier B.V.

1. Introduction

PRRSV is a member of the Arteriviridae family, along with equine arteritis virus (EAV), lactate dehydrogenase elevating virus (LDV) of mice, and simian hemorrhagic fever virus (SHFV). The PRRSV genome is a positive-sense, single-stranded RNA of approximately 15 kb that encodes 10 open reading frames (ORF): ORF1a, ORF1b, ORF2a, ORF2b, and ORFs 3–7, which includes ORF5 and ORF5a (Firth et al., 2011; Johnson et al., 2011). ORF1 (a and b) code for two large polyproteins that are cleaved into 14 nonstructural proteins (Fang and Snijder, 2010). There are seven structural proteins encoded by ORF2a, ORF2b, ORF3-7 and ORF5a: glycoprotein (GP) 2, small envelope (E), GP3, GP4, GP5, membrane (M), nucleocapsid (N) proteins, and ORF5a protein (Dea et al., 2000; Meulenberg et al., 1995). GP5 and M can form a dimer, and GP2, GP3, and GP4 can form a hetero-trimer that has been shown to facilitate viral entry into the host cell (Mardassi et al., 1996; Wissink et al., 2005). PRRSV's are grouped into 2 genotypes; European (Type I) and North American (Type II) and there are significant genetic differences between the two genotypes. In addition, there is significant heterogeneity among strains within a genotype, giving rise to a significant number of antigenically distinct viruses, although a clear antigenic classification scheme has not been developed. Thus, protection against heterologous viruses with a vaccine is highly sought.

PRRSV infection is persistent, as numerous research studies have shown that virus can be isolated from lymphoid tissue months after the initial infection (Allende et al., 2000). However, the implementation of herd closure and farm stabilization protocols using exposure to wildtype PRRSV has shown that the virus can be eliminated from an individual animal and herd (Linhares et al., 2014; Torremorell et al., 2002). Therefore, the pig immune system is capable of mounting a response that eventually resolves the infection, eliminating the virus from the animal entirely. However, clearance and disease resolution takes a significant amount of time – most herd closure protocols indicate more than 200 days. Ultimately, this tells us that the pig eventually "sees" PRRSV in the context necessary to develop protective immunity and eliminate the virus from the body. This context most likely involves proper innate immune activation to adequately direct development of protective adaptive immunity. The portions of the virus that the immune system must target are eventually recognized and the immune cells necessary to mediate clearance are induced. Although it takes a long time for clearance, natural infection and subsequent convalescent immunity can be used to correlate specific immune parameters (T cell or antibody) to particular PRRSV epitopes that are involved in protection. While we can dissect this from immunity that develops post-exposure, the question remains, why does it take so long to get a protective immune response that can clear PRRSV infection?

The following review is focused on type 2 PRRSV and is structured based on the different arms of the immune response (antibody, cell-mediated, and innate) with the intent of outlining factors associated with protection or a lack thereof. It is well accepted that neutralizing antibody is a key component of sterilizing immunity (Osorio et al., 2002). For vaccine-based immunity, the rapid induction of neutralizing antibody is the ultimate goal, as it would provide protection against infection. Induction of antibody secretion from B cells requires T cell help, and T cells are required for killing virally infected cells; however, we know very little mechanistically about cell-mediated immunity against PRRSV. The final section relates to innate immunity, and is presented last because it aims to tie together findings described for adaptive immunity against PRRSV. As mentioned above, we know that pigs do eventually "see" PRRSV and can clear the infection. Thus, identifying the epitopes and immune cells necessary for clearance and pairing those antigens in the context of "proper" innate immune activation will be necessary to find solutions for enhancing PRRSV immunity, and developing strategies to significantly decrease the time it takes to develop protective immunity.

2. Antibody-mediated immune response to PRRSV

2.1. PRRSV-specific antibody response during infection

Early after PRRSV exposure a vigorous anti-PRRSV antibody response can be measured, with initial detection at 7–9 days post-infection (PI). However, there is no evidence that this early antibody response plays a role in the protection against PRRSV infection (Labarque et al., 2000; Yoon et al., 1994). The antibodies that appear during the early PI period do not neutralize PRRSV *in vitro* (Yoon et al., 1994) and when used in passive protection experiments, early PI antibodies (*i.e.* serum antibodies collected at 21 days PI) do not mediate passive protection against challenge with virulent antibody-matched PRRSV but rather seemingly enhance the virulence of the infection (Lopez et al., 2007). Serum antibodies with PRRSV-neutralizing activity appear only at later PI times, specifically at periods equal or higher than 28 days PI (Meier et al., 2000, 2003; Yoon et al., 1994).

The kinetics of antibody development, especially antibodies directed to the major structural proteins N, M and GP5 of PRRSV, has been studied in experimentally challenged pigs (Loemba et al., 1996; Nelson et al., 1994). PRRSV-specific IgM is detected at 7 days PI, with titers peaking between 14 and 21 days PI and decreasing to undetectable levels around 40 days PI. Anti-PRRSV IgG peaks at day 21 to 28 days PI, and levels remain elevated through the persistent phase of the infection. The earliest antibodies detected are directed against the 15 kDa N protein, followed by the 19 kDa M protein then the 26 kDa GP5 envelope glycoprotein (Loemba et al., 1996). Interestingly, NSP2 contains a cluster of non-neutralizing epitopes, suggesting an immunodominant role for this major nonstructural

(NS) protein (de Lima et al., 2006; Oleksiewicz et al., 2001). The most commonly used PRRSV serologic test (IDEXX Labs, Portland Maine, US) detects antibodies mainly against the N protein. These antibodies appear around the first week PI and persist for several months, but do not correlate with protection (Lopez et al., 2007; Lopez and Osorio, 2004). Although the N protein is a commonly used diagnostic antigen, it has been shown that several of the NS proteins induce antibodies that, based on kinetics and persistence, rival with N as a diagnostic antigen and maybe a better target (Brown et al., 2009; Johnson et al., 2007; Mulupuri et al., 2008).

2.2. Antibody enhancement of infectivity: . . .its importance?

PRRSV is primarily a macrophage-tropic virus, and given what is known for other macrophage-tropic viruses, it was initially plausible to propose a role for antibody-dependent enhancement (ADE) of infectivity for PRRSV. The occurrence of ADE *in vitro* and *in vivo* has been reported (Gu et al., 2015; Yoon et al., 1996, 1997). These reports contributed to the establishment of the notion that anti-PRRSV antibodies, particularly the early antibodies that do not neutralize virus and precede the neutralizing response both in time of appearance and robustness, could enhance disease caused by PRRSV and be deleterious for the host. While it appears possible to demonstrate the occurrence of ADE on cultures of swine macrophages with PRRSV *in vitro*, the real role of ADE *in vivo*, if any exists, remains unclear and most likely holds little significance for the overall pathogenesis of PRRS. The actual occurrence of ADE in PRRSV-infected macrophages is, at best, controversial, with different laboratories reporting different success at detecting ADE (Delputte et al., 2004; Yoon et al., 1996). Such contrast between experimental results of different labs has been ascribed to "virus strain or swine macrophage differences" (Delputte et al., 2004), a contention which *per se* limits significantly the putative overall pathogenic importance of ADE. Regarding *in vivo* proof for the occurrence of ADE, only two experiments can be cited. In one case it was reported that passive transfer of sub-neutralizing titers of PRRSV followed by PRRSV challenge resulted in enhanced viremia 1–2 logs over the groups receiving only virus, without inducing any other clinical exacerbation (Yoon et al., 1996). Another report described that passive transfer of salt-concentrated, non-neutralizing IgGs (IgGs collected at 21 days PI) followed by PRRSV challenge led to increased rectal temperatures and caused increased interstitial pneumonia (Lopez et al., 2007). However, the authors could not rule out the possibility that the increased pathology observed was caused by the presence of pro-inflammatory cytokines co-salted out with the IgG fractions collected from the 21 days PI donors sera rather than by the non-neutralizing IgGs. It is known that significant amounts of pro-inflammatory mediators are likely contained in the serum of the PRRSV-infected animals during the first weeks post infection (Van Reeth et al., 1999; van Reeth and Nauwynck, 2000). In summary it would appear that the degree of pathogenicity that ADE contributes after PRRSV infection could not be distinguished from the overall pathology caused by the introduction of serum cytokines from PRRSV infected pigs, which would have co-precipitated with the IgG. Overall, the contribution of ADE to PRRS pathogenesis is unlikely and has been critically discussed at in a recent review (Murtaugh and Genzow, 2011).

2.3. Role of neutralizing antibodies in protection

The early observation that virus-specific antibodies in the sera of PRRSV – convalescent pigs displayed the ability to neutralize infectious PRRSV in the absence of complement attracted the interest of many researchers (Nelson et al., 1994; Yoon et al., 1994). However, at the time initial systematic studies into PRRSV pathogenesis were being carried out in the early 1990s, data showing the simultaneous presence of PRRSV and PRRSV-specific antibodies in serum of infected pigs was interpreted as an indication that the neutralizing antibodies (NAb) did not play a role in protection (Albina, 1997; Collins, 1998; Loemba et al., 1996; Molitor, 1993; Molitor et al., 1997; Snijder and Meulenberg, 2001). At the same time, the idea that PRRSV antibodies produced early following infection, which were present in the absence of neutralizing antibodies, could exacerbate PRRSV replication in macrophages *in vitro* and *in vivo* (Yoon et al., 1996, 1997) led to the general conclusion that anti-PRRSV antibodies played a non-protective but deleterious role in the disease (reviewed by Snijder and Meulenberg, 2001). Due to the slow and irregular appearance of PRRSV neutralizing antibodies following PRRSV infection, not much credit was given to the role of neutralizing antibodies in PRRSV protection and/or clearance. Overall, literature related to the role of NAb against PRRSV infection has been, if anything, confusing. For example, reports describing the persistence of infectious PRRSV in lymphoid tissues (*e.g.* tonsils) for weeks PI in animals with high titers of circulating homologous PRRSV-NAb was interpreted as consistent with the idea that PRRSV NAb are incapable of clearing the virus (Murtaugh et al., 2002; Wills et al., 1997). However, other data indicated that NAb prevented the appearance of viremia (Osorio et al., 2002). Likewise, immunization with PRRSV proteins GP5 and M (Bastos et al., 2004; Pirzadeh and Dea, 1997, 1998) confers some degree of protection against infection, and this protection correlates to the appearance of serum NAb.

Serum transfer experiments conducted by Osorio et al. have provided unequivocal evidence that PRRSV NAb alone can fully prevent transplacental PRRSV infection of piglets and prevent PRRSV infection in the pregnant females (Osorio et al., 2002). The transfer of NAb provided sterilizing immunity to both the dam and piglets *in utero* against subsequent challenge since PRRSV could not be detected in lymphoid organs by virus isolation, RT-PCR, or swine bio-assay in either the dams or the offspring. Along the same line, we later observed that passive transfer of NAb to piglets directly at the time of or prior to challenge protected against PRRSV infection, though protection was dependent on the amount of antibody transferred (Lopez et al., 2007). Such passive transfer experiments in piglets also indicated significant differences from the results obtained in gestating sows. Passive transfer of NAb to piglets under concentrations that would attain an endpoint of around 1:8 did not provide sterilizing immunity to all the piglets. We observed transmission of PRRSV to naïve penmates from piglets that had passively received NAb and were challenged, although viremia was not detected. Likewise, additional experiments indicated that higher concentrations of antibodies transferred (*i.e.* to attain an end-point titer of 1:32) were capable of providing sterilizing immunity in piglets, as had been reported with passive transfer of NAb into gestating sows (required end-point titer of 1:8). These paradoxical results are likely the consequence of differences in susceptibility and overall capacity for PRRSV replication in adult sows *versus* piglets, with piglet target cells (macrophages) being much more permissive to PRRSV replication and consequently the ensuing viral loads in tissues is higher in young pigs than in sows. This data suggest that a vaccine may need to elicit higher titers of NAb in younger pigs to be effective in that target population. However, these points have not been clearly demonstrated and warrant further investigation.

A major confounding factor impeding an accurate evaluation of the role of NAb in PRRSV protection is the fact that many authors attempted interpreting the value of NAb using post-exposure correlations between the development of NAb and resolution of infection as opposed to prophylactic capacity of NAb to prevent PRRSV infection. When analyzing the NAb response upon natural wild-type PRRSV infection, it was demonstrated that NAb were not produced rapidly enough to limit the establishment of a chronic infection (Nelson et al., 1994). In addition, the kinetics of appearance of antibodies to the major candidate target for NAb (GP5) did not coincide

with the clearance of viremia (Mulupuri et al., 2008), and it was observed that PRRSV persisted in tissues even after the appearance of NAb (Allende et al., 2000). Collectively, these observations led to the conclusion that NAb do not contribute to control of PRRSV following infection. The ability of prophylactic anti-PRRSV NAb to provide protection against subsequent challenge/exposure is a completely separate experimental question than correlating the appearance of NAb during infection with viral clearance, as the former is a question of whether vaccine-induced NAb (separate from other arms of the immune system) can provide protection against disease and the later a question on the mechanism of resolution following natural infection. Delineating these two concepts is important for drawing conclusions on the role of different immune parameters in providing protection against PRRSV. It is worth noting that the source of NAb for prophylactic passive transfer studies was derived from experimentally challenged pigs, indicating that during the course of infection, pigs can produce antibodies that can prevent infection.

Overall, experiments on the prophylactic administration of NAb established that a specific titer of NAb is required to prevent subsequent infection. In that respect, an immunization regimen that gives rise to protective levels of NAb in blood, subsequently, the serum NAb titer can serve as a correlate of protection (Plotkin and Gilbert, 2012). Recent publications analyzing the response of a large number of animals to different PRRSV vaccination regimens (Li et al., 2014) indicate that NAb are a possible correlate of vaccine-induced protection for PRRSV. However, it should be kept in mind that: (1) Protective NAb titers in blood (*i.e.* 1:8–1:32) are hard to obtain with just one application of currently available PRRSV vaccines (Han et al., 2014; Roca et al., 2012; Trus et al., 2014; Zuckermann et al., 2007); (2) Most of NAb are specific for the vaccine strain (homologous), as significant titers of cross-neutralizing antibodies are rare (Vu et al., 2011; Zhou et al., 2012); and (3) The anamnestic induction of NAb observed after heterologous challenge in some previously sensitized (vaccinated or previously infected) animals indicates a good prognosis of broad heterologous protection, but the anamnestic NAb response only seems to occur when a certain period of time has elapsed between primary sensitization and subsequent secondary heterologous challenge. The necessary length of time appears to be 2–3 months, if the challenge is one month or less, the anamnestic NAb response is not detected (Osorio et al., 1998; Scortti et al., 2006; Zuckermann et al., 2007).

2.4. Where on the PRRSV virion are neutralizing epitopes located?

The overall serum NAb response against PRRSV has been repeatedly studied and characterized by different groups, although little is known about the location of the most prominent neutralizing epitopes on the PRRS virion. The initial investigations directed at locating neutralizing epitopes on the PRRS virion focused on the envelope glycoproteins and the M protein, which likely interact with host cell proteins and mediate viral penetration of the cell. Other structural proteins on the PRRSV envelope, such as small envelope (E) protein and the ORF5a gene product have already been ruled out as inducers of neutralizing antibodies (Robinson et al., 2013; Wu et al., 2001). Early papers reported that monoclonal antibodies specific for GP5 and GP4 (van Nieuwstadt et al., 1996) possessed PRRSV-neutralizing capacity, which led to significant investigation on these two glycoproteins as the main targets of serum NAbs, with an emphasis placed on GP5. Numerous initial studies proposed that a major neutralizing epitope(s) was located on GP5 (Pirzadeh and Dea, 1997, 1998; Weiland et al., 1999; Yang et al., 2000). Besides the neutralizing epitope on GP5, at least another neutralizing epitope was recognized by a monoclonal antibody on the envelope's GP4 (van Nieuwstadt et al., 1996; Yang et al., 2000) and another on the envelope protein M (Yang et al., 2000). Recently, a single amino acid in the M protein was shown to be necessary for polyclonal swine antibody to mediate broadly neutralizing activity (Trible et al., 2015). Initial reverse vaccinology studies and additional reports proposed that the main neutralizing epitope on GP5 targeted by the NAb was located on the GP5 ectodomain (Ostrowski et al., 2002; Plagemann et al., 2002). The minimum antigenic area of this GP5 epitope comprises amino acids 37 to 44 as the core of the main neutralizing epitope (Ostrowski et al., 2002). This data established the notion that the main neutralizing epitope is located on the ectodomain of GP5. This notion would be consistent with other arteriviridae, as the neutralizing epitope of EAV and LDV is in the ectodomain of GL and VP-3P, respectively.

The GP5 neutralizing epitope was assumed to be a linear epitope; however, data now shows that the neutralizing epitope in GP5 is conformational rather than linear (Fan et al., 2015; Li and Murtaugh, 2012; Pirzadeh and Dea, 1998). The conformational nature of the GP5 neutralizing epitope was first described by Pirzadeh and Dea (1998) who showed that immunizing against PRRSV with a DNA vaccine encoding GP5 elicited the production of serum NAbs, while immunization with recombinant GP5 protein produced in bacteria from the same ORF5 DNA construct failed to induce the production of NAbs. More recently, two independent reports have concluded that the GP5 ectodomain does not contain a linear neutralizing epitope in PRRSV type 1 (Vanhee et al., 2011) or in PRRSV type 2 (Li and Murtaugh, 2012) strains. In summary, the original observation that DNA immunization with the single GP5 gene alone can evoke antibodies that efficiently neutralize PRRSV infectivity still holds true and has been confirmed by multiple subsequent independent reports that used GP5 as possible subunit candidate to make experimental vaccines (based on GP5 alone or in GP5/M expressed as a dimer) (Jiang et al., 2007; Kim et al., 2013; Pirzadeh and Dea, 1998; Vanhee et al., 2011; Xu et al., 2012; Zheng et al., 2007; Zhou et al., 2010). In all these cases GP5 alone or GP5/M expressed in multiple different vaccine platforms have elicited PRRSV-neutralizing antibodies. While no consistent evidence for sequential epitopes in the ectodomain of GP5 exists, we should assume that the neutralizing epitope(s) that exist(s) on the PRRSV GP5 is (are) conformational (Fan et al., 2015), and that the M protein, when forming the dimer with GP5, contributes to the conformational nature of the neutralization epitope on GP5.

More recently, two important pieces of information have had a significant impact on our view of the role of specific PRRSV glycoproteins as targets for antibody-mediated virus neutralization. First, one major discovery has been the unequivocal recognition that GP5 *per se* does not determine the host cell tropism of PRRSV, thus suggesting that GP5 does not interact with the main cellular receptor for PRRSV. Instead, the cluster of minor PRRSV glycoproteins GP2-GP3-GP4 interacts with the host cell to initiate infection (Tian et al., 2012). Second, the notion that GP2, GP3 and GP4 interact with each other to form a multi-protein complex that is present in a scarce number on the virus envelope and that at least GP2 and GP4 would be the proteins that interact with the main membrane and endosomal receptor for PRRSV penetration (CD163; see Fig. 1) (Das et al., 2010). Such information has stimulated significant interest in these minor glycoproteins and their role as antibody targets for neutralization of PRRSV. Peptide scanning technology led to the identification of peptides in GP2, GP3 and GP4 that define actual linear neutralizing epitopes in these three minor glycoproteins but, interestingly, none in GP5 (Vanhee et al., 2011). If the glycoproteins that interact with the main cellular receptor for PRRSV contain conserved residues that contact or interact with the host cell for entry, these epitopes would be candidates for vaccine antigen for induction of broadly neutralizing antibodies, which may be of significance in cross-protection. Further work in this area is warranted to determine if this is actually the case.

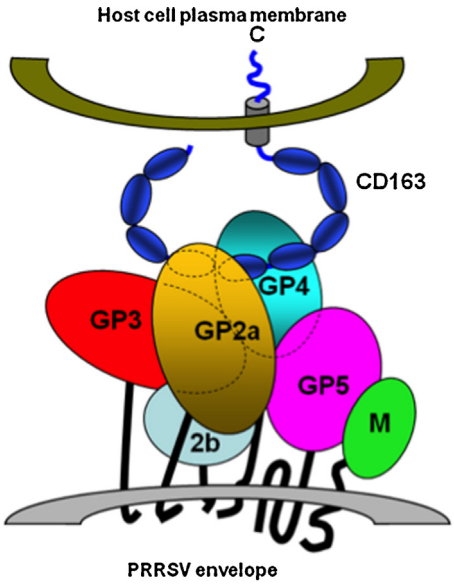

Fig. 1. A preliminary model of PRRSV envelope protein complex and its interaction with CD163 on the host cell plasma membrane (Das et al., 2010).Copyright © American Society for Microbiology, J. Virol. 84, 2010, 1731–1740, doi:10.1128/JVI.01774-09.

2.5. Glycan shielding for immune evasion

As persistence of PRRSV infection in individual animals is one of the biggest obstacles to control the disease in the field, all the possible mechanisms that could explain this persistence have been given great pathogenic significance. Since the initial reports that the N-glycan moieties in GP5 of type-II PRRSV are important for the virus to escape the effect of NAbs (Ansari et al., 2006; Faaberg et al., 2006), glycan shielding has been postulated to be a primary mechanism to explain evasion from the neutralizing antibody response, ensuring *in vivo* persistence of virus, such as had been previously reported for simian and human immunodeficiency virus, influenza A virus, and Hepatitis C virus and Ebola virus (Francica et al., 2010; Liu et al., 2007; Reitter et al., 1998; Wang et al., 2009a; Wei et al., 2003). Furthermore, experiments with GP3 would confirm the previous findings observed in GP5, suggesting that also the N-glycan in GP3 of type-II PRRSV would also be important in protecting the virus from antibody neutralization (Vu et al., 2011). Recent reports involving deglycosylation experiments of PRRSV type II strains have provided support to the conclusion that glycosylation of GP5 downstream of the putative neutralizing epitope renders PRRSV resistant to neutralization (Wei et al., 2013a,b) though the same effect on GP3 could not be confirmed (Wei et al., 2013b). Regardless of whether glycosylation of GP3 contributes to the overall glycan shielding of neutralizing epitopes, the overall body of hypo-glycosylation experiments with GP3 by Vu et al. firmly confirmed the important role of GP3 in the induction of neutralizing antibodies, a concept that had been already suggested by previous investigators (Cancel-Tirado et al., 2004; Jiang et al., 2008; Kim and Yoon, 2008; Plana Duran et al., 1997). More recent work by Vanhee et al. (2011) clearly demonstrated that GP3 contains important linear neutralizing epitopes.

It is also important to mention here that the use of hypo-glycosylated versions of PRRSV generated by reverse genetics with the intent of enhancing of the ability of live virus vaccines to elicit neutralizing antibodies (Ansari et al., 2006) is severely limited by the short life span of the hypo-glycoyslated phenotype *in vivo*, as virus replication *in vivo* quickly leads to reversion to wildtype of the glycan shielded version of the GPs (Osorio and Pattnaik, University of Nebraska, unpublished data). However, the possibility of significant induction of serum NAbs by immunization with recombinant or inactivated hypo-glycosylated forms of GP5 or GP3 administered as adjuvanted, non-replicating immunogens deserves consideration for additional study, as has been recently published (Linhares et al., 2014).

2.6. Induction of cross-neutralizing antibody and viral protein targets

Passive transfer experiments demonstrated that NAb are a *bona fide* parameter of PRRSV protective immunity and an important mediator of protection against PRRSV. Many different PRRSV vaccine candidates are now being evaluated by protection against homologous/heterologous challenge plus their ability to induce cross-neutralizing antibody. While passive protection through prophylactic administration of NAb has demonstrated their value for prevention of infection, protection is only consistently provided under homologous conditions (*i.e.* conditions of close identity between the NAb specificity and the challenge strain). As already discussed, initial studies aimed at identifying the location of neutralizing epitopes in PRRSV positioned GP5 as the primary immunogen target for cross-protection. GP5 is known to form a heterodimer with non-glycosylated M protein in the virion envelope, which enhances immunogenicity and production of NAb. This explains the worldwide use, alone or in combination, of GP5 and M as immunogens for different platforms and constructs tested as subunit vaccine candidates against PRRSV. These attempts have included, amongst others, viral vectors, DNA vaccines as well as genetic adjuvants and other immunomodulators. Regardless of the platform and formulation, it has become obvious that the use of GP5 and M subunits alone, although immunogenic and somewhat protective, are not sufficient to provide complete protection. Vaccines containing only GP5/M subunits provide limited protection against homologous challenge, and provide less protection than that attained by modified live virus vaccine. Thus GP5 alone, or even formulated with M protein, is unlikely to serve as a vaccine immunogen for homologous or heterologous protection.

A significant advancement in delineating the role immunity against other PRRSV glycoproteins plays in protection derives from studies on the interactions amongst the four PRRSV envelope glycoproteins. Das et al. (2010) looked at either the interaction of PRRSV surface glycoproteins with each other or with the main cellular receptor CD163. They were able to show strong GP4-GP5 interaction and, to a lesser extent, interaction between GP5 with each of the other minor glycoproteins. The GP2 and GP4 proteins, on their part, were found to interact with all the other GPs, resulting in the formation of a multi-protein complex (see Fig. 1). Remarkably, these results showed that the GP2 and GP4 proteins specifically and exclusively, interact with the CD163 molecule (Das et al., 2010). Recent reports by Wei et al. have confirmed the CD163 interaction with GP4 but were unable to confirm GP2 participation in the interaction (Wei et al., 2013b). Overall, Das et al. concluded that GP4 is critical for mediating inter-glycoprotein interactions and along with GP2, serves as proteins that are responsible for mediating interactions with CD163 for virus entry into host cells. Other groups have reported similar findings working with both European (Martinez-Lobo et al., 2011) and US strains (Hesse et al., 1996). A study out of China using chimeric viruses obtained by reverse genetics, seem to confirm Das et al.'s original contention suggesting that the PRRSV cluster of GP2-3-4 is responsible for interaction with CD163. In Fig. 1, CD163 is shown as a structure with extracellular region having repeating units (9 SRCR domains of CD163) projecting from the plasma membrane of a host cell. Although multi-protein complexes like the one proposed by Das et al. can be detected in experiments with transfected cells, the molar ratio of the proteins in such complexes is still unknown.

Although GP5 is the major glycoprotein on the envelope and may be distributed uniformly throughout the viral envelope, one can hypothesize that only few of the complexes (possibly two to three per particle) as shown in Fig. 1 may be responsible for binding to CD163. Cryo-electron microscopic studies published by Dokland et al. (Spilman et al., 2009) suggest that only two to three large protein complexes can be seen on the viral envelope and thus provide additional support for the hypothesis and for the model.

The view and the possibility that GP2 and GP4 may indeed be responsible for stimulating antibodies that would block their interaction with the main host cell receptor resurfaces interest in these two proteins, which for a while were rather neglected based on the primary interest in GP5, which was generally believed to be the major PRRSV immunogen for induction of NAb. Although the original finding that GP5 induces neutralizing antibodies still holds, further evidence collected at laboratories at the University of Nebraska (Ansari et al., 2006) would indicate that, rather than evoking broadly reactive (pan-neutralizing) antibodies (Robinson et al., 2015), it would appear that GP5 evokes primarily strain-specific antibodies. It should be mentioned here that a recent publication would assign to M protein, rather than GP5 itself, the ability to induce cross-neutralizing antibodies (Trible et al., 2015). Evidence for the strain specific character induced by GP5 alone comes from experiments using reverse genetics to generate a strain of PRRSV in which GP5 is deglycosylation (clone FL12). The glycan shielding ability of PRRSV showed that GP5, in its deglycosylated form, results in a significant of PRRSV-NAb, whereas the glycosylated form of GP5 does not stimulate significant production of NAb (Ansari et al., 2006). However, the titers were only increased to homologous virus and were not significantly increased against other heterologous strains of PRRSV (Vu et al., 2011). Thus, it is plausible to conclude that antibodies targeting the receptor-binding region of GP2 and GP4 would prevent entry of the virus into the host cell and serve to neutralize infection. Likewise, mutations in the regions of GP2 and/or GP4 that directly interact with the cellular receptor would render the virus incapable of infection and such mutations would not be selected for during an infection. Consistent with this view, a hypothesis could be made that antibodies directed against glycoprotein epitopes conserved across different PRRSV strains (those necessary for viral attachment and entry) would be able to neutralize multiple PRRSV strains, or in other words would be cross-neutralizing.

One major piece of evidence that supports the conclusion that certain PRRSV epitopes are capable of inducing the production cross-neutralizing antibodies comes from the observation that pigs vaccinated with MLV vaccine are protected against secondary heterologous challenge (Osorio et al., 1998). Specifically, in the fraction of pigs that initially respond to the MLV vaccine with the production of serum NAb to the vaccine antigen there is a robust and strain-specific increase in serum NAb titers following heterologous challenge that correlates with protection against the secondary challenge (Osorio et al., 1998; Scortti et al., 2006). This phenomenon can be interpreted as evidence that protective cross-neutralization can occur with PRRSV, not that it always occurs (Martinez-Lobo et al., 2011; Osorio et al., 1998). This data suggests that the MLV primes the immune system, but the secondary challenge is required for NAb to be detected systemically. The mechanism by which the MLV primes for cross-protection is unclear – it could be memory B cells, memory T cells, or a combination of both. However, the induction of the anamnestic response suggests that the secondary challenge virus replicates for the response, though it may not result in a detectable viremia. Further research in this area is warranted to understand what epitopes the NAb are directed against, as well as the immune memory compartment primed by the MLV. Understanding this mechanism my lead to understanding how to prime against PRRSV.

Collectively, these data suggest that NAb, if present at the time of challenge in a significant enough titer, can provide sterilizing immunity against challenge. Anti-PRRSV antibodies derived through active immunity can provide protection when delivered prophylactically through passive transfer. The specific epitopes that the NAb recognize have not been completely defined, but recent work suggests an important role for antibodies directed against minor glycoproteins and the complexes they generate on the viral surface. Prior exposure, either natural or with use of MLV vaccine, primes the pig immune system for a robust secondary antibody response that is indicative of protection. What is unclear is why it takes so long for peripheral NAb to appear following challenge/MLV vaccination and what the role of NAb is in clearance following infection. Shielding of epitopes may be a mechanism by which PRRSV evades the host immune system; however, the pig eventually develops detectable serum NAb or is primed for an anamnestic response that can provide protection against heterologous infection.

3. Cell-mediated immunity against PRRSV

3.1. PRRSV T cell immunity

T cells play a critical role in many aspects of anti-PRRSV immunity due to their central role in development and regulation of antigen-specific immune responses, including education and activation of B cells, determination of the cytokine milieu in the environment of antigen presentation, cytotoxic effector functions to destroy infected cells, and regulation of immune responses to control inflammation and monitor for autoimmune reactions. The expansive toolkit of functional and biochemical assays that are used to characterize T cell responses in mice are largely unavailable in swine due to a paucity of reagents, inability to clone antigen-specific T cells, and inability to expand and immortalize antigen-specific T cells. However, that which is known is reviewed here.

3.2. General features of T cell response

T cell subpopulations in swine are characterized primarily by cluster of differentiation (CD) marker phenotyping for CD3, CD4, and CD8, and by secretion of interferon-gamma (IFN-γ). Notably, swine have a significant population of mature (extrathymic) CD4/CD8 double-positive T cells that represent memory T cells (Zuckermann and Husmann, 1996) and are thus likely to play a role in memory responses. These cells are capable of producing IFN-γ and localize to sites of inflammation, suggesting that they play a role in mediating protective immunity (Zuckermann, 1999). Working within these limitations, it is known that a substantial, transient decrease in CD4+ T cells occurs in blood at about 3–7 days after infection, with a return to normal levels by 7–14 days after infection (Nielsen and Botner, 1997; Shimizu et al., 1996). Increased CD8+ T cells are frequently observed, usually after 4–5 weeks of infection (Albina et al., 1998; Dwivedi et al., 2012; Kawashima et al., 1999; Shimizu et al., 1996). CD8+ T cells also are increased in infected lungs, whereas CD4+ cells are fewer or rare (Samsom et al., 2000; Tingstedt and Nielsen, 2004). Increased CD8+ and CD4/CD8 double-positive T cells also are observed in lymphoid tissues (Gomez-Laguna et al., 2009). The T cell response to PRRSV also includes γδ T cells, but little is known about their significance (Olin et al., 2005).

In piglets infected *in utero*, reduced numbers of circulating CD4+ T cells are observed at birth and one week of age, but by 14 days, their numbers have returned to normal, whereas CD8+ T cells are substantially increased (Feng et al., 2002; Nielsen et al., 2003). *In*

utero infection also is associated with mRNA expression of IL-6, IL-10 and IFN-γ (Feng et al., 2003). Antigen-specific lymphocyte proliferation is first detected at four weeks post-infection (PI), peaks at 7 weeks PI, and declines after 11 weeks PI. Experiments that utilized blocking antibodies to specific porcine leukocyte antigens demonstrated that the main effector cell type proliferating was CD4+ T-cells (Bautista and Molitor, 1997). The *in vivo* T cell response to PRRSV was shown by detection of a specific, dose-dependent delayed-type hypersensitivity (DTH) reaction in infected pigs after intradermal administration of UV-inactivated virus (Bautista and Molitor, 1997).

The evolution of PRRSV-specific T cell responses over time following infection has been primarily assessed by the IFN-γ ELISPOT assay which, depending on how the assay is performed, gives a measure of the number of NK cells, helper T cells and/or cytotoxic T cells producing IFN-γ. Peripheral PRRSV-specific T cells were observed at 2 weeks after infection, with extensive variation over time and among animals (Xiao et al., 2004a). No significant differences were observed in PRRSV-specific T cells in lymphoid tissues examined during viremic or post-viremic infection, and there was no correlation between PRRSV-specific T cell frequencies and viral loads in lymphoid tissues (Xiao et al., 2004a). In nearly all cases, the frequency of specific IFN-γ secreting T cells was low regardless of age (Klinge et al., 2009).

Interferon-γ secretion also has been used as an indicator of T_{H1} polarization of helper T cell responses in PRRSV infection to identify candidate treatments that potentiate antiviral immunity (Meier et al., 2004; Xiao et al., 2004b). However, the significance of IFN-γ secretion as an indicator of helper T cell polarization is uncertain in swine since the phenotype of secreting cells usually is not known, and multiple lymphocyte populations are capable of IFN-γ secretion. While there have been reports on the TH1-TH2-like T cell phenotypes in pigs (Dawson et al., 2005; Ebner et al., 2014) a thorough characterization of the T_{H1}-T_{H2} paradigm in swine and its relevance to PRRSV immunity have not been established (Murtaugh et al., 2009).

3.3. Cytotoxic T Lymphocyte (CTL) activity

At 35 days after vaccination, a low virus-specific IFN-γ secreting CD8+ T cell response has been observed that is consistent with a CTL response (Ferrari et al., 2013). Immune activation was influenced by isolate but not route of administration (Ferrari et al., 2013). However, the cytotoxic effector function of CD8-expressing T cells has not been strongly linked to control of primary PRRSV infection. Temporary depletion of CD8+ T cells at the time of infection did not lead to an increase in infection, suggesting that cytotoxic T cells do not have a functional role in control of acute infection (Lohse et al., 2004). Similarly, CTL activity was not detected against PRRSV-infected macrophages until after viremia was cleared (Costers et al., 2009). Memory CTL proliferation was observed at 14 days after infection but CTL activity was not detected until 49 days after infection (Costers et al., 2009). These data suggest that CTL may be involved in the clearance of PRRSV-infected cells in tissues. However, the effect of PRRSV infection on CD8+ T cell frequencies in lymphoid tissues has not been established. An extensive survey of PRRSV-specific T cell frequencies in lung and lymphoid tissues during acute and prolonged infection showed no significant change (Xiao et al., 2004a), whereas, in another study, prolonged presence of PRRSV in lymphoid tissues was associated with elevated levels of highly positive CD8+ T cells (Lamontagne et al., 2003).

3.4. Regulatory T cell activity

PRRSV infection is reported to increase the frequency of putative regulatory T cells (Tregs) that produce TGF-β (Silva-Campa et al., 2012). Type 1 PRRSV strains can induce IL-10 production in infected dendritic cells, but co-culture with lymphocytes does not induce Treg cells or TGF-β production (Silva-Campa et al., 2010). By contrast, Type 2 PRRSV infection of dendritic cells can increase Tregs and induce TGF-β production (Silva-Campa et al., 2009). Recent work indicates that monocyte-derived dendritic cells infected with either Type I or Type 2 PRRSV do not increase the frequency or proliferation of CD4+ Foxp3+ regulatory T cells in an *in vitro* co-culture system (Rodriguez-Gomez et al., 2015). PRRSV induction of Tregs with suppressive activity occurs both *in vitro* and *in vivo* (Wongyanin et al., 2010). The induced Tregs suppress mitogenic proliferation of peripheral blood mononuclear cells. Early induction of Tregs by PRRSV infection of dendritic cells would provide a mechanism for facilitating establishment of viral infection. The inconsistency in observations might be due to variation in subpopulations of T lymphocytes that express the transcription factor FoxP3, or to immunosuppressive cytokine expression differences (Cecere et al., 2012).

An alternative to IL-10 secretion or Treg induction as an explanation for the difficulty in resolving PRRSV infection is lymphoid apoptosis. Using histology and viral antigen expression, Gomez-Laguna et al. showed that mainly lymphocytes in B cell and T cell areas of lymphoid tissues were apoptotic in pigs infected with a type 2 PRRSV (Gómez-Laguna et al., 2013). This loss of cells may result in the low number of PRRSV-specific T cells detected during infection. However, a direct link between lymphoid apoptosis and lack of PRRSV-specific T cells has not been clearly established. In addition, the marked lymphadenopathy associated with PRRSV infection has not been explained. Clearly, additional research is needed to resolve these questions.

3.5. Memory response

The role of memory T cells in anti-PRRSV immunity has not been studied extensively. It has been reported that a recall response, mainly dependent on CD4+ cells and SLA-II, is present from 4 weeks after infection and remains for more than 3 months (Lopez Fuertes et al., 1999). The majority of studies evaluating T cell responses to PRRSV have investigated the response during the acute infection rather than following clearance of viremia. During infection, it is assumed that primarily T effector cells are detected as opposed to memory cells. The distinction between effector *versus* memory T cells is difficult in swine due to the lack of phenotypic markers and functional characterization of individual T cell populations. Clearly, the evaluation of vaccine immunogenicity and efficacy warrants the measurement of PRRSV-specific memory T cells; however, memory T cells have not been clearly characterized in pigs.

3.6. Viral epitope targets of T cell immunity

PRRSV antigens that are targets of cell-mediated immunity have been investigated using various immune assays commonly used to evaluate antigen-specific responses. Incubation of PBMC from PRRSV-infected, but not non-infected pigs, in the presence of envelope glycoproteins (GP) 2 and 5, and matrix (M) protein induces proliferative responses (Bautista et al., 1999). Further analysis of GP5 identified immunodominant epitopes, including in conserved amino acid regions at residues 117–131 and 149–163, using an IFN-γ secreting-cell ELISPOT assay (Burgara-Estrella et al., 2013; Vashisht et al., 2008). Conserved T cell epitopes were identified in N and GP5 of Type 1 PRRSV, with the GP5 epitope corresponding to epitopes reported in Type 2 PRRSV (Diaz et al., 2009). IFN-γ ELISPOT also was used to identify four conserved T cell peptides in the matrix (membrane) protein of a Chinese highly pathogenic PRRSV strain (Wang et al., 2011). In the nonstructural protein repertoire, conserved epitopes were identified in nsp2, nsp9 and nsp10

in Type 2 PRRSV that stimulated antigen-specific proliferation and IFN-γ secretion (Burgara-Estrella et al., 2013; Parida et al., 2012). Peptides stimulating IL-10 secretion were also observed, some of which inhibited IFN-γ responses of PBMC stimulated with mitogen. It was noted that low IFN-γ responding pigs tended to be homozygous for SLA haplotypes, but the significance of this observation was not pursued (Burgara-Estrella et al., 2013).

An outbred pig model has been developed to evaluate antigen-specific T cell responses to PRRSV. Swine were immunized with plasmid constructs expressing porcine granulocyte-macrophage colony-stimulating factor (GM-CSF) and PRRSV M or N to generate a source of potentially antigen-specific T cells (Wang et al., 2009b). Dendritic cells derived from the same pigs were loaded with the recombinant proteins and used as MHC-matched autologous antigen-presenting cells (APC) to stimulate PBMC from the same pig. T cell proliferation and IFN-γ synthesis were induced in immunized pigs only in response to M and N, with M being more stimulatory. By contrast, serum antibodies were produced only to N (Jeong et al., 2010). This approach, using T cells and MHC-matched autologous APCs, to identify T cell epitopes is likely to be useful in future studies.

3.7. Major gaps in PRRSV T cell immunity

The inability of pigs to achieve rapid sterilizing immunity to PRRSV may be due in large part to inadequate T cell responses, since T cells play a central role in direct effector responses to infection, overall immune activation and modulation of appropriate T cell and B cell responses, in regulation of response intensity and duration, and maintenance of immune memory. The essential role of T cells in resistance to viral infection and disease are known primarily from studies of defined cell populations that are specifically expanded and maintained in cell culture, and from genetic deletion of defined cell populations in animals. Examination of cellular and animal responses to infection in these experimental systems has resulted in detailed dissections of cellular properties and molecular mechanisms that explain key features of rapidly sterilizing immunity in a variety of viral infections of animals. These features do not operate the same way in pigs infected with PRRSV; however, the ability to perform a detailed investigation to identify the molecular mechanisms gone awry is not possible.

Thus, it is essential to develop a T cell biology toolkit for swine to investigate antigen-specific expansion and growth of normal porcine helper and cytotoxic T cells, as well as immortalization of antigen-specific helper and cytotoxic T cells. At the same time, it is essential to develop MHC class I and class II antigen-presentation culture systems to functionally assess helper and cytotoxic T cell functions *in vitro* under conditions representative of PRRS disease in pregnant sows and growing pigs. Further enlargement of the immune reagents toolkit is essential for phenotypic characterization of T cell [subpopulations that is the basis for describing T cell subsets]. New and better methods must be found to characterize the responses of activated cells in terms of biologically active secreted molecules, and alterations in expression of surface molecules that can change their ability to respond to the local environment. Genetic tools are needed to introduce and delete targeted gene functions to test potential causal relationships, in place of association studies that are often times difficult to reproduce.

Key investments in basic porcine cellular immunology are essential for unraveling the mechanisms of protection against PRRS. The benefit of these investments accrues not only to better control and prevent PRRS, but also is directly applicable to other viral diseases of swine, which are numerous and ever-expanding.

4. The innate immune response to PRRSV

The most effective innate anti-viral immune response is the production of type I interferons (IFNs) IFN-α and IFN-β by virus-infected cells, which induces an antiviral state through the expression of numerous interferon-stimulated genes (Baum and Garcia-Sastre, 2010). Although most cells types are able to produce type I IFN in response to a viral infection, this cytokine is most efficiently produced by leukocytes such as macrophages and dendritic cells. A prominent subset of the latter is the plasmacytoid dendritic cell (pDC), which produces more IFN-α than macrophages on a per cell basis (Fitzgerald-Bocarsly, 2002; Fitzgerald-Bocarsly et al., 2008). Notably, pDCs can recognize endocytosed viral molecules without being infected *via* Toll-like receptor 7 (TLR7), and thus due to their prodigious ability to produce IFN-α, pDCs can generate a systemic wave of type I IFN production (Takeuchi and Akira, 2009). Since pDCs are mainly found in secondary lymphoid tissues and in the blood, it has been proposed that pDCs have a vital role during systemic viral infections where the virus has tropisms for secondary lymphoid organs (Swiecki and Colonna, 2010). Macrophages, and primarily alveolar macrophages (AMϕ), are the main host cell for PRRSV replication. Within 2–4 days of respiratory inoculation with PRRSV, the infection becomes systemic resulting in viremia that persists for 21 days or longer. After this period, PRRSV can be detected in most secondary lymphoid tissues for months. Thus, based on the distribution of PRRSV in the body and the known triggering signals for a type I IFN response, there are two types of cells that could potentially mediate a type I IFN in response to PRRSV infection – macrophages and pDCs.

The seminal study by Albina et al. (1998) indicated that the IFN-α response of swine to genotype I PRRSV was meager and could even be actively suppressed (Albina et al., 1998). Since then, a significant amount of research has focused on the possible mechanisms, at the molecular level, by which PRRSV might inhibit the IFN-α response. Since this topic has been recently reviewed extensively by others (Sun et al., 2012), this review we will mainly focus on what is known about the IFN-α response of swine to PRRSV either *in vivo* or by porcine cells *in vitro*. In this regard, it seems clear that unlike other swine respiratory viruses that are capable of stimulating significant production IFN-α, such as porcine respiratory coronavirus (PRCV) and swine influenza virus (SIV), PRRSV elicits a moderate to negligible IFN-α response in the respiratory tract. Production of IFN-α in the lungs of pigs acutely infected with PRRSV was either almost undetectable, or >10-fold lower than that induced by PRCV (Buddaert et al., 1998; Jung et al., 2009; Van Reeth et al., 1999).

Evidence indicative of the engagement of virus-sensing pattern recognition receptors (PRRs) in lung cells of PRRSV-infected pigs was reported as the increase in the transcription of several viral sensing TLRs (Liu et al., 2009; Xiao et al., 2010a,b). However, these observations were not confirmed in *ex vivo* experiments using precision-cut lung slices exposed to PRRSV (Dobrescu et al., 2014). While RIG-I and MDA5 transcription in AMϕ infected with PRRSV was found to be increased, the level of expression was not as strong as that induced by SIV (Dobrescu et al., 2014). While the transcription of type I IFN genes in AMϕ and monocyte-derived dendritic cells has been described (Genini et al., 2008; Loving et al., 2007), protein analysis was not always performed. Additional studies using quantitative real-time PCR and ELISA analysis has shown that even though transcription of type I IFN genes occurs, actual secretion of the corresponding cytokine fails to occur (Dobrescu et al., 2014; Miller et al., 2009; Zhang et al., 2012). These observations have led to the suggestion that the production of type I IFN by AMϕ might be regulated at the post-transcriptional level (Wang and Christopher-Hennings, 2012). The poor IFN-α response detected in the lungs of PRRSV-infected pigs is consistent with the reported poor IFN-α response of porcine AMϕ to PRRSV infection

in vitro (Albina et al., 1998; Dobrescu et al., 2014; Lee et al., 2004). The limited type I IFN response could be due to the ability of virus to actively block the response or to the rapid destruction of infected AMϕ, which is known to occur in the lungs of PRRSV-infected pigs (Weesendorp et al., 2013). However, the underlying mechanism is unclear.

The IFN-α response of porcine pDC to PRRSV, as compared to other viruses that are able to stimulate a strong response by this cell type, has been characterized from moderate (Baumann et al., 2013) to negligible (Calzada-Nova et al., 2010). Although the modest response of porcine pDC to PRRSV reportedly occurs through the TLR7 pathway (Baumann et al., 2013), PRRSV has been shown to have the capacity to strongly inhibit the IFN-α response of pDCs to other porcine viruses, such as transmissible gastroenteritis virus (TGEV), and moderately inhibit the same cytokine response to synthetic immune-stimulatory DNAs (Baumann et al., 2013; Calzada-Nova et al., 2011). In contrast to TGEV, which can stimulate the activation and differentiation of porcine pDC through the up-regulation of IRF-7 expression and acquisition of dendritic cell-type morphology concomitant with an increase in CD80/86 expression, exposure of pDCS to PRRSV did not induce any of these activation pathways (Calzada-Nova et al., 2011). The importance of the lack of pDC activation by PRRSV resides in the fact that pDCs appear to play a role in promoting cytotoxic T cell responses that require help or co-stimulation (Swiecki and Colonna, 2010). Since pDCs are not permissive to PRRSV and live PRRSV is not required to suppress their function, the inhibitory effect of PRRSV on the pDC's ability to produce IFN-α is unlikely due to the killing of pDCs by PRRSV. Rather, it appears that PRRSV alters pDC function through a negative signal delivered at the cell surface (Calzada-Nova et al., 2011). A negative signal could be mediated *via* engagement of cell surface receptors on pDCs that are known to negatively regulate type I IFN secretion (Swiecki and Colonna, 2010).

4.1. Influence of innate immunity on the adaptive immune response

The production of IFN-α by pDCs has an autocrine effect that promotes functional and phenotypic activation events necessary for the optimal expression of co-stimulatory molecules and subsequent ability of pDCs to induce naïve T cell differentiation into IFN-γ SCs (Cella et al., 2000; Fitzgerald-Bocarsly, 2002; Honda et al., 2003; Kadowaki et al., 2000; Montoya et al., 2002). At this point, the cells express co-stimulatory molecules that promote the differentiation of naïve T cells into IFN-γ secreting cells (Cella et al., 2000; Fitzgerald-Bocarsly, 2002; Honda et al., 2003; Kadowaki et al., 2000) and cytotoxic T lymphocytes (Swiecki and Colonna, 2010). Thus, it has become increasingly evident that the link between innate and adaptive immunity in viral infections occurs through the interaction of dendritic cells with type I interferon (Montoya et al., 2002; Tough, 2004) and the dendritic cell controlled polarization of T-cell function (Kapsenberg, 2003).

Accordingly, the apparent lack of an adequate IFN-α response of swine upon exposure to PRRSV likely contributes significantly to the inadequate development of a specific cell-mediated immune response (Meier et al., 2003). Usually, virus-infected cells secrete type I IFN and the released cytokine interacts with a subset of naïve T cells to promote their conversion into virus-specific IFN-γ secreting cells (Biron, 2001; Cella et al., 2000; Cousens et al., 1999; Kadowaki et al., 2000; Levy et al., 2003). In contrast, as we have described above, the IFN-α response of swine upon exposure to PRRSV is meager at best. The lack of efficient stimulation of IFN-α production by host cells after pathogen exposure would be expected to have a significant impact on the nature of the host's adaptive immune response, since IFN-α up-regulates IFN-γ gene expression, and thus controls the dominant pathway that promotes the development of adaptive immunity, namely, T cell-mediated IFN-γ responses and peak antiviral immune defenses (Cousens et al., 1997; Levy et al., 2003). Under experimental conditions the presence of IFN-α at the time of PRRSV infection or vaccination has been found to significantly alter both the innate and the adaptive immune response (Brockmeier et al., 2012; Royaee et al., 2004). Given this prominent role for IFN-α in altering PRRSV-specific immune responses it seems likely that, under field conditions, the individually variable presence of spontaneously produced IFN-α in the blood and tissues of pigs may be responsible for the significant level of individual variation that has been observed in the kinetics and intensity of the cell-mediated immune response following vaccination with a modified live virus (Royaee et al., 2004). This is supported by the observation of a statistically significant ($p < 0.001$) correlation ($R^2 = 0.6$) between the frequency of IFN-α and IFN-γ-s in vaccinated swine (Royaee et al., 2004). Regardless, there is clear data indicating a significant difference in viral clearance and adaptive immune responses when IFN-α is present before or at the time of PRRSV infection (Brockmeier et al., 2009, 2012).

4.2. Approaches to improve the stimulation of protective immunity to PRRSV

Given the influence that the innate immune response can have on the subsequent development of the adaptive immune response, much effort has been directed at compensating for the apparent inadequate innate cytokine stimulation elicited following PRRSV infection. Novel adjuvants have been used during immunization to attempt to overcome the deficit in innate stimulation, including the administration of IL-12. In combination with live or killed PRRSV vaccine, it resulted in increased lymphoproliferative responses to the vaccine virus (Wee et al., 2001), as well as an enhanced IFN-γ response to a modified-live PRRSV vaccine (Foss et al., 2002). Similarly, an injection of IFN-α provided exogenously in the form of an expressible cDNA or a replication-defective adenovirus vector was found to exert an enhancing effect on the induction of antigen-specific IFN-γ response to PRRSV (Brockmeier et al., 2012; Meier et al., 2004). Remarkably, no significant alteration in the development of the humoral immune response was observed with either of these treatments. The interventions at the initiation of PRRSV immunization did not alter the usual rapid onset of anti-PRRSV antibody production and delayed appearance of serum virus neutralization antibodies (Brockmeier et al., 2012; Labarque et al., 2000; Meier et al., 2003; Ostrowski et al., 2002). PRRSV engineered to express IFN have been shown to limit the replication of co-infecting PRRSV, but the effects on PRRSV-specific adaptive immunity *in vivo* have not been elucidated (Sang et al., 2012, 2014). However, a recent study utilizing a strain of PRRSV that induced a strong type I IFN response was able to show an increase in virus neutralizing antibodies both in time to development and levels (Wang et al., 2013). Thus, the mechanism and/or pathway involved in type I IFN induction may have an effect on subsequent immune responses.

It has been observed that the delivery of IFN-α cDNA has a pronounced and sustained effect on the intensity of the cell-mediated immune response (Meier et al., 2004). Likewise, the introduction of the type I interferon agonist poly I:C, a synthetic double-stranded RNA, during vaccination temporarily amplified the number of PRRSV-specific IFN-γ SCs. However, polyI:C was not as efficient as an IFN-α encoding plasmid delivered with vaccine at enhancing the IFN-γ response to PRRSV. The observation that the inclusion of either IL-12 or IFN-α during immunization increased the intensity of the IFN-γ response to PRRSV validates the proposed role of these two innate cytokines in directing the *in vivo* differentiation of swine Th1 cells, and helps explain the poor virus-specific IFN-γ response that develops as a result of the exposure of pigs to PRRSV

(Meier et al., 2003; Xiao et al., 2004a). Co-administration of adjuvant with modified-live PRRSV has also been shown to enhance the early IFN-γ cellular immunity, though the connection to innate immune activation was not made (Mair et al., 2015). The plausible role of IFN-γ in mediating protective immunity against PRRSV is supported by the ability of this cytokine to inhibit PRRSV replication in the simian MARC-145 cell line (Rowland et al., 2001) as well as in porcine AMϕ (Bautista and Molitor, 1997). The connection between IFN-γ and protective immunity against PRRSV *in vivo* has been implied from the correlation between the frequency of virus-specific IFN-γ secreting cells and the clinical outcome upon PRRSV infection (Charerntantanakul et al., 2006; Lowe et al., 2005; Martelli et al., 2009). A major focus for assessing the improvement of vaccine efficacy has been the stimulation of strong T cell mediated IFN-γ response upon immunization. Although further studies are needed to demonstrate a direct cause and effect between protective immunity and IFN-γ, at the very least, measurement of cell-mediated immunity by measuring the frequency of IFN-γ secreting cells serves as a gauge for stimulation of a Th1-like immune response. The use of additional technologies to measure virus-specific CTLs might enable the establishment of a stronger correlation between protective immunity and CMI and thus facilitate the development of highly efficacious biologics. Ultimately however, the best measurement of protective immunity triggered by an effective vaccine is a pig that remains healthy and immunity is able to minimize the replication of PRRSV in a challenge infection model.

5. Moving forward

The central strategy for control and elimination of animal infectious diseases is vaccination to induce, without any adverse effects, rapid and sterilizing immunity that prevents infection upon exposure. In the case of PRRSV, this goal has not been achieved for reasons that have not yet been deduced. The purpose of this review is to highlight what is known about PRRSV immunity and focus on key areas that may help enhance vaccination strategies to achieve rapid and more complete sterilizing immunity against PRRSV. In moving forward with PRRSV immunology research, we have identified two directed research areas to achieve the goal.

First is research directed at identifying the mechanism(s) involved in preventing the pig from developing rapid and sterilizing immunity following infection (*i.e.* delayed development of convalescent immunity soon after infection). This will require a basic, yet mechanistic, understanding of PRRSV pathogenesis and identification of PRRSV proteins that alter induction of the host innate immune response. This research area in particular is full of contention, disagreement and conflicting results. Specifically, there are many conflicting reports on the ability of PRRSV infection to alter immune responses to antigens or infectious agents delivered simultaneously with PRRSV infection. Nevertheless, it is widely accepted that PRRSV is a predisposing agent for development of the porcine respiratory disease complex (PRDC), as PRRSV infection leaves pig herds susceptible to a variety of secondary infections. The association of PRRSV with secondary infections highlights the challenge of determining if PRRSV suppresses immune resistance other virulent pathogens, and/or weakens physiological control mechanisms to endogenous microorganisms such that they cause disease in the presence, but not the absence, of PRRSV. Until more is known about how the virus itself alters basic host immunity, it seems that further development of modified-live vaccines derived from virulent field viruses would be unlikely to yield markedly better protection than what is currently achieved. The mechanism(s) of attenuation for currently available commercial modified-live vaccines are not known, and induction of immunity is relatively slow, similar to natural infection. Thus, research focused on the mechanism(s) by which PRRSV affects the host immune system, including innate responses, T cell development, antigen presentation, and induction of PRRSV-specific lymphocytes, is needed to explain the inability of the pig to develop rapid and sterilizing immunity following infection.

Secondly, research directed at identification of protective immunogens and epitopes, and elucidation of immune mechanisms of antigen-specific protection, is essential for rational development of vaccines that provide rapid protection against PRRSV. PRRSV immunogens, epitopes and protective mechanisms unquestionably exist since sterilizing immunity to natural infection is achieved when given sufficient time. A mechanistic understanding of immunity to primary infection might be gained through dissection of immunity in adult animals that achieve sterile protection. At present, the majority of research has been conducted in growing swine that have not completely eliminated viral infection. Thus, timing of adaptive immunity evaluation will be critical for clear determination of convalescent protective immunity. Antigen delivery systems that deliver key immunogens and epitopes might be an avenue for both basic research (clearly identify protective epitopes) and for PRRSV vaccines (to achieve rapid protection and reduced susceptibility to secondary infections). Overall, the delivery of the protective epitopes or immunogens outside the context of PRRSV infection, *i.e.* in the absence of viral pathogenesis, may help reveal protective mechanisms more easily.

To achieve the goal of developing a PRRSV vaccine that rapidly induces protective immunity, the two primary research areas as outlined above would ideally be pursued simultaneously using both natural viral infection and model antigen delivery systems. PRRSV immunity research must move beyond observational studies to identification of mechanisms and immune functions to significantly advance beyond our current stage of knowledge. This will require a full understanding of the modulating effect that PRRSV has on the innate immune response *in vivo*. In addition, methods for a mechanistic evaluation of immune cell function, such as culture and immortalization of porcine antigen-specific T and B lymphocytes are needed. The ability to evaluate immune cell function requires a sustained commitment and investment that is independent of any particular pathogen. Such an investment will be extremely beneficial for understanding PRRSV immunity, as well as immunity to other swine infectious diseases. Collectively, this approach will move us forward in the challenge against PRRS, but also against other emerging diseases of swine.

Acknowledgements

Authors gratefully acknowledge support from the National Pork Board grant #13-241 to CLL, MPM, FAO and FAZ; the USDA NIFA multistate project MIN 63-084 and MIN63-112 to MPM; the USDA-AFRI-NIFA 2011-02917 and 2013-01035 to FAO.

References

Albina, E., 1997. Porcine reproductive and respiratory syndrome: ten years of experience (1986–1996) with this undesirable viral infection. Vet. Res. 28, 305–352.

Albina, E., Piriou, L., Hutet, E., Cariolet, R., L'Hospitalier, R., 1998. Immune responses in pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV). Vet. Immunol. Immunopathol. 61, 49–66.

Allende, R., Laegreid, W.W., Kutish, G.F., Galeota, J.A., Wills, R.W., Osorio, F.A., 2000. Porcine reproductive and respiratory syndrome virus: description of persistence in individual pigs upon experimental infection. J. Virol. 74, 10834–10837.

Ansari, I.H., Kwon, B., Osorio, F.A., Pattnaik, A.K., 2006. Influence of N-linked glycosylation of porcine reproductive and respiratory syndrome virus GP5 on virus infectivity, antigenicity, and ability to induce neutralizing antibodies. J. Virol. 80, 3994–4004.

Bastos, R.G., Dellagostin, O.A., Barletta, R.G., Doster, A.R., Nelson, E., Zuckermann, F., Osorio, F.A., 2004. Immune response of pigs inoculated with *Mycobacterium*

bovis BCG expressing a truncated form of GP5 and M protein of porcine reproductive and respiratory syndrome virus. Vaccine 22, 467–474.
Baum, A., Garcia-Sastre, A., 2010. Induction of type I interferon by RNA viruses: cellular receptors and their substrates. Amino Acids 38, 1283–1299.
Baumann, A., Mateu, E., Murtaugh, M.P., Summerfield, A., 2013. Impact of genotype 1 and 2 of porcine reproductive and respiratory syndrome viruses on interferon-alpha responses by plasmacytoid dendritic cells. Vet. Res. 44, 33.
Bautista, E.M., Molitor, T.W., 1997. Cell-mediated immunity to porcine reproductive and respiratory syndrome virus in swine. Viral Immunol. 10, 83–94.
Bautista, E.M., Suarez, P., Molitor, T.W., 1999. T cell responses to the structural polypeptides of porcine reproductive and respiratory syndrome virus. Arch. Virol. 144, 117–134.
Biron, C.A., 2001. Interferons alpha and beta as immune regulators – a new look. Immunity 14, 661–664.
Brockmeier, S.L., Lager, K.M., Grubman, M.J., Brough, D.E., Ettyreddy, D., Sacco, R.E., Gauger, P.C., Loving, C.L., Vorwald, A.C., Kehrli Jr., M.E., Lehmkuhl, H.D., 2009. Adenovirus-mediated expression of interferon-alpha delays viral replication and reduces disease signs in swine challenged with porcine reproductive and respiratory syndrome virus. Viral Immunol. 22, 173–180.
Brockmeier, S.L., Loving, C.L., Nelson, E.A., Miller, L.C., Nicholson, T.L., Register, K.B., Grubman, M.J., Brough, D.E., Kehrli Jr., M.E., 2012. The presence of alpha interferon at the time of infection alters the innate and adaptive immune responses to porcine reproductive and respiratory syndrome virus. Clin. Vaccine Immunol.: CVI 19, 508–514.
Brown, E., Lawson, S., Welbon, C., Gnanandarajah, J., Li, J., Murtaugh, M.P., Nelson, E.A., Molina, R.M., Zimmerman, J.J., Rowland, R.R., Fang, Y., 2009. Antibody response to porcine reproductive and respiratory syndrome virus (PRRSV) nonstructural proteins and implications for diagnostic detection and differentiation of PRRSV types I and II. Clin. Vaccine Immunol.: CVI 16, 628–635.
Buddaert, W., Van Reeth, K., Pensaert, M., 1998. In vivo and in vitro interferon (IFN) studies with the porcine reproductive and respiratory syndrome virus (PRRSV). Adv. Exp. Med. Biol. 440, 461–467.
Burgara-Estrella, A., Díaz, I., Rodríguez-Gómez, I.M., Essler, S.E., Hernández, J., Mateu, E., 2013. Predicted peptides from non-structural proteins of porcine reproductive and respiratory syndrome virus are able to induce IFN-γ and IL-10. Viruses 5, 663–677.
Calzada-Nova, G., Schnitzlein, W., Husmann, R., Zuckermann, F.A., 2010. Characterization of the cytokine and maturation responses of pure populations of porcine plasmacytoid dendritic cells to porcine viruses and toll-like receptor agonists. Vet. Immunol. Immunopathol. 135, 20–33.
Calzada-Nova, G., Schnitzlein, W.M., Husmann, R.J., Zuckermann, F.A., 2011. North American porcine reproductive and respiratory syndrome viruses inhibit type I interferon production by plasmacytoid dendritic cells. J. Virol. 85, 2703–2713.
Cancel-Tirado, S.M., Evans, R.B., Yoon, K.J., 2004. Monoclonal antibody analysis of porcine reproductive and respiratory syndrome virus epitopes associated with antibody-dependent enhancement and neutralization of virus infection. Vet. Immunol. Immunopathol. 102, 249–262.
Cecere, T.E., Todd, S.M., Leroith, T., 2012. Regulatory T cells in arterivirus and coronavirus infections: do they protect against disease or enhance it? Viruses 4, 833–846.
Cella, M., Facchetti, F., Lanzavecchia, A., Colonna, M., 2000. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. Nat. Immunol. 1, 305–310.
Charerntantanakul, W., Platt, R., Johnson, W., Roof, M., Vaughn, E., Roth, J.A., 2006. Immune responses and protection by vaccine and various vaccine adjuvant candidates to virulent porcine reproductive and respiratory syndrome virus. Vet. Immunol. Immunopathol. 109, 99–115.
Collins, J.E., 1998. Porcine reproductive and respiratory syndrome: the disease. In: Proceedings of the 15th IPVS Congress, Birmingham, England, 5–9 July, pp. 149–156.
Costers, S., Lefebvre, D.J., Goddeeris, B., Delputte, P.L., Nauwynck, H.J., 2009. Functional impairment of PRRSV-specific peripheral CD3+ CD8high cells. Vet. Res. 40, 46.
Cousens, L.P., Orange, J.S., Su, H.C., Biron, C.A., 1997. Interferon-alpha/beta inhibition of interleukin 12 and interferon-gamma production in vitro and endogenously during viral infection. Proc. Natl. Acad. Sci. U. S. A. 94, 634–639.
Cousens, L.P., Peterson, R., Hsu, S., Dorner, A., Altman, J.D., Ahmed, R., Biron, C.A., 1999. Two roads diverged: interferon alpha/beta- and interleukin 12-mediated pathways in promoting T cell interferon gamma responses during viral infection. J. Exp. Med. 189, 1315–1328.
Das, P.B., Dinh, P.X., Ansari, I.H., de Lima, M., Osorio, F.A., Pattnaik, A.K., 2010. The minor envelope glycoproteins GP2a and GP4 of porcine reproductive and respiratory syndrome virus interact with the receptor CD163. J. Virol. 84, 1731–1740.
Dawson, H.D., Beshah, E., Nishi, S., Solano-Aguilar, G., Morimoto, M., Zhao, A., Madden, K.B., Ledbetter, T.K., Dubey, J.P., Shea-Donohue, T., Lunney, J.K., Urban Jr., J.F., 2005. Localized multigene expression patterns support an evolving Th1/Th2-like paradigm in response to infections with Toxoplasma gondii and Ascaris suum. Infect. Immun. 73, 1116–1128.
de Lima, M., Pattnaik, A.K., Flores, E.F., Osorio, F.A., 2006. Serologic marker candidates identified among B-cell linear epitopes of Nsp2 and structural proteins of a North American strain of porcine reproductive and respiratory syndrome virus. Virology 353, 410–421.
Dea, S., Gagnon, C.A., Mardassi, H., Pirzadeh, B., Rogan, D., 2000. Current knowledge on the structural proteins of porcine reproductive and respiratory syndrome (PRRS) virus: comparison of the North American and European isolates. Arch. Virol. 145, 659–688.
Delputte, P.L., Meerts, P., Costers, S., Nauwynck, H.J., 2004. Effect of virus-specific antibodies on attachment, internalization and infection of porcine reproductive and respiratory syndrome virus in primary macrophages. Vet. Immunol. Immunopathol. 102, 179–188.
Diaz, I., Pujols, J., Ganges, L., Gimeno, M., Darwich, L., Domingo, M., Mateu, E., 2009. In silico prediction and ex vivo evaluation of potential T-cell epitopes in glycoproteins 4 and 5 and nucleocapsid protein of genotype-I (European) of porcine reproductive and respiratory syndrome virus. Vaccine 27, 5603–5611.
Dobrescu, I., Levast, B., Lai, K., Delgado-Ortega, M., Walker, S., Banman, S., Townsend, H., Simon, G., Zhou, Y., Gerdts, V., Meurens, F., 2014. In vitro and ex vivo analyses of co-infections with swine influenza and porcine reproductive and respiratory syndrome viruses. Vet. Microbiol. 169, 18–32.
Dwivedi, V., Manickam, C., Binjawadagi, B., Linhares, D., Murtaugh, M.P., Renukaradhya, G.J., 2012. Evaluation of immune responses to porcine reproductive and respiratory syndrome virus in pigs during early stage of infection under farm conditions. Virol. J. 9, 45.
Ebner, F., Rausch, S., Scharek-Tedin, L., Pieper, R., Burwinkel, M., Zentek, J., Hartmann, S., 2014. A novel lineage transcription factor based analysis reveals differences in T helper cell subpopulation development in infected and intrauterine growth restricted (IUGR) piglets. Dev. Comp. Immunol. 46, 333–340.
Faaberg, K.S., Hocker, J.D., Erdman, M.M., Harris, D.L., Nelson, E.A., Torremorell, M., Plagemann, P.G., 2006. Neutralizing antibody responses of pigs infected with natural GP5 N-glycan mutants of porcine reproductive and respiratory syndrome virus. Viral Immunol. 19, 294–304.
Fan, B., Liu, X., Bai, J., Zhang, T., Zhang, Q., Jiang, P., 2015. The amino acid residues at 102 and 104 in GP5 of porcine reproductive and respiratory syndrome virus regulate viral neutralization susceptibility to the porcine serum neutralizing antibody. Virus Res. 204, 21–30.
Fang, Y., Snijder, E.J., 2010. The PRRSV replicase: exploring the multifunctionality of an intriguing set of nonstructural proteins. Virus Res. 154, 61–76.
Feng, W.H., Tompkins, M.B., Xu, J.S., Brown, T.T., Laster, S.M., Zhang, H.X., McCaw, M.B., 2002. Thymocyte and peripheral blood T lymphocyte subpopulation changes in piglets following in utero infection with porcine reproductive and respiratory syndrome virus. Virology 302, 363–372.
Feng, W.H., Tompkins, M.B., Xu, J.S., Zhang, H.X., McCaw, M.B., 2003. Analysis of constitutive cytokine expression by pigs infected in-utero with porcine reproductive and respiratory syndrome virus. Vet. Immunol. Immunopathol. 94, 35–45.
Ferrari, L., Martelli, P., Saleri, R., De Angelis, E., Cavalli, V., Bresaola, M., Benetti, M., Borghetti, P., 2013. Lymphocyte activation as cytokine gene expression and secretion is related to the porcine reproductive and respiratory syndrome virus (PRRSV) isolate after in vitro homologous and heterologous recall of peripheral blood mononuclear cells (PBMC) from pigs vaccinated and exposed to natural infection. Vet. Immunol. Immunopathol. 151, 193–206.
Firth, A.E., Zevenhoven-Dobbe, J.C., Wills, N.M., Go, Y.Y., Balasuriya, U.B., Atkins, J.F., Snijder, E.J., Posthuma, C.C., 2011. Discovery of a small arterivirus gene that overlaps the GP5 coding sequence and is important for virus production. J. Gen. Virol. 92, 1097–1106.
Fitzgerald-Bocarsly, P., 2002. Natural interferon-alpha producing cells: the plasmacytoid dendritic cells. Biotechniques (Suppl.), 16–20, 22, 19–24.
Fitzgerald-Bocarsly, P., Dai, J., Singh, S., 2008. Plasmacytoid dendritic cells and type I IFN: 50 years of convergent history. Cytokine Growth Factor Rev. 19, 3–19.
Foss, D.L., Zilliox, M.J., Meier, W., Zuckermann, F., Murtaugh, M.P., 2002. Adjuvant danger signals increase the immune response to porcine reproductive and respiratory syndrome virus. Viral Immunol. 15, 557–566.
Francica, J.R., Varela-Rohena, A., Medvec, A., Plesa, G., Riley, J.L., Bates, P., 2010. Steric shielding of surface epitopes and impaired immune recognition induced by the ebola virus glycoprotein. PLoS Pathog. 6, e1001098.
Genini, S., Delputte, P.L., Malinverni, R., Cecere, M., Stella, A., Nauwynck, H.J., Giuffra, E., 2008. Genome-wide transcriptional response of primary alveolar macrophages following infection with porcine reproductive and respiratory syndrome virus. J. Gen. Virol. 89, 2550–2564.
Gomez-Laguna, J., Salguero, F.J., De Marco, M.F., Pallares, F.J., Bernabe, A., Carrasco, L., 2009. Changes in lymphocyte subsets and cytokines during European porcine reproductive and respiratory syndrome: increased expression of IL-12 and IL-10 and proliferation of CD4(−)CD8(high). Viral Immunol. 22, 261–271.
Gómez-Laguna, J., Salguero, F.J., Fernández de Marco, M., Barranco, I., Rodríguez-Gómez, I.M., Quezada, M., Carrasco, L., 2013. Type 2 Porcine Reproductive and Respiratory Syndrome Virus infection mediated apoptosis in B- and T-cell areas in lymphoid organs of experimentally infected pigs. Transbound. Emerg. Dis. 60, 273–278.
Gu, W., Guo, L., Yu, H., Niu, J., Huang, M., Luo, X., Li, R., Tian, Z., Feng, L., Wang, Y., 2015. Involvement of CD16 in antibody-dependent enhancement of porcine reproductive and respiratory syndrome virus infection. J. Gen. Virol.
Han, K., Seo, H.W., Park, C., Chae, C., 2014. Vaccination of sows against type 2 Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) before artificial insemination protects against type 2 PRRSV challenge but does not protect against type 1 PRRSV challenge in late gestation. Vet. Res. 45, 12.
Hesse, R., Couture, L., Lau, M., Wunder, K., Wasmoen, T., 1996. Efficacy of Prime Pac PRRS in controlling PRRS reproductive disease: heterologous challenge. In:

Proceedings 27th Annual Meetg American Association of Swine Practitioners, Nashvill, TN, USA, pp. 107–110.
Honda, K., Sakaguchi, S., Nakajima, C., Watanabe, A., Yanai, H., Matsumoto, M., Ohteki, T., Kaisho, T., Takaoka, A., Akira, S., Seya, T., Taniguchi, T., 2003. Selective contribution of IFN-alpha/beta signaling to the maturation of dendritic cells induced by double-stranded RNA or viral infection. Proc. Natl. Acad. Sci. U. S. A. 100, 10872–10877.
Jeong, H.J., Song, Y.J., Lee, S.W., Lee, J.B., Park, S.Y., Song, C.S., Ha, G.W., Oh, J.S., Oh, Y.K., Choi, I.S., 2010. Comparative measurement of cell-mediated immune responses of swine to the M and N proteins of porcine reproductive and respiratory syndrome virus. Clin. Vaccine Immunol.: CVI 17, 503–512.
Jiang, W., Jiang, P., Wang, X., Li, Y., Du, Y., 2007. Influence of porcine reproductive and respiratory syndrome virus GP5 glycoprotein N-linked glycans on immune responses in mice. Virus Genes 35, 663–671.
Jiang, W., Jiang, P., Wang, X., Li, Y., Du, Y., 2008. Enhanced immune responses of mice inoculated recombinant adenoviruses expressing GP5 by fusion with GP3 and/or GP4 of PRRS virus. Virus Res. 136, 50–57.
Johnson, C.R., Griggs, T.F., Gnanandarajah, J., Murtaugh, M.P., 2011. Novel structural protein in porcine reproductive and respiratory syndrome virus encoded by an alternative ORF5 present in all arteriviruses. J. Gen. Virol. 92, 1107–1116.
Johnson, C.R., Yu, W., Murtaugh, M.P., 2007. Cross-reactive antibody responses to nsp1 and nsp2 of Porcine reproductive and respiratory syndrome virus. J. Gen. Virol. 88, 1184–1195.
Jung, K., Renukaradhya, G.J., Alekseev, K.P., Fang, Y., Tang, Y., Saif, L.J., 2009. Porcine reproductive and respiratory syndrome virus modifies innate immunity and alters disease outcome in pigs subsequently infected with porcine respiratory coronavirus: implications for respiratory viral co-infections. J. Gen. Virol. 90, 2713–2723.
Kadowaki, N., Antonenko, S., Lau, J.Y., Liu, Y.J., 2000. Natural interferon alpha/beta-producing cells link innate and adaptive immunity. J. Exp. Med. 192, 219–226.
Kapsenberg, M.L., 2003. Dendritic-cell control of pathogen-driven T-cell polarization. Nat. Rev. Immunol. 3, 984–993.
Kawashima, K., Narita, M., Yamada, S., 1999. Changes in macrophage and lymphocyte subpopulations of lymphoid tissues from pigs infected with the porcine reproductive and respiratory syndrome virus (PRRSV). Vet. Immunol. Immunopathol. 71, 257–262.
Kim, W.I., Kim, J.J., Cha, S.H., Wu, W.H., Cooper, V., Evans, R., Choi, E.J., Yoon, K.J., 2013. Significance of genetic variation of PRRSV ORF5 in virus neutralization and molecular determinants corresponding to cross neutralization among PRRS viruses. Vet. Microbiol. 162, 10–22.
Kim, W.I., Yoon, K.J., 2008. Molecular assessment of the role of envelope-associated structural proteins in cross neutralization among different PRRS viruses. Virus Genes 37, 380–391.
Klinge, K.L., Vaughn, E.M., Roof, M.B., Bautista, E.M., Murtaugh, M.P., 2009. Age-dependent resistance to Porcine reproductive and respiratory syndrome virus replication in swine. Virol. J. 6, 177.
Labarque, G.G., Nauwynck, H.J., Van Reeth, K., Pensaert, M.B., 2000. Effect of cellular changes and onset of humoral immunity on the replication of porcine reproductive and respiratory syndrome virus in the lungs of pigs. J. Gen. Virol. 81, 1327–1334.
Lamontagne, L., Page, C., Larochelle, R., Magar, R., 2003. Porcine reproductive and respiratory syndrome virus persistence in blood, spleen, lymph nodes, and tonsils of experimentally infected pigs depends on the level of CD8high T cells. Viral Immunol. 16, 395–406.
Lee, S.M., Schommer, S.K., Kleiboeker, S.B., 2004. Porcine reproductive and respiratory syndrome virus field isolates differ in in vitro interferon phenotypes. Vet. Immunol. Immunopathol. 102, 217–231.
Levy, D.E., Marie, I., Prakash, A., 2003. Ringing the interferon alarm: differential regulation of gene expression at the interface between innate and adaptive immunity. Curr. Opin. Immunol. 15, 52–58.
Li, J., Murtaugh, M.P., 2012. Dissociation of porcine reproductive and respiratory syndrome virus neutralization from antibodies specific to major envelope protein surface epitopes. Virology 433, 367–376.
Li, X., Galliher-Beckley, A., Pappan, L., Trible, B., Kerrigan, M., Beck, A., Hesse, R., Blecha, F., Nietfeld, J.C., Rowland, R.R., Shi, J., 2014. Comparison of host immune responses to homologous and heterologous type II porcine reproductive and respiratory syndrome virus (PRRSV) challenge in vaccinated and unvaccinated pigs. Biomed Res. Int. 2014, 416727.
Linhares, D.C., Cano, J.P., Torremorell, M., Morrison, R.B., 2014. Comparison of time to PRRSv-stability and production losses between two exposure programs to control PRRSv in sow herds. Prev. Vet. Med. 116, 111–119.
Liu, C.H., Chaung, H.C., Chang, H.L., Peng, Y.T., Chung, W.B., 2009. Expression of Toll-like receptor mRNA and cytokines in pigs infected with porcine reproductive and respiratory syndrome virus. Vet. Microbiol. 136, 266–276.
Liu, M., Chen, H., Luo, F., Li, P., Pan, Q., Xia, B., Qi, Z., Ho, W.Z., Zhang, X.L., 2007. Deletion of N-glycosylation sites of hepatitis C virus envelope protein E1 enhances specific cellular and humoral immune responses. Vaccine 25, 6572–6580.
Loemba, H.D., Mounir, S., Mardassi, H., Archambault, D., Dea, S., 1996. Kinetics of humoral immune response to the major structural proteins of the porcine reproductive and respiratory syndrome virus. Arch. Virol. 141, 751–761.
Lohse, L., Nielsen, J., Eriksen, L., 2004. Temporary CD8+ T-cell depletion in pigs does not exacerbate infection with porcine reproductive and respiratory syndrome virus (PRRSV). Viral Immunol. 17, 594–603.
Lopez Fuertes, L., Domenech, N., Alvarez, B., Ezquerra, A., Dominguez, J., Castro, J.M., Alonso, F., 1999. Analysis of cellular immune response in pigs recovered from porcine respiratory and reproductive syndrome infection. Virus Res. 64, 33–42.
Lopez, O.J., Oliveira, M.F., Garcia, E.A., Kwon, B.J., Doster, A., Osorio, F.A., 2007. Protection against porcine reproductive and respiratory syndrome virus (PRRSV) infection through passive transfer of PRRSV-neutralizing antibodies is dose dependent. Clin. Vaccine Immunol.: CVI 14, 269–275.
Lopez, O.J., Osorio, F.A., 2004. Role of neutralizing antibodies in PRRSV protective immunity. Vet. Immunol. Immunopathol. 102, 155–163.
Loving, C.L., Brockmeier, S.L., Sacco, R.E., 2007. Differential type I interferon activation and susceptibility of dendritic cell populations to porcine arterivirus. Immunology 120, 217–229.
Lowe, J.E., Husmann, R., Firkins, L.D., Zuckermann, F.A., Goldberg, T.L., 2005. Correlation of cell-mediated immunity against porcine reproductive and respiratory syndrome virus with protection against reproductive failure in sows during outbreaks of porcine reproductive and respiratory syndrome in commercial herds. J. Am. Vet. Med. Assoc. 226, 1707–1711.
Mair, K.H., Koinig, H., Gerner, W., Hohne, A., Bretthauer, J., Kroll, J.J., Roof, M.B., Saalmuller, A., Stadler, K., Libanova, R., 2015. Carbopol improves the early cellular immune responses induced by the modified-life vaccine Ingelvac PRRS MLV. Vet. Microbiol. 176, 352–357.
Mardassi, H., Massie, B., Dea, S., 1996. Intracellular synthesis, processing, and transport of proteins encoded by ORFs 5–7 of porcine reproductive and respiratory syndrome virus. Virology 221, 98–112.
Martelli, P., Gozio, S., Ferrari, L., Rosina, S., De Angelis, E., Quintavalla, C., Bottarelli, E., Borghetti, P., 2009. Efficacy of a modified live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine in pigs naturally exposed to a heterologous European (Italian cluster) field strain: clinical protection and cell-mediated immunity. Vaccine 27, 3788–3799.
Martinez-Lobo, F.J., Diez-Fuertes, F., Simarro, I., Castro, J.M., Prieto, C., 2011. Porcine Reproductive and Respiratory Syndrome Virus isolates differ in their susceptibility to neutralization. Vaccine 29, 6928–6940.
Meier, W., Wheeler, J.G., Husmann, R.J., Osorio, F.A., Zuckermann, F., 2000. Charcateristics of the immune response of pigs to PRRS virus. Vet. Res. 31, 41.
Meier, W.A., Galeota, J., Osorio, F.A., Husmann, R.J., Schnitzlein, W.M., Zuckermann, F.A., 2003. Gradual development of the interferon-gamma response of swine to porcine reproductive and respiratory syndrome virus infection or vaccination. Virology 309, 18–31.
Meier, W.A., Husmann, R.J., Schnitzlein, W.M., Osorio, F.A., Lunney, J.K., Zuckermann, F.A., 2004. Cytokines and synthetic double-stranded RNA augment the T helper 1 immune response of swine to porcine reproductive and respiratory syndrome virus. Vet. Immunol. Immunopathol. 102, 299–314.
Meulenberg, J.J., Bende, R.J., Pol, J.M., Wensvoort, G., Moormann, R.J., 1995. Nucleocapsid protein N of Lelystad virus: expression by recombinant baculovirus, immunological properties, and suitability for detection of serum antibodies. Clin. Diagn. Lab. Immunol. 2, 652–656.
Miller, L.C., Lager, K.M., Kehrli Jr., M.E., 2009. Role of Toll-like receptors in activation of porcine alveolar macrophages by porcine reproductive and respiratory syndrome virus. Clin. Vaccine Immunol.: CVI 16, 360–365.
Molitor, T., 1993. Immune response to PRRS virus. In: Proc. Allen D. Leman Confer., St. Paul, p. 20.
Molitor, T.W., Bautista, E.M., Choi, C.S., 1997. Immunity to PRRSV: double-edged sword. Vet. Microbiol. 55, 265–276.
Montoya, M., Schiavoni, G., Mattei, F., Gresser, I., Belardelli, F., Borrow, P., Tough, D.F., 2002. Type I interferons produced by dendritic cells promote their phenotypic and functional activation. Blood 99, 3263–3271.
Mulupuri, P., Zimmerman, J.J., Hermann, J., Johnson, C.R., Cano, J.P., Yu, W., Dee, S.A., Murtaugh, M.P., 2008. Antigen-specific B-cell responses to porcine reproductive and respiratory syndrome virus infection. J. Virol. 82, 358–370.
Murtaugh, M.P., Genzow, M., 2011. Immunological solutions for treatment and prevention of porcine reproductive and respiratory syndrome (PRRS). Vaccine 29, 8192–8204.
Murtaugh, M.P., Johnson, C.R., Xiao, Z., Scamurra, R.W., Zhou, Y., 2009. Species specialization in cytokine biology: is interleukin-4 central to the T(H)1-T(H)2 paradigm in swine? Dev. Comp. Immunol. 33, 344–352.
Murtaugh, M.P., Xiao, Z., Zuckermann, F., 2002. Immunological responses of swine to porcine reproductive and respiratory syndrome virus infection. Viral Immunol. 15, 533–547.
Nelson, E.A., Christopher-Hennings, J., Benfield, D.A., 1994. Serum immune responses to the proteins of porcine reproductive and respiratory syndrome (PRRS) virus. J. Vet. Diagn. Invest. 6, 410–415.
Nielsen, J., Botner, A., 1997. Hematological and immunological parameters of 4 1/2-month old pigs infected with PRRS virus. Vet. Microbiol. 55, 289–294.
Nielsen, J., Botner, A., Tingstedt, J.E., Aasted, B., Johnsen, C.K., Riber, U., Lind, P., 2003. In utero infection with porcine reproductive and respiratory syndrome virus modulates leukocyte subpopulations in peripheral blood and bronchoalveolar fluid of surviving piglets. Vet. Immunol. Immunopathol. 93, 135–151.
Oleksiewicz, M.B., Botner, A., Toft, P., Normann, P., Storgaard, T., 2001. Epitope mapping porcine reproductive and respiratory syndrome virus by phage display: the nsp2 fragment of the replicase polyprotein contains a cluster of B-cell epitopes. J. Virol. 75, 3277–3290.
Olin, M.R., Batista, L., Xiao, Z., Dee, S.A., Murtaugh, M.P., Pijoan, C.C., Molitor, T.W., 2005. Gammadelta lymphocyte response to porcine reproductive and respiratory syndrome virus. Viral Immunol. 18, 490–499.

Osorio, F.A., Galeota, J.A., Nelson, E., Brodersen, B., Doster, A., Wills, R., Zuckermann, F., Laegreid, W.W., 2002. Passive transfer of virus-specific antibodies confers protection against reproductive failure induced by a virulent strain of porcine reproductive and respiratory syndrome virus and establishes sterilizing immunity. Virology 302, 9–20.

Osorio, F.A., Zuckermann, F., Wills, R., Meier, W., Christian, S., Galeota, J., Doster, A., 1998. PRRSV: comparison of commercial vaccines in their ability to induce protection against current PRRSV strains of high virulence. In: Allen D. Leman Swine Conference, vol. 25, pp. 176–182.

Ostrowski, M., Galeota, J.A., Jar, A.M., Platt, K.B., Osorio, F.A., Lopez, O.J., 2002. Identification of neutralizing and nonneutralizing epitopes in the porcine reproductive and respiratory syndrome virus GP5 ectodomain. J. Virol. 76, 4241–4250.

Parida, R., Choi, I.S., Peterson, D.A., Pattnaik, A.K., Laegreid, W., Zuckermann, F.A., Osorio, F.A., 2012. Location of T-cell epitopes in nonstructural proteins 9 and 10 of type-II porcine reproductive and respiratory syndrome virus. Virus Res.

Pirzadeh, B., Dea, S., 1997. Monoclonal antibodies to the ORF5 product of porcine reproductive and respiratory syndrome virus define linear neutralizing determinants. J. Gen. Virol. 78 (Pt 8), 1867–1873.

Pirzadeh, B., Dea, S., 1998. Immune response in pigs vaccinated with plasmid DNA encoding ORF5 of porcine reproductive and respiratory syndrome virus. J. Gen. Virol. 79 (Pt 5), 989–999.

Plagemann, P.G., Rowland, R.R., Faaberg, K.S., 2002. The primary neutralization epitope of porcine respiratory and reproductive syndrome virus strain VR-2332 is located in the middle of the GP5 ectodomain. Arch. Virol. 147, 2327–2347.

Plana Duran, J., Climent, I., Sarraseca, J., Urniza, A., Cortes, E., Vela, C., Casal, J.I., 1997. Baculovirus expression of proteins of porcine reproductive and respiratory syndrome virus strain Olot/91. Involvement of ORF3 and ORF5 proteins in protection. Virus Genes 14, 19–29.

Plotkin, S.A., Gilbert, P.B., 2012. Nomenclature for immune correlates of protection after vaccination. Clin. Infect. Dis.: Off. Publ. Infect. Dis. Soc. Am. 54, 1615–1617.

Reitter, J.N., Means, R.E., Desrosiers, R.C., 1998. A role for carbohydrates in immune evasion in AIDS. Nat. Med. 4, 679–684.

Robinson, S.R., Figueiredo, M.C., Abrahante, J.E., Murtaugh, M.P., 2013. Immune response to ORF5a protein immunization is not protective against porcine reproductive and respiratory syndrome virus infection. Vet. Microbiol. 164, 281–285.

Robinson, S.R., Li, J., Nelson, E.A., Murtaugh, M.P., 2015. Broadly neutralizing antibodies against the rapidly evolving porcine reproductive and respiratory syndrome virus. Virus Res. 203, 56–65.

Roca, M., Gimeno, M., Bruguera, S., Segales, J., Diaz, I., Galindo-Cardiel, I.J., Martinez, E., Darwich, L., Fang, Y., Maldonado, J., March, R., Mateu, E., 2012. Effects of challenge with a virulent genotype II strain of porcine reproductive and respiratory syndrome virus on piglets vaccinated with an attenuated genotype I strain vaccine. Vet. J. 193, 92–96.

Rodriguez-Gomez, I.M., Kaser, T., Gomez-Laguna, J., Lamp, B., Sinn, L., Rumenapf, T., Carrasco, L., Saalmuller, A., Gerner, W., 2015. PRRSV-infected monocyte-derived dendritic cells express high levels of SLA-DR and CD80/86 but do not stimulate PRRSV-naive regulatory T cells to proliferate. Vet. Res. 46, 54.

Rowland, R.R., Robinson, B., Stefanick, J., Kim, T.S., Guanghua, L., Lawson, S.R., Benfield, D.A., 2001. Inhibition of porcine reproductive and respiratory syndrome virus by interferon-gamma and recovery of virus replication with 2-aminopurine. Arch. Virol. 146, 539–555.

Royaee, A.R., Husmann, R.J., Dawson, H.D., Calzada-Nova, 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. Vet. Immunol. Immunopathol. 102, 199–216.

Samsom, J.N., de Bruin, T.G., Voermans, J.J., Meulenberg, J.J., Pol, J.M., Bianchi, A.T., 2000. Changes of leukocyte phenotype and function in the broncho-alveolar lavage fluid of pigs infected with porcine reproductive and respiratory syndrome virus: a role for CD8(+) cells. J. Gen. Virol. 81, 497–505.

Sang, Y., Rowland, R.R., Blecha, F., 2014. Antiviral regulation in porcine monocytic cells at different activation states. J. Virol. 88, 11395–11410.

Sang, Y., Shi, J., Sang, W., Rowland, R.R., Blecha, F., 2012. Replication-competent recombinant porcine reproductive and respiratory syndrome (PRRS) viruses expressing indicator proteins and antiviral cytokines. Viruses 4, 102–116.

Scortti, M., Prieto, C., Simarro, I., Castro, J.M., 2006. Reproductive performance of gilts following vaccination and subsequent heterologous challenge with European strains of porcine reproductive and respiratory syndrome virus. Theriogenology 66, 1884–1893.

Shimizu, M., Yamada, S., Kawashima, K., Ohashi, S., Shimizu, S., Ogawa, T., 1996. Changes of lymphocyte subpopulations in pigs infected with porcine reproductive and respiratory syndrome (PRRS) virus. Vet. Immunol. Immunopathol. 50, 19–27.

Silva-Campa, E., Cordoba, L., Fraile, L., Flores-Mendoza, L., Montoya, M., Hernandez, 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., Flores-Mendoza, L., Resendiz, M., Pinelli-Saavedra, A., Mata-Haro, V., Mwangi, W., Hernandez, J., 2009. Induction of T helper 3 regulatory cells by dendritic cells infected with porcine reproductive and respiratory syndrome virus. Virology 387, 373–379.

Silva-Campa, E., Mata-Haro, V., Mateu, E., Hernandez, J., 2012. Porcine reproductive and respiratory syndrome virus induces CD4+ CD8+ CD25+ Foxp3+ regulatory T cells (Tregs). Virology 430, 73–80.

Snijder, E.J., Meulenberg, J.M., 2001. In: Kniper, D., et al. (Eds.), Arteriviruses in Fields Virology, vol. 1, 4th ed. Publ. by Lippincott Williams & Wilkins, Philadelphia, pp. 1205–1220.

Spilman, M.S., Welbon, C., Nelson, E., Dokland, T., 2009. Cryo-electron tomography of porcine reproductive and respiratory syndrome virus: organization of the nucleocapsid. J. Gen. Virol. 90, 527–535.

Sun, Y., Han, M., Kim, C., Calvert, J.G., Yoo, D., 2012. Interplay between interferon-mediated innate immunity and porcine reproductive and respiratory syndrome virus. Viruses 4, 424–446.

Swiecki, M., Colonna, M., 2010. Unraveling the functions of plasmacytoid dendritic cells during viral infections, autoimmunity, and tolerance. Immunol. Rev. 234, 142–162.

Takeuchi, O., Akira, S., 2009. Innate immunity to virus infection. Immunol. Rev. 227, 75–86.

Tian, D., Wei, Z., Zevenhoven-Dobbe, J.C., Liu, R., Tong, G., Snijder, E.J., Yuan, S., 2012. Arterivirus minor envelope proteins are a major determinant of viral tropism in cell culture. J. Virol. 86, 3701–3712.

Tingstedt, J.E., Nielsen, J., 2004. Cellular immune responses in the lungs of pigs infected in utero with PRRSV: an immunohistochemical study. Viral Immunol. 17, 558–564.

Torremorell, M., Moore, C., Christianson, W.T., 2002. Establishment of a herd negative for porcine reproductive and respiratory syndrome virus (PRRSV) from PRRSV-positive sources. J. Swine Health Prod. 10, 153–160.

Tough, D.F., 2004. Type I interferon as a link between innate and adaptive immunity through dendritic cell stimulation. Leukemia Lymphoma 45, 257–264.

Trible, B.R., Popescu, L.N., Monday, N., Calvert, J.G., Rowland, R.R., 2015. A single amino Acid deletion in the matrix protein of porcine reproductive and respiratory syndrome virus confers resistance to a polyclonal Swine antibody with broadly neutralizing activity. J. Virol. 89, 6515–6520.

Trus, I., Bonckaert, C., van der Meulen, K., Nauwynck, H.J., 2014. Efficacy of an attenuated European subtype 1 porcine reproductive and respiratory syndrome virus (PRRSV) vaccine in pigs upon challenge with the East European subtype 3 PRRSV strain Lena. Vaccine 32, 2995–3003.

van Nieuwstadt, A.P., Meulenberg, J.J., van Essen-Zanbergen, A., Petersen-den Besten, A., Bende, R.J., Moormann, R.J., Wensvoort, G., 1996. Proteins encoded by open reading frames 3 and 4 of the genome of Lelystad virus (Arteriviridae) are structural proteins of the virion. J. Virol. 70, 4767–4772.

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. Res. Vet. Sci. 67, 47–52.

van Reeth, K., Nauwynck, H., 2000. Proinflammatory cytokines and viral respiratory disease in pigs. Vet. Res. 31, 187–213.

Vanhee, M., Van Breedam, W., Costers, S., Geldhof, M., Noppe, Y., Nauwynck, H., 2011. Characterization of antigenic regions in the porcine reproductive and respiratory syndrome virus by the use of peptide-specific serum antibodies. Vaccine 29, 4794–4804.

Vashisht, K., Erlandson, K.R., Firkins, L.D., Zuckermann, F.A., Goldberg, T.L., 2008. Evaluation of contact exposure as a method for acclimatizing growing pigs to porcine reproductive and respiratory syndrome virus. J. Am. Vet. Med. Assoc. 232, 1530–1535.

Vu, H.L., Kwon, B., Yoon, K.J., Laegreid, W.W., Pattnaik, A.K., Osorio, F.A., 2011. Immune evasion of porcine reproductive and respiratory syndrome virus through glycan shielding involves both glycoprotein 5 as well as glycoprotein 3. J. Virol. 85, 5555–5564.

Wang, C.C., Chen, J.R., Tseng, Y.C., Hsu, C.H., Hung, Y.F., Chen, S.W., Chen, C.M., Khoo, K.H., Cheng, T.J., Cheng, Y.S., Jan, J.T., Wu, C.Y., Ma, C., Wong, C.H., 2009a. Glycans on influenza hemagglutinin affect receptor binding and immune response. Proc. Natl. Acad. Sci. U. S. A. 106, 18137–18142.

Wang, R., Xiao, Y., Opriessnig, T., Ding, Y., Yu, Y., Nan, Y., Ma, Z., Halbur, P.G., Zhang, Y.J., 2013. Enhancing neutralizing antibody production by an interferon-inducing porcine reproductive and respiratory syndrome virus strain. Vaccine 31, 5537–5543.

Wang, X., Christopher-Hennings, J., 2012. Post-transcriptional control of type I interferon induction by porcine reproductive and respiratory syndrome virus in its natural host cells. Viruses 4, 725–733.

Wang, X., Li, J., Jiang, P., Li, Y., Zeshan, B., Cao, J., Wang, X., 2009b. GM-CSF fused with GP3 and GP5 of porcine reproductive and respiratory syndrome virus increased the immune responses and protective efficacy against virulent PRRSV challenge. Virus Res. 143, 24–32.

Wang, Y.X., Zhou, Y.J., Li, G.X., Zhang, S.R., Jiang, Y.F., Xu, A.T., Yu, H., Wang, M.M., Yan, L.P., Tong, G.Z., 2011. Identification of immunodominant T-cell epitopes in membrane protein of highly pathogenic porcine reproductive and respiratory syndrome virus. Virus Res. 158, 108–115.

Wee, G.J., Cao, M.J., Liu, W., Kwang, J., 2001. Efficacy of porcine reproductive and respiratory syndrome virus vaccine and porcine interleukin-12. Vet. Therap.: Res. Appl. Vet. Med. 2, 112–119.

Weesendorp, E., Stockhofe-Zurwieden, N., Popma-De Graaf, D.J., Fijten, H., Rebel, J.M., 2013. Phenotypic modulation and cytokine profiles of antigen presenting cells by European subtype 1 and 3 porcine reproductive and respiratory syndrome virus strains in vitro and in vivo. Vet. Microbiol. 167, 638–650.

Wei, X., Decker, J.M., Wang, S., Hui, H., Kappes, J.C., Wu, X., Salazar-Gonzalez, J.F., Salazar, M.G., Kilby, J.M., Saag, M.S., Komarova, N.L., Nowak, M.A., Hahn, B.H., Kwong, P.D., Shaw, G.M., 2003. Antibody neutralization and escape by HIV-1. Nature 422, 307–312.

Wei, Z., Lin, T., Sun, L., Li, Y., Wang, X., Gao, F., Liu, R., Chen, C., Tong, G., Yuan, S., 2013a. N-linked glycosylation of GP5 of porcine reproductive and respiratory syndrome virus is critically important for virus replication in vivo. J. Virol. 86, 9941–9951.

Wei, Z., Tian, D., Sun, L., Lin, T., Gao, F., Liu, R., Tong, G., Yuan, S., 2013b. Influence of N-linked glycosylation of minor proteins of porcine reproductive and respiratory syndrome virus on infectious virus recovery and receptor interaction. Virology 429, 1–11.

Weiland, E., Wieczorek-Krohmer, M., Kohl, D., Conzelmann, K.K., Weiland, F., 1999. Monoclonal antibodies to the GP5 of porcine reproductive and respiratory syndrome virus are more effective in virus neutralization than monoclonal antibodies to the GP4. Vet. Microbiol. 66, 171–186.

Wills, R.W., Zimmerman, J.J., Yoon, K.J., Swenson, S.L., McGinley, M.J., Hill, H.T., Platt, K.B., Christopher-Hennings, J., Nelson, E.A., 1997. Porcine reproductive and respiratory syndrome virus: a persistent infection. Vet. Microbiol. 55, 231–240.

Wissink, E.H., Kroese, M.V., van Wijk, H.A., Rijsewijk, F.A., Meulenberg, J.J., Rottier, P.J., 2005. Envelope protein requirements for the assembly of infectious virions of porcine reproductive and respiratory syndrome virus. J. Virol. 79, 12495–12506.

Wongyanin, P., Buranapraditkun, S., Chokeshai-Usaha, K., Thanawonguwech, R., Suradhat, S., 2010. Induction of inducible CD4+ CD25+ Foxp3+ regulatory T lymphocytes by porcine reproductive and respiratory syndrome virus (PRRSV). Vet. Immunol. Immunopathol. 133, 170–182.

Wu, W.H., Fang, Y., Farwell, R., Steffen-Bien, M., Rowland, R.R., Christopher-Hennings, J., Nelson, E.A., 2001. A 10-kDa structural protein of porcine reproductive and respiratory syndrome virus encoded by ORF2b. Virology 287, 183–191.

Xiao, S., Jia, J., Mo, D., Wang, Q., Qin, L., He, Z., Zhao, X., Huang, Y., Li, A., Yu, J., Niu, Y., Liu, X., Chen, Y., 2010a. Understanding PRRSV infection in porcine lung based on genome-wide transcriptome response identified by deep sequencing. PLoS ONE 5, e11377.

Xiao, S., Wang, Q., Jia, J., Cong, P., Mo, D., Yu, X., Qin, L., Li, A., Niu, Y., Zhu, K., Wang, X., Liu, X., Chen, Y., 2010b. Proteome changes of lungs artificially infected with H-PRRSV and N-PRRSV by two-dimensional fluorescence difference gel electrophoresis. Virol. J. 7, 107.

Xiao, Z., Batista, L., Dee, S., Halbur, P., Murtaugh, M.P., 2004a. The level of virus-specific T-cell and macrophage recruitment in porcine reproductive and respiratory syndrome virus infection in pigs is independent of virus load. J. Virol. 78, 5923–5933.

Xiao, Z., Trincado, C.A., Murtaugh, M.P., 2004b. Beta-glucan enhancement of T cell IFNgamma response in swine. Vet. Immunol. Immunopathol. 102, 315–320.

Xu, X.G., Wang, Z.S., Zhang, Q., Li, Z.C., Ding, L., Li, W., Wu, H.Y., Chang, C.D., Lee, L.H., Tong, D.W., Liu, H.J., 2012. Baculovirus as a PRRSV and PCV2 bivalent vaccine vector: baculovirus virions displaying simultaneously GP5 glycoprotein of PRRSV and capsid protein of PCV2. J. Virol. Methods 179, 359–366.

Yang, L., Frey, M.L., Yoon, K.J., Zimmerman, J.J., Platt, K.B., 2000. Categorization of North American porcine reproductive and respiratory syndrome viruses: epitopic profiles of the N, M, GP5 and GP3 proteins and susceptibility to neutralization. Arch. Virol. 145, 1599–1619.

Yoon, I.J., Joo, H.S., Goyal, S.M., Molitor, T.W., 1994. A modified serum neutralization test for the detection of antibody to porcine reproductive and respiratory syndrome virus in swine sera. J. Vet. Diagn. Invest. 6, 289–292.

Yoon, K.J., Wu, L.L., Zimmerman, J.J., Hill, H.T., Platt, K.B., 1996. Antibody-dependent enhancement (ADE) of porcine reproductive and respiratory syndrome virus (PRRSV) infection in pigs. Viral Immunol. 9, 51–63.

Yoon, K.J., Wu, L.L., Zimmerman, J.J., Platt, K.B., 1997. Field isolates of porcine reproductive and respiratory syndrome virus (PRRSV) vary in their susceptibility to antibody dependent enhancement (ADE) of infection. Vet. Microbiol. 55, 277–287.

Zhang, H., Guo, X., Nelson, E., Christopher-Hennings, J., Wang, X., 2012. Porcine reproductive and respiratory syndrome virus activates the transcription of interferon alpha/beta (IFN-alpha/beta) in monocyte-derived dendritic cells (Mo-DC). Vet. Microbiol. 159, 494–498.

Zheng, Q., Chen, D., Li, P., Bi, Z., Cao, R., Zhou, B., Chen, P., 2007. Co-expressing GP5 and M proteins under different promoters in recombinant modified vaccinia virus ankara (rMVA)-based vaccine vector enhanced the humoral and cellular immune responses of porcine reproductive and respiratory syndrome virus (PRRSV). Virus Genes 35, 585–595.

Zhou, J.X., Xue, J.D., Yu, T., Zhang, J.B., Liu, Y., Jiang, N., Li, Y.L., Hu, R.L., 2010. Immune responses in pigs induced by recombinant canine adenovirus 2 expressing the glycoprotein 5 of porcine reproductive and respiratory syndrome virus. Vet. Res. Commun. 34, 371–380.

Zhou, L., Ni, Y.Y., Pineyro, P., Sanford, B.J., Cossaboom, C.M., Dryman, B.A., Huang, Y.W., Cao, D.J., Meng, X.J., 2012. DNA shuffling of the GP3 genes of porcine reproductive and respiratory syndrome virus (PRRSV) produces a chimeric virus with an improved cross-neutralizing ability against a heterologous PRRSV strain. Virology 434, 96–109.

Zuckermann, F.A., 1999. Extrathymic CD4/CD8 double positive T cells. Vet. Immunol. Immunopathol. 72, 55–66.

Zuckermann, F.A., Garcia, E.A., Luque, I.D., Christopher-Hennings, J., Doster, A., Brito, M., Osorio, F., 2007. Assessment of the efficacy of commercial porcine reproductive and respiratory syndrome virus (PRRSV) vaccines based on measurement of serologic response, frequency of gamma-IFN-producing cells and virological parameters of protection upon challenge. Vet. Microbiol. 123, 69–85.

Zuckermann, F.A., Husmann, R.J., 1996. Functional and phenotypic analysis of porcine peripheral blood CD4/CD8 double-positive T cells. Immunology 87, 500–512.