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Novel strategies and approaches to develop the next generation of vaccines against porcine reproductive and respiratory syndrome virus (PRRSV)

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Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically important swine pathogen. Since its discovery in the early 1990s, tremendous progresses have been made in understanding the molecular biology and pathogenesis of PRRSV. Although modified live-attenuated vaccines (MLVs) and inactivated vaccines against PRRSV have been available for more than a decade, the disease remains difficult to control. The efficacies of these vaccines especially against heterologous strains remain questionable: the MLVs were generally effective against homologous strains but variable in success against heterologous strains, and the outcomes of inactivated vaccines in the field are not very promising. With the development of PRRSV reverse genetics systems and the acquisition of new understanding on anti-PRRSV immunity, rational design of the next generation of PRRSV vaccines can now be explored. In this review, we discussed the recent advances in anti-PRRSV immunity and vaccinology, the recent progresses in PRRSV vaccine development particularly the reverse genetics system-based vaccine development, and provided a perspective on potential novel strategies and approaches that may be applicable to the development of the next generation of PRRSV vaccines.

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1. The disease, virus and current vaccines

Porcine reproductive and respiratory syndrome (PRRS) is an economically important disease characterized by reproductive failure in sows and respiratory disease in pigs of all ages (Botner, 1997; Done and Paton, 1995). Pigs suffered from the disease first manifest acute illness including anorexia and lethargy, and sometimes pyrexia, dyspnea, and cyanosis of extremities. Reproductive failure in sows, including stillbirths, mummifications,
weak born piglets and high preweaning mortality, occurs concurrently or soon after the acute illness (Mengeling et al., 1998; Meulenberg, 2000; Rowland, 2007). The causative agent of PRRS, porcine reproductive and respiratory syndrome virus (PRRSV), is an enveloped, single-stranded, positive-sense RNA virus (Collins et al., 1992; Dea et al., 2000; Meulenberg, 2000; Wensvoort et al., 1991).

PRRSV belongs to the family *Arteriviridae* in the order of the *Nidovirales* that include members of the *Coronaviridae* and *Roniviridae* families (Gorbalenya et al., 2006). The family *Arteriviridae* also comprises three other viruses: equine arteritis virus (EAV), simian hemorrhagic fever virus (SHFV), and lactate dehydrogenase elevating virus (LDV). Members of the *Arteriviridae* family have distinct host range for each virus: pigs for PRRSV, horses and donkeys for EAV, monkeys for SHFV, and mice for LDV (Snijder and Meulenberg, 1998). The four viruses all target macrophages of the respective host during infection. Two receptors on porcine alveolar macrophage (PAM), porcine sialoadhesin (pCD169) and pCD163, have been identified for PRRSV entry and uncoating, respectively (Calvert et al., 2007; Van Gorp et al., 2008; Vanderheijden et al., 2003). PRRSV isolates are divided into two distinct genotypes: the European (Type I) and North American (Type II) (Meng et al., 1995). The two genotypes of PRRSV cause the same disease symptoms but are antigenically different (Kapur et al., 1996; Meng et al., 1995; Meng, 2000; Meulenberg, 2000; Nelsen et al., 1999; Ropp et al., 2004; Stadejek et al., 2006).

The PRRSV genome is approximately 15 kb in length and consists of a 5′- untranslated region (UTR), nine open reading frames (ORFs), ORF1a, ORF1b, ORF2a, ORF2b, ORF3 and ORF7, followed by a 3′-UTR and a poly(A) tail (Dea et al., 2000; Meng et al., 1994; Meng, 2000). The ORF1a and ORF1b located in the 5′-proximal part consist of approximately 75% of the genome and encode 13 putative non-structural proteins (nsp), nsp1, nsp1p, nsp1p, and nsp2 to nsp12 that are cleaved by proteolytic processing (Meulenberg et al., 1997; Snijder and Meulenberg, 1998; Ziebuhr et al., 2000). Nsp4 is the main proteinase (3C-like serine proteinase, 3CLpro) mediating most of the nsp processing (Ziebuhr et al., 2000). Cleavages of the nsp3/nsp4, nsp4/nsp5, and nsp11/nsp12 junctions by the nsp4 were confirmed (Tian et al., 2009). Nsp1α, nsp1β, and nsp2 are three accessory cysteine proteinases responsible for autocatalytic processing of a single cleaved site (den Boon et al., 1995; Ziebuhr et al., 2000). Nsp2 contains several immunodominant B-cell epitopes that are dispensable for virus replication but somehow modulate the host immune response (Chen et al., 2010b; Oleksiewicz et al., 2001). The ORF1b-encoded nsp 9–11 comprises the putative domains of viral RNA-dependent RNA polymerase (RdRp), helicase (Bautista et al., 2002) and uridylyl-specific endoribonuclease (NendoU), respectively (Nedialkova et al., 2009; Snijder and Meulenberg, 1998). The functional activities of helicase in nsp10 and NendoU in nsp11 of PRRSV have been experimentally verified (Bautista et al., 2002; Nedialkova et al., 2009). Recently, nsp1α, nsp1β, nsp2, nsp4 and nsp11 were shown to inhibit the activation of interferon beta (IFN-β) promoter with different intensity, indicating that some PRRSV nsp5 are able to subvert host innate immunity by attenuating type I IFN response (Beura et al., 2010; Chen et al., 2010a).

The 3′-proximal part of the genome encodes seven PRRSV structural proteins that are translated from a 3′-coterminal nested set of six subgenomic mRNAs (Meng et al., 1996; Meulenberg et al., 1995). ORF2a and ORF5–3 encode four membrane-associated N-glycosylated proteins (GP2a, GP3, GP4 and GP5) whereas ORF2b and ORF6 encode two non-glycosylated membrane proteins (E and M) (Dea et al., 2000; Meulenberg et al., 1995). The last ORF (ORF7) encodes for a nucleocapsid protein (N) encapsidating the viral RNA genome (Spilman et al., 2009). The GP5 protein is the most abundant enveloped glycoprotein containing major neutralizing epitopes (Ostrowski et al., 2002; Pirzadeh and Dea, 1997; Plagemann, 2004a,b; Plagemann et al., 2002). The GP5 and M proteins form a disulfide-linked heterodimer that serves as a ligand for the PRRSV internalization receptor, pCD169, on alveolar macrophages (Van Brederom et al., 2010). Recently, interactions of the other three minor envelope glycoproteins (GP2a, GP3, GP4) with GP5 were also detected by co-immunoprecipitation assay (Das et al., 2010). GP2a, GP3 and GP4 form a heterotrimeric complex that is required for assembly of infectious PRRSV virions (Wissink et al., 2005), but only GP2a and GP4 were found to interact with pCD163 PRRSV receptor (Das et al., 2010). Overall, the recent findings on PRRSV non-structural and structural proteins provide new insights on PRRSV biology and vaccine development.

Since its recognition in the late 1980s, PRRS has devastated the swine industry worldwide. PRRS outbreaks frequently occur worldwide and cause tremendous economic losses. A recent example is the emergence of a variant strain of PRRSV associated with porcine high fever syndrome (PHFS) in China (Tian et al., 2007), which resulted in significant economic losses in China and Vietnam. In the United States alone, it is estimated that annual losses associated with PRRSV can reach up to half a billion dollars (Neumann et al., 2005). Two types of commercial vaccines are currently available against PRRSV, modified live-attenuated vaccines (MLVs) and inactivated vaccines (Kimman et al., 2009). MLVs against PRRSV became available in 1994 and were generally effective against homologous strains but variable in success (less effective or sometimes ineffective) against heterologous strains. The current MLVs have been associated with numerous problems such as shedding of vaccine virus, persistent infection, incomplete protection, and reversion to virulence. For example, acute PRRS outbreak occurred in Danish herds vaccinated with an MLV, and the outbreak was linked to reversion of the vaccine virus to a pathogenic phenotype (Botner et al., 1997; Storgaard et al., 1999). Vaccine-derived PRRSV strains were isolated from non-symptomatic persistently-infected pigs (Key et al., 2001, 2003; Zimmerman et al., 2006), and an MLV vaccine-derived isolate was found to cause disease in experimentally-infected pigs (Opiessnig et al., 2002). MLV derived from a single PRRSV vaccine strain do not fully protect pigs against heterologous PRRSV infection (Kimman et al., 2009; Larbarque et al., 2004). Commercial inactivated vaccines, except for farm-specific autogenous products, are not available in the United States. The outcomes of the use of inactivated vaccines in other countries are not promising. Given the degree of genetic diversity observed among PRRSV strains, it is unlikely that a vaccine based on a single strain will confer effective protection against the antigenically and genetically diversified field strains currently circulating in swine herds worldwide. The effectiveness of a vaccine against heterologous strains will largely depend on the antigenic and genetic relatedness of the virus strain to which the vaccinated animals were exposed. The observed genetic diversity among field isolates will continue to be the major obstacle for PRRS control. Therefore, the design of future vaccines must take the antigenic and genetic diversity of PRRSV into consideration or PRRS will remain difficult to control.

In this article, we will first review the recent advances in anti-PRRSV immunity and vaccinology. In depth reviews of PRRSV biology and pathogenesis can be found elsewhere (Kimman et al., 2009; Lopez and Osorio, 2004; Mateu and Diaz, 2008; Murtaugh et al., 2002). We will then discuss the recent progresses in PRRSV vaccine development, particularly the use of reverse genetics system for PRRSV vaccine development. Finally, we will provide a perspective on potential novel strategies and approaches that may be applicable to the development of the next generation of PRRSV vaccines.
2. Key issues relevant to anti-PRRSV immunity

2.1. Correlation of clearance of PRRSV infection and the level of host immunity

PRRSV-infected pigs survived from the acute stage of infection that last up to 1 month usually develop persistent infection with low level of viral loads. The time of complete PRRSV clearance was estimated to be at least 150 days that is beyond the market lifetime of a pig (Allende et al., 2000). Although the mechanisms underlying the failure to promptly clear PRRSV infection are poorly understood, it appears that a major reason is the inability of pigs to develop effective protective immune responses (Lunney et al., 2010; Mateu and Diaz, 2008).

It has been demonstrated that PRRSV neutralizing antibodies play a critical role in clearance of virus and are able to completely protect pigs against PRRSV re-infection (Lopez and Osorio, 2004; Murtough et al., 2002; Osorio et al., 2002). However, neutralizing antibodies against the major structural component, GP5, appear later than non-neutralizing antibodies induced by other structural proteins and ns2 after PRRSV infection. As a consequence, infected pigs do not efficiently resolve virus infection at the early phase (Lopez and Osorio, 2004). There are three possible explanations for the delayed appearance of neutralizing antibodies. First, GP5 contains an immunodominant epitope (epitope A or “decoy” epitope) in its ectodomain inducing an early and strong non-neutralizing immune response that diminish or mask the immune responses elicited by a downstream nearby neutralizing epitope (epitope B) (Osorio et al., 2002; Ostrowski et al., 2002; Yang et al., 2000). Secondly, there exist high manose glycans at the N-linked glycosylation sites flanking epitope B on GP5 that diminish the sensitivity of PRRSV virions to neutralizing antibodies and thus impair the immunogenicity of the nearby epitope B (Ansari et al., 2006; Faaberg et al., 2006; Plagemann et al., 2002). Thirdly, PRRSV employs various strategies to inhibit the initial innate immune responses, in particular, the production of type I IFN, which subsequently dampens the host adaptive immune responses including the humoral immune response (Mateu and Diaz, 2008; Murtough et al., 2002; Royae et al., 2004). Therefore, an ideal PRRSV vaccine should be able to induce more rapid and robust responses of neutralizing antibodies without suppressing the anti-PRRSV innate immunity.

2.2. The roles of dendritic cells and regulatory T cells in PRRSV infection

Dendritic cells (DCs) are professional antigen-presenting cells (APC) located throughout the peripheral immune system and play a critical role in linking innate detection and capture of virus to induction of adaptive immune responses (Palucka and Banchereau, 1999). Invading foreign pathogens trigger the migration of immature DCs from the blood into tissues where they detect and capture the pathogens. Activated DCs uptake pathogen antigens, process into immunogenic peptides through MHC molecules and further present to T cells (Banchereau and Steinman, 1998). Many viruses that cause persistent infection, such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV), exploit or eliminate DC function to evade innate and adaptive immunity (Liu et al., 2009). For examples, DCs serve as the “Trojan horses” that efficiently sequester HIV and then transfer HIV to target CD4+ T cells, thus facilitating persistent virus transmission (Wu and KewalRamani, 2006). This process is mediated by the interaction of HIV gp120 protein with a C-type lectin, DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin), on DCs (van Kooyk and Geijtenbeek, 2003).

Whether PRRSV targets porcine DCs to exercise its immune evasion strategies may have been understood. Current researches on PRRSV and DCs interactions mainly focused on whether the production of interferons and specific inflammatory cytokines in DCs that may activate and prime differentiation of distinct types of T cells are down- or up-regulated in response to PRRSV exposure. Plasmacytoid DCs (pDCs) are considered as key effector DCs that express high level of type I IFN in the early antiviral innate immunity (Gilliet et al., 2008). Most recently, using novel fluorescent-activated cell sorting procedures, porcine pDCs characterized by CD4<sup>high</sup>CD172<sup>low</sup> phenotype have been isolated from porcine peripheral blood mononuclear cells (PBMC) (Caldaza-Nova et al., 2009). It was shown that porcine pDCs exposed to PRRSV in vitro did not secret IFN-α, IL-6, IL-8, IL-12, IFN-γ, or TNF-α (Caldaza-Nova et al., 2009). PRRSV also appeared to be capable of infecting porcine myeloid DCs (mDCs) including bone marrow-derived DCs (BMDCs) and monocyte-derived DCs (MDDCs) in vitro and altering the expression level of various cytokines and CD markers, which has been speculated to contribute to the suppression of anti-PRRSV protective immune responses (Chang et al., 2008; Charrentantankul et al., 2006; Flores-Mendoza et al., 2008; Loving et al., 2007; Wang et al., 2007). In addition, the porcine DC-SIGN (pDC-SIGN) gene was recently cloned from in vitro cultured MDDCs and found to enhance the in vitro transmission of PRRSV to target cells (Huang et al., 2009a). It will be interesting to see whether pDC-SIGN functions as the attachment receptor on specific subsets of porcine DCs to disseminate PRRSV in the course of infection in vivo. To aid in designing novel strategies and vaccines that can efficiently modulate the pig immune system against PRRSV infection, future studies are warranted to identify and dissect the distinct signaling pathways and molecules as well as relevant viral components involved in the interplay between DCs and PRRSV, and to investigate the role of DCs in facilitating PRRSV transmission.

Regulatory T cells (Tregs) characterized by CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> phenotype are also crucial players in balancing innate and adaptive immune responses (Sakaguchi et al., 2009). Adaptive (inducible) Tregs that are induced by DCs-presenting microbial antigens secret high amounts of IL-10 or TGF-β to suppress excessive inflammatory responses and thus reduce damage to host tissues (Sakaguchi et al., 2009). However, many pathogens including HIV, HBV and HCV also exploit inducible Tregs to subvert protective immune responses, allowing the establishment of persistent infection (Belkaid, 2007). Recently, two studies independently showed that porcine inducible Tregs were indeed induced when co-cultured with porcine MDDCs exposed to type II PRRSV in vitro, as indicated by increased numbers of CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> cells and enhanced expression of Foxp3<sup>+</sup> mRNA (Silva-Campa et al., 2009; Wongyanin et al., 2010). These inducible Tregs displayed a suppressive activity on PHA-stimulated PBMC. Moreover, the expression of both mRNA and protein of TGF-β, but not IL-10, was up-regulated in co-cultured cells infected with PRRSV, indicating that the PRRSV-induced Tregs belong to T helper 3 (Th3) subset (Silva-Campa et al., 2009). Interestingly, using the same experimental protocols, no CD25<sup>+</sup>Foxp3<sup>+</sup> T cells and TGF-β were induced by type I PRRSV-infected MDDCs, suggesting that PRRSV-induced Th3 response may be type- or strain-dependent, at least in vitro (Silva-Campa et al., 2010). These findings may provide the basis for a potential strategy that improves PRRSV vaccine efficacy by inhibiting PRRSV-induced Tregs activity. However, whether PRRSV infection primes Tregs activation, leading to immunosuppression of pigs in vivo, remains to be determined in future studies.

2.3. Other major obstacles for the development of a more effective PRRSV vaccine

PRRSV utilized different means to subvert the host immune responses during the later stage of infection. Due to the low fidelity of viral RdRp, mutations are introduced into the PRRSV genome, resulting in the existence of antigenic heterogeneity on epitopes

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that are recognized by neutralizing antibodies and T cells. This leads to the escape of host immune surveillance and makes it difficult for the host to generate protective immune responses to eliminate the virus, especially heterologous strains (Meng, 2000). In addition, PRRSV-infected PAM may sequester the virus so that there are no viral proteins expressed on the plasma membrane, which makes the viral information become invisible to the host immune system. As a consequence, the infected cells are refractory to antibody-dependent and complement-mediated lysis (Costers et al., 2006). It is reasonable to hypothesize that, as discussed above, other host non- or semi-permissive cells such as pDCs and mDCs likely have a similar property to help PRRSV remain in a persistent state.

One of the unusual characteristics of PRRSV-inducing immunity is that PRRSV-specific antibodies of maternal origin, or conferred by vaccination, can facilitate the entry of virus into target cells, leading to increased infectivity (Yoon et al., 1996, 1997). This phenomenon, known as antibody-dependent enhancement (ADE) of viral infection, is also considered as an obstacle to PRRSV vaccine development, although the underlying mechanism remains elusive (Christianson et al., 1993; Shibata et al., 1998; Tirado and Yoon, 2003). Recent studies of ADE mechanisms from other RNA viruses have suggested that, in addition to enhancing viral uptake, ADE may suppress the innate antiviral response, especially type I IFN system of the host (Mahalingam and Lidbury, 2002). Moreover, ADE enables the viruses to benefit from the anti-inflammatory and immunosuppressive environment created by autocrine and paracrine IL-10 production (Berlato et al., 2002; Ito et al., 1999; Mahalingam and Lidbury, 2002). Since production of IL-10 were significantly increased in bronchoalveolar lavage fluids of PRRSV-infected pigs (Thanawongnuwech et al., 2004), it is speculated that, in the course of ADE of PRRSV infection, stronger up-regulation of IL-10 would occur in PAM, which may contribute to suppressing the innate antiviral response, especially type I IFN system of the host (Mahalingam and Lidbury, 2002). The RNA-launched system has been reported for recovering both genotypes of PRRSV of different geographic origins, and has been widely used for functional studies of viral genes (Fang et al., 2006; Li et al., 2008; Truong et al., 2004; Yoo et al., 2004; Zhou et al., 2009).

3. Recent advances in reverse genetics system-based PRRSV vaccine development

3.1. PRRSV subunit vaccines and reverse genetics system-based MLVs

Experimental subunit vaccine systems based upon plasmid DNA, bacteria, baculovirus, adenovirus, fowlpox virus and pseudorabies virus, carrying several PRRSV antigens including GP5, M, and/or GP3, have been developed and evaluated against PRRSV (Jiang et al., 2004, 2006, 2007, 2008; Shen et al., 2007). In some of these cases, animals immunized with GP5 together with M or GP3 antigen developed higher titers of neutralizing antibodies to PRRSV, and produced stronger lymphocyte proliferation responses compared to animals immunized with GP5, M or GP3 alone. It remains to be seen whether such subunit vaccines will be better than the existing MLVs and killed products.

Reverse genetics system is defined as the generation of viruses possessing a genome derived from cloned cDNAs (infectious clone) (Boyer and Haenni, 1994). It not only provides a powerful tool for dissecting the role of viral proteins in virus life cycle and pathogenicity, but also opens the door to develop live-attenuated virus vaccines by introducing attenuating mutations on viral genome. Both efficient humoral and cellular immunities against the engineered virus are induced, which increases the likelihood of protection from viral disease (Neumann et al., 2002). Such vaccines can be designed to be more stable and safer than conventional live-attenuated viruses generated by continuous blind cell passages.

A major limitation for developing effective subunit vaccines and reverse genetics-based MLVs is the heterogenic nature of the PRRSV strains worldwide. The current experimental subunit vaccines and reverse genetics-based MLVs are mostly based upon a single strain of PRRSV. It remains doubtful that such a vaccine will provide sufficient level of heterologous protection in the field.

3.2. PRRSV reverse genetics systems

The first reverse genetics system for both European genotype (Lelystad virus) and the North American genotype (strain VR2332) of PRRSV was RNA-launched, using a low-copy-number DNA plasmid as the cloning vector and template for generation of in vitro RNA transcripts that are directly transfected into susceptible cells to recover the virus (Meulenberg et al., 1998; Nielsen et al., 2003). T7 or SP6 promoter was inserted immediately upstream the full-length viral genomic cDNA to drive the in vitro transcription reaction (Choi et al., 2006; Nielsen et al., 2003). The RNA-launched system has been reported for recovering both genotypes of PRRSV of different geographic origins, and has been widely used for functional studies of viral genes (Fang et al., 2006; Li et al., 2008; Truong et al., 2004; Yoo et al., 2004; Zhou et al., 2009).

A DNA-launched reverse genetics system was reported for recovering a type II PRRSV strain P129 by direct transfection of cells with the full-length viral genomic cDNA clone under the cytomegalovirus (CMV) promoter recognized by the cellular RNA polymerases II (Lee et al., 2005). The DNA-launched system could be improved by introduction of self-cleaving ribozyme elements at both termini of the viral genomic cDNA, which was expected to retain the authentic terminal nucleotide sequences of the viral genome (Huang et al., 2009b). The rescue efficacy of a type I PRRSV (strain SD01-08) expressing GFP with this system was approximately 10- to 50-fold higher than the RNA-launched system and the traditional DNA-launched system without the engineered ribozyme elements, as determined by reporter GFP level in transfected cells and the peak titer of the recovery virus (Huang et al., 2009b). This improved reverse genetics system has also been applied to rescuing a type II PRRSV strain VR2385 with higher efficacy (Ni et al., unpublished data). With the availability of the improved DNA-launched system, future production of the “seed virus stock” for reverse genetics-based MLV will have a reduced cost and time by bypassing the need of an RNA in-vitro-transcription step.

3.3. Chimeric infectious cDNA clones for attenuation of PRRSV

Understanding the molecular basis of PRRSV virulence is critical for the generation of attenuated PRRSV vaccines. A series of PRRSV infectious cDNA clones were constructed where specific genomic regions of an attenuated type II PRRSV vaccine strain, Prime Pac (PP18) (Kwon et al., 2006), were systematically swapped with their counterparts in the backbone of a highly virulent infectious cDNA clone of PRRSV, FL12 (Kwon et al., 2008; Truong et al., 2004). Transfection of the respective chimeric constructs into cells resulted in rescue of viable viruses. Pregnant sows were inoculated with the chimeric viruses, along with PP18 and FL12, at 90 days of gestation and the viability of their offspring at birth and weaning was evaluated. The results showed that the virulence determinants possibly locate in all nsps (except nsp9) and structural ORFs, confirming the multigenic character of PRRSV virulence as deduced from sequence comparisons. In particular, nsp3–8 and ORF5 appeared to comprise major virulence determinants, whereas other virulence determinants may be resided in nsp1–3, nsp10–12 and ORF2 (Kwon et al., 2008). In addition, when chimeric viruses were used to infect PAM in vitro to assess the genetic determinants affecting the host
immune responses, nsp1–8 in ORF1a was identified to play a regulatory role (Gudmundsdottir and Risatti, 2009).

A similar study was also conducted using chimeric PRRSV infectious cDNA clones derived from two distinct type II PRRSV strains, the attenuated Ingelvac PRRS MLV and the virulent strain MN184 (Wang et al., 2008). Two reciprocal chimeric cDNA clones with the region of 5′-UTR/ORF1 from one genome fused to ORF2–7′-UTR from the other genome displayed similar antibody responses to the two parent viruses, but had much less severe pathogenicity when compared to the MN184 group, indicating that the virulent determinants were presented throughout the viral genome (Wang et al., 2008). The two reciprocal chimeric viruses as well as a chimera with the ORF5–6 (encoding GP5 and M) of strain MN184 cloned in the backbone of Ingelvac PRRS MLV were able to protect pigs against challenge with two heterologous strains, SDSU73 and JA-142, respectively (Ellingson et al., 2010; Wang et al., 2008). The results suggest that MLVs based on the backbone of a traditionally cell culture-attenuated vaccine strain can be developed in the future using chimeric infectious cDNA clones. Moreover, it appears that a simple exchange of the ORF5–6 region from a field strain could provide an improved vaccine efficacy for heterologous protection. It will be interesting to see whether chimeric constructs between type I and type II PRRSV can rescue the recombinant viruses.

3.4. Development of PRRSV gene-deletion marker vaccines

A disadvantage of current PRRSV MLVs is that pigs vaccinated with MLVs cannot be distinguished from those naturally-infected with field strains of PRRSV by serological assays. Generation of a marker or DIVA (differentiating infected from vaccinated animals) PRRSV vaccine based on a deletion marker (i.e., an immunogenic marker absent from the vaccine strain but present in field strains) on the viral genome by reverse genetics system will allow for differentiation and be of great value for the control and eventual elimination of PRRSV. The candidate gene used for generating a deletion marker is nsp2 since it appears to tolerate large deletions and is not genetically stable. Nevertheless, the results suggested that the type I or type II PRRSV strains of type I PRRSV, as measured by the ES4 epitope-specific ELISA (Fang et al., 2008), suggesting that the ES4 epitope-deleted mutant could be useful as a PRRSV marker vaccine to distinguish type I PRRSV strains. However, since the ES4 epitope is not conserved between type I and type II PRRSV, it did not react with serum samples of pigs infected with four representative field strains of type I PRRSV, as measured by the ES4 epitope-specific ELISA (Fang et al., 2008). A disadvantage of current PRRSV MLVs is that pigs vaccinated with MLVs cannot be distinguished from those naturally-infected with field strains of PRRSV by serological assays. Generation of a marker or DIVA (differentiating infected from vaccinated animals) PRRSV vaccine based on a deletion marker (i.e., an immunogenic marker absent from the vaccine strain but present in field strains) on the viral genome by reverse genetics system will allow for differentiation and be of great value for the control and eventual elimination of PRRSV. The candidate gene used for generating a deletion marker is nsp2 since it appears to tolerate large deletions and is not genetically stable. Nevertheless, the results suggested that the type I or type II PRRSV strains of type I PRRSV, as measured by the ES4 epitope-specific ELISA (Fang et al., 2008), suggesting that the ES4 epitope-deleted mutant could be useful as a PRRSV marker vaccine to distinguish type I PRRSV strains. However, since the ES4 epitope is not conserved between type I and type II PRRSV, it did not react with serum samples of pigs infected with four representative field strains of type I PRRSV, as measured by the ES4 epitope-specific ELISA (Fang et al., 2008), indicating that the introduced marker in the nsp2 region is not genetically stable. Nevertheless, the results suggested that it is possible to develop a double-marker MLV containing both the positive (GFP) and negative (deleted ES4 epitope) markers, allowing serological differentiation between vaccinated pigs and those infected with field strains alone or those co-infected with MLV and field strains (Fang et al., 2008).

Amino acid sequence analysis of the ES4 epitope region revealed that it contains seven small B-cell epitopes, two of which are highly conserved among all the type I PRRSV strains with known nsp2 sequence. Accordingly, the anti-ES4 antibodies could be detected in serum samples of pigs infected with four representative field strains of type I PRRSV, as measured by the ES4 epitope-specific ELISA (Fang et al., 2008), suggesting that the ES4 epitope-deleted mutant could be useful as a PRRSV marker vaccine to distinguish type I PRRSV strains. However, since the ES4 epitope is not conserved between type I and type II PRRSV, it did not react with serum samples of pigs infected with four representative field strains of type I PRRSV, as measured by the ES4 epitope-specific ELISA (Fang et al., 2008), indicating that the introduced marker in the nsp2 region is not genetically stable. Nevertheless, the results suggested that it is possible to develop a double-marker MLV containing both the positive (GFP) and negative (deleted ES4 epitope) markers, allowing serological differentiation between vaccinated pigs and those infected with field strains alone or those co-infected with MLV and field strains (Fang et al., 2008).

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4. Potential novel strategies and approaches for developing the next generation of PRRSV vaccines

4.1. Vaccines targeting porcine dendritic cells

The central role of DCs in host immunity indicates that efficacious vaccinations rely on how efficient the vaccine interacts with DCs. DCs are now being considered as the target in the design of novel vaccines by enhancing the immunogenicity of vaccines (McCullough and Summerfield, 2009; Steinman and Banchereau, 2007). Development of vaccine delivery systems capable of targeting the vaccine antigen and the agonist to DCs are recognized as the research priority (“Advances in immunology and vaccine discovery”: Report of the United States–European Union Commission Workshop; http://www.theavii.org/EU-US_Report.doc). DC-targeting immunization approaches have been validated in both primate and murine models (Steinman and Banchereau, 2007). For examples, Tacken et al. demonstrated that antibody-mediated targeting of antigen to human DCs via DC-SIGN effectively induces antigen-specific naive and memory T-cell responses. The antigen KLH cross-linked to a humanized DC-SIGN antibody (hD1) specifically bound to DC-SIGN and was rapidly internalized and translocated to the lysosomal compartment. The targeting efficiency of hD1–KLH to DCs is 100-fold higher than that of KLH alone (Tacken et al., 2005).

Recently, Revilla et al. demonstrated that this new avenue could be applied to pigs by using pCD169 (sialoadhesin) as the antigen-delivery target on porcine MDDCs (Revilla et al., 2009). Using T cells from pigs immunized with antigen (mouse Ig) as responder cells, and MDDCs with or without the expression of pCD169, they com-
pared the T cell proliferative response induced by an anti-pCD169 Mab with that of a non-targeting control Mab. The anti-pCD169 Mab bound to pCD169 is internalized rapidly and induced recall T cell proliferative responses at concentrations 100-fold lower than the control Mab in vitro, indicating that targeting antigen to pCD169 is an effective strategy to increase the immunogenicity of antigen (Revilla et al., 2009). Since pCD169 has been identified as the entry receptor for PRRSV through interaction with GP5-M heterodimers (Van Breedam et al., 2010), it will be very interesting to see whether targeting the PRRSV antigens directly to pCD169 will increase the vaccine efficacy. Other potential DC receptors used for targeting PRRSV antigens may include several porcine Toll-like receptors (TLRs), such as TLR3 and TLR7 that were involved in PRRSV infection (Chuang et al., 2010; Sang et al., 2008), and C-type lectin receptors such as pDC-SIGN (Huang et al., 2009a), pLangerin (Nfon et al., 2008) and pCLEC4G (Huang and Meng, 2009).

4.2. Vaccines suppressing regulatory T cell induction

As mentioned above, inducible CD25+Foxp3+ Tregs appeared to be involved in inhibiting T–cell activity during PRRSV persistent infection and thus may be an obstacle for vaccination with PRRSV MLV. If this phenomenon is confirmed in vivo, suppressing Tregs induction to enhance vaccine efficacy will be important for developing the next generation of PRRSV vaccines based on attenuated virus. In human and mouse models, many Tregs–depleting strategies have been investigated. For example, it has been reported that depletion of natural Tregs using anti–CD25 Mab before vaccination with a vaccine against herpes simplex virus type 1 enhanced CD8+ T cell response to the virus (Toka et al., 2004). Administration of anti–IL-10R antibody before immunization with a murine leishmaniasis vaccine can improve vaccine efficacy (Stober et al., 2005). However, these approaches may be cost–prohibitive for porcine vaccines. Future studies are needed to identify the viral components associated with Tregs induction, and then design recombinant MLVs by reverse genetics approaches that can abolish the potential risks associated with Tregs induction.

4.3. Vaccines inducing type I IFN response

MLVs can be generated by deletion of essential factors associated with pathogenicity using reverse genetics techniques. In some coronaviruses, the deletion of nsp genes has been shown to confer attenuation in their natural hosts (de Haan et al., 2002; Haijema et al., 2004; Ortego et al., 2003). The major role of nsp1 in mouse hepatitis virus (MHV) and SARS–coronavirus (SARS-CoV) is to counter the antiviral type I IFN response by inhibiting IFN–dependent signaling or the downstream events (Watthelet et al., 2007; Zust et al., 2007). A mutant MHV containing a deletion in nsp1 gene was shown to grow normally in vitro, but was strongly attenuated in different animal models due to induction of a strong type I interferon response that limits their replication (Rich and Garcia-Sastre, 2009).

As described previously, several PRRSV nsps (nsp1a, nsp1β, nsp2, nsp4 and nsp11) are involved in suppressing antiviral type I IFN response (Beura et al., 2010). Among them, nsp1β appeared to have a strong inhibitory activity (Beura et al., 2010; Chen et al., 2010a). However, Beura et al. suggested that, since nsp1β is a multifunctional viral protein, it may not be feasible to structurally separate proteinase function from its IFN antagonistic function, and therefore an nsp1β mutant virus lacking anti–IFN activity may not be rescued (Beura et al., 2010). Therefore, selection of other nsps as deletion targets may be more appropriate for searching a suitable MLV candidate. Nevertheless, genetic manipulations of viruses to restore type I IFN response may provide a novel strategy for the development of the next generation of PRRSV vaccines.

Acknowledgements

The authors’ work on PRRSV is supported in part by a grant from USDA PRRS–CAP NIFA 2008–55620–19132 and from Fort Dodge Animal Health Inc (now Pfizer Animal Health Inc). Due to limited scope of this review, we apologize to those whose important PRRSV publications are not cited here.

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