Porcine Reproductive and Respiratory Syndrome Virus (PRRSV): Pathogenesis and Interaction with the Immune System

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Abstract

This review addresses important issues of porcine reproductive and respiratory syndrome virus (PRRSV) infection, immunity, pathogenesis, and control. Worldwide, PRRS is the most economically important infectious disease of pigs. We highlight the latest information on viral genome structure, pathogenic mechanisms, and host immunity, with a special focus on immune factors that modulate PRRSV infections during the acute and chronic/persistent disease phases. We address genetic control of host resistance and probe effects of PRRSV infection on reproductive traits. A major goal is to identify cellular/viral targets and pathways for designing more effective vaccines and therapeutics. Based on progress in viral reverse genetics, host transcriptomics and genomics, and vaccinology and adjuvant technologies, we have identified new areas for PRRS control and prevention. Finally, we highlight the gaps in our knowledge base and the need for advanced molecular and immune tools to stimulate PRRS research and field applications.

PRRS VIRION AND GENOME STRUCTURE

Porcine reproductive and respiratory syndrome (PRRS) has been one of the most economically significant swine diseases worldwide for over two decades. It has been estimated to cost the US swine industry at least $600 million annually (1). The etiologic agent, porcine reproductive and respiratory syndrome virus (PRRSV), is an enveloped, positive-stranded RNA virus, which belongs to the order Nidovirales, family Arteriviridae, including equine arteritis virus, mouse lactate dehydrogenase-elevating virus, and simian hemorrhagic fever virus (reviewed in 2). The PRRS virion appears to be a roughly spherical or oval-shaped particle of 50–60 nm in diameter with a relatively smooth external appearance (**Figure 1*b***) (reviewed in 3). The viral genome RNA is packed by nucleocapsid proteins. Surrounding the nucleocapsid, surface glycoproteins (GPs) and membrane proteins are inserted into the lipid-bilayered envelope to form the virion particles (**Figure 1*a***). The PRRSV genome RNA is a positive-stranded, 3′-polyadenylated molecule approximately 15 kb in length, which contains 11 known open reading frames (ORFs). The replicase gene consists of the large ORFs 1a and 1b, which are situated in the 5′-proximal three quarters of the polycistronic genome (**Figure 2**). In contrast to the more conserved ORF1b region, the size of ORF1a is quite variable owing to the hypervariability in the central region of nonstructural protein 2 (nsp2) (reviewed in 4). The ORF1a and ORF1b regions encode two large nonstructural polyproteins, pp1a and pp1ab, with expression of the latter depending on a −1 ribosomal frame shift signal in the ORF1a/ORF1b overlap region. Following their synthesis from the genomic mRNA template, the pp1a and pp1ab replicase polyproteins are processed into at least 14 nonstructural proteins (nsps) by 4 ORF1a-encoded proteinases residing in nsp1α, nsp1β, nsp2, and nsp4 (**Table 1**). Recently, a new ORF (TF) and −1/−2 programmed ribosomal frameshift signal were discovered in the central region of ORF1a, which expresses two novel proteins, nsp2TF and nsp2N (5, 6). The 3′-end of the viral genome contains eight relatively small genes, and these genes have both 5′- and 3′-terminal sequences overlapping with neighboring genes (**Figure 2**), with the

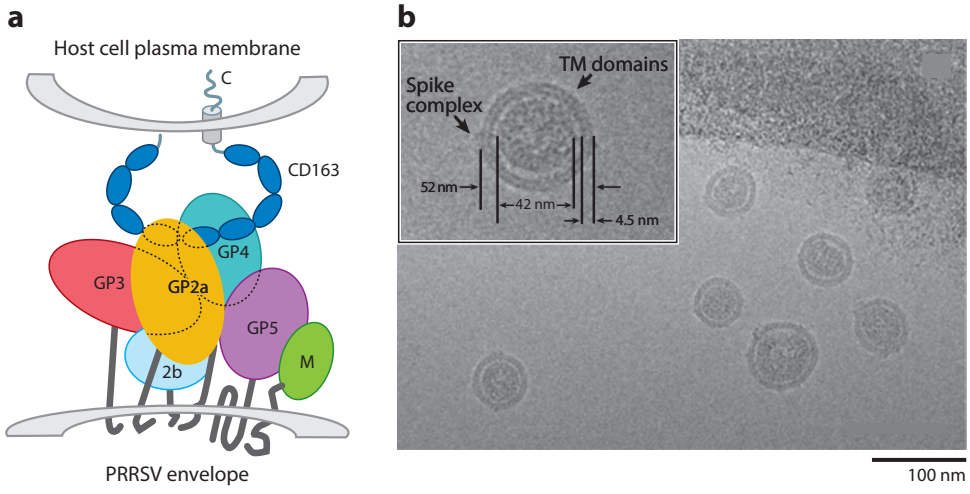


Figure 1

Porcine reproductive and respiratory syndrome virus (PRRSV) structure. (*a*) A model of the PRRSV envelope protein complex and its interaction with CD163 on the host cell plasma membrane (based on Reference 15). (*b*) Cryoelectron microscope image of PRRSV virion; inset shows a typical particle with pertinent dimensions indicated (based on Reference 3).

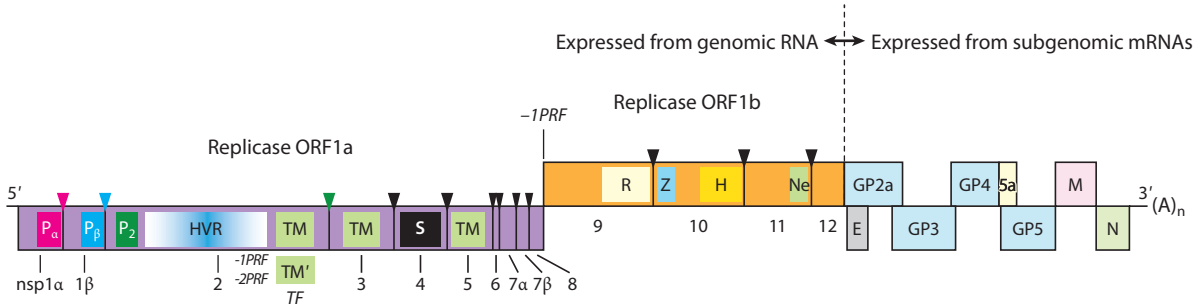


Figure 2

Porcine reproductive and respiratory syndrome virus (PRRSV) genome organization. The replicase-associated polyproteins pp1a and pp1ab are translated from viral genomic RNA and further processed into 14 nonstructural proteins (nsps). A short transframe (TF) open reading frame (ORF) underlying the nsp2-coding region is expressed via −2 programmed ribosomal frameshifting (PRF) to yield nsp2TF (5), whereas the −1 PRF at the same shift site yields a truncated nsp2 variant, nsp2N (6). ORF1b is expressed via −1 PRF to extend pp1a into pp1ab. Papain-like cysteine protease (PLP)1α (Pα), PLP1β (Pβ), and PLP2 (P2) are three proteinases residing in nsp1α, nsp1β, and nsp2, whereas the main serine proteinase (S) is embedded in nsp4. The cleavage sites for Pα (*pink triangle*), Pβ (*light blue triangle*), P2 (*green triangle*), and S (*black triangles*) are shown. A hypervariable region (HVR) resides in nsp2. ORF1a encodes four transmembrane (TM) domains. ORF1b encodes four highly conserved domains: RNA-dependent RNA polymerase (R), multinuclear zinc-binding domain (Z), RNA helicase (H), and NendoU endoribonuclease domain (Ne). ORFs 2–7 are expressed from six subgenomic mRNAs encoding eight structural proteins, including minor envelope proteins (GP2a, GP3, GP4, E, and ORF5a), major envelope proteins (GP5 and M), and the nucleocapsid protein (N).

exception of ORF4/ORF5 of type 2 PRRSV. These genes encode four membrane-associated glycoproteins (GP2a, GP3, GP4, and GP5), three unglycosylated membrane proteins (E, ORF5a, and M), and a nucleocapsid protein (N) (**Table 1**) (reviewed in 2).

PRRS VIRAL INFECTIVITY

Swine are the only known natural host of PRRSV; moreover, the virus has a very restricted tropism for cells of the monocytic lineage. The fully differentiated porcine alveolar macrophage serves as a primary cell target for PRRSV infection (7). In addition, dendritic cells were reported to be able to support PRRSV replication (8). Among many different cell lines tested, only the African green monkey kidney cell line MA-104, and derivatives such as MARC-145, are fully permissive to PRRSV replication in vitro (9). PRRSV enters host cells through standard clathrin-mediated endocytosis (**Figure 3**). The viral genome is released into the cytosol following endosome acidification and membrane fusion (10). The PRRSV receptor-mediated viral entry has been studied extensively (reviewed in 11). To date, CD163 has been determined to be the major receptor that mediates viral internalization and disassembly. It was reported that sialoadhesin (CD169) may serve as the receptor to mediate viral internalization via interaction with the ectodomains of GP5/M heterodimer (12). However, a recent study using the CD169 gene knockout pig demonstrated that an intact sialoadhesin (CD169) is not required for attachment and/or internalization of the PRRSV (13). By screening a swine macrophage cDNA library for receptor function, CD163, a member of the scavenger receptor cysteine-rich family, was identified as a key factor in the initiation of PRRSV infection (14). Overexpression of CD163 renders a variety of nonpermissive cell lines susceptible to PRRSV infection. Two minor structural proteins, GP2a and GP4, were determined as viral attachment proteins that mediate virus entry into susceptible host cells by interacting with CD163 (**Figure 1**) (15). In addition, equine arteritis virus minor structural proteins (GP2a, GP3, GP4, and E) swapped into a chimeric PRRSV extended the cell tropism of

Table 1 Characteristics and functions of porcine reproductive and respiratory syndrome virus (PRRSV) proteins

Gene	Protein	Protein length		Known or predicted properties/functions
		Type I[a]	Type II[b]	
ORF1a	nsp1α	180	180	Contains protease PLPα[c]; zinc-finger protein; regulator of sg mRNA synthesis; potential interferon (IFN) antagonist
	nsp1β	205	203	Contains protease PLPβ; potential IFN antagonist
	nsp2	1078	1196	Contains protease PLP2; deubiquitinating enzyme; potential IFN antagonist; transmembrane protein involved in membrane modification forming replication complex
ORF1a′-TF	nsp2TF[c]	902	1019	Contains PLP2 domain
	nsp2N*	733	850	Contains PLP2 domain
ORF1a	nsp3	230	230	Transmembrane (TM) domain protein involved in membrane modification; forming replication complex
	nsp4	203	204	Main protease SP; apoptosis inducer; potential IFN antagonist
	nsp5	170	170	TM protein possibly involved in membrane modification
	nsp6	16	16	?
	nsp7α	149	149	Recombinant nsp7 is highly antigenic
	nsp7β	120	110	
	nsp8	45	45	N-terminal domain of nsp9
ORF1b*	nsp9	685	685	RNA-dependent RNA polymerase
	nsp10	442	441	RNA NTPase/helicase; contains putative zinc-binding domain
	nsp11	224	223	Uridylate-specific endoribonuclease (NendoU)
	nsp12	152	153	?
ORF2a	GP2a	249	256	Minor glycosylated structural protein; essential for virus infectivity; incorporated into virion as a multimeric complex with GP3-4; viral attachment protein
ORF2b	E	70	73	Minor unglycosylated and myristoylated structural protein; essential for virus infectivity; incorporated into virion as a multimeric complex; possesses ion-channel-like properties and may function as a viroporin in the envelope
ORF3	GP3	265	254	Minor glycosylated structural protein; essential for virus infectivity; highly antigenic and may be involved in viral neutralization; incorporated into virions as a multimeric complex with GP2a and GP4; a subset of GP3 could be secreted as a non-virion-associated soluble protein
ORF4	GP4	183	178	Minor glycosylated structural protein; essential for virus infectivity; formation of GP2a-3-4 complex to incorporate into virion; viral attachment protein and may involve in viral neutralization
ORF5	GP5	201	200	Major glycosylated structural protein; TM protein with a variable number of potential N-glycosylation sites; the most variable structural protein; formation of a disulfide-linked heterodimer with M protein
ORF5a	ORF5a	43	51	Minor unglycosylated, hydrophobic structural protein; essential for virus viability; incorporated into virion as a multimeric complex

(*Continued*)

Table 1 (*Continued*)

Gene	Protein	Protein length		Known or predicted properties/functions
		Type I[a]	Type II[b]	
ORF6	M	173	174	Major unglycosylated structural protein; highly conserved; GP5-M heterodimerization is crucial for virus infectivity; plays a key role in virus assembly and budding
ORF7	N	128	123	Unglycosylated and phosphorylated structural protein; component of the viral capsid; highly antigenic; potential IFN antagonist

[a]Protein sizes refer to the sequence of type 1 PRRSV strain Lelystad (GenBank accession # M96262).
[b]Protein sizes refer to type 2 PRRSV strain VR-2332 (GenBank accession # AY150564).
[c]PLP, papain-like cysteine protease; SP, serine protease.
[d]Nsp2TF is expressed through an alternative transframe (TF) open reading frame (ORF) underlying the nsp2-coding region by −2 ribosomal frameshifting (5), whereas the −1 ribosomal frameshift at the same position yields a truncated nsp2 variant, nsp2N (6). ORF1b is expressed by −1 ribosomal frameshifting.

PRRSV (16). This provides further genetic evidence to affirm that the minor GPs are the prime viral determinants of host cell binding and possibly also fusion and entry.

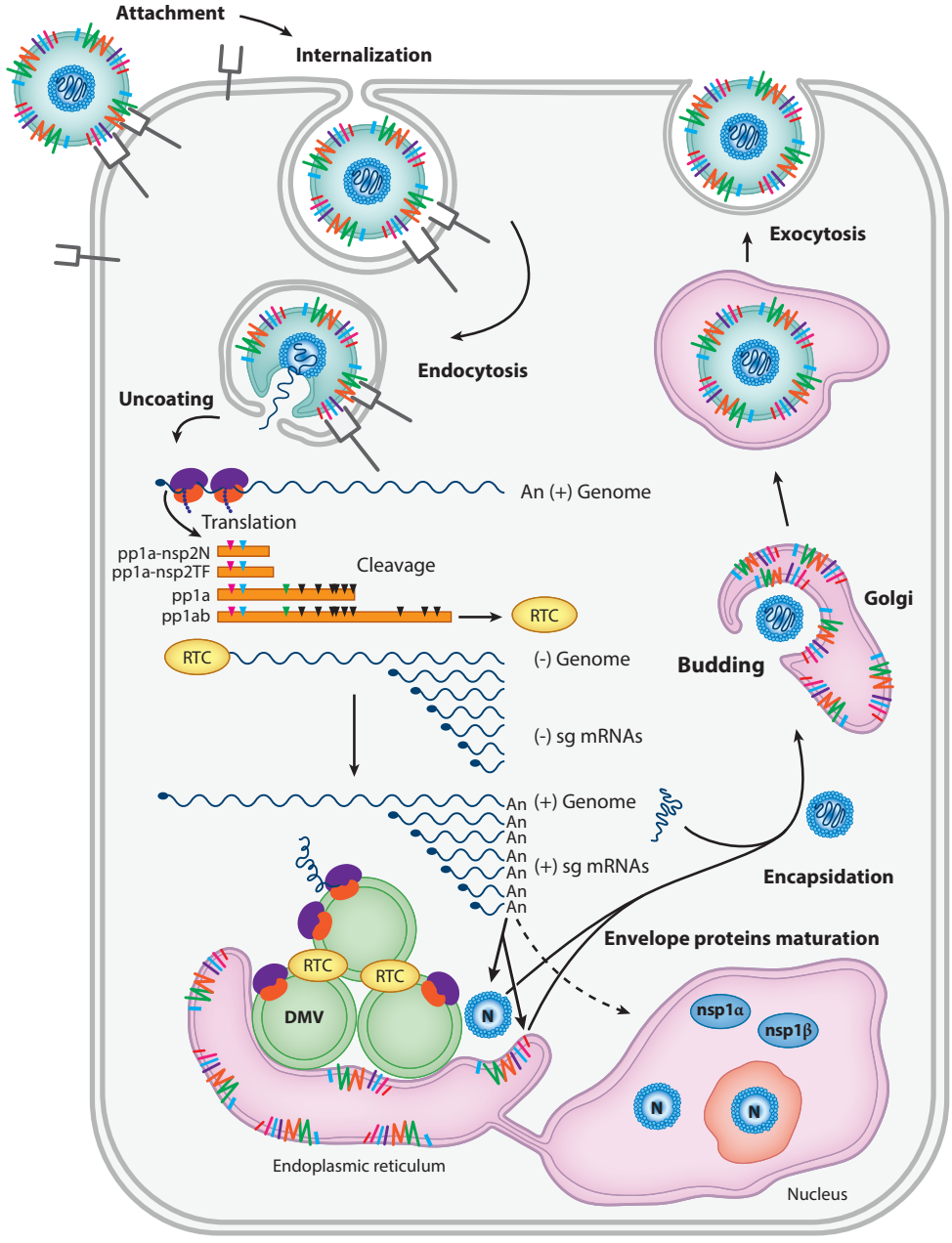
PRRSV infection can be divided into at least three distinct stages: acute infection, persistence, and extinction, which are each unique in terms of immunology, virology, and clinical disease (**Figure 4**). The first stage is represented by acute infection, during which the lung serves as a preferential site of infection. PRRSV replicates mainly in macrophages and dendritic cells in lungs and the upper respiratory tract, resulting in viremia by 6–12 h postinfection (pi). Serum viremia may last for several weeks despite the presence of circulating antibodies. During the second stage of persistent infection, virus replication subsides to the point where virus is no longer detected in blood and lungs and pigs no longer exhibit overt signs of clinical disease. At this stage, viral replication is primarily localized in lymphoid organs, including tonsil and lymph nodes but not spleen (17–19). Continuous virus replication in regional lymph nodes accounts for the efficient transmission of virus to naïve pigs via oral-nasal secretions and semen (20). Subsequently, virus replication gradually decays until the virus becomes extinct in the host. The eventual disappearance of virus represents the final stage of infection. It is not known exactly when virus disappears, but replication can be maintained for as long as 250 days after infection (21). Therefore, PRRSV replication does not establish a steady-state equilibrium but gradually declines over time, with the lymphoid organs as the site of the last vestige of virus replication before viral extinction (18). However, it should be noted that in the context of the typical swine production setting, during which pigs are maintained for 250 days, PRRSV establishes a "life-long" infection.

VIRAL PATHOGENESIS AND EVOLUTION

PRRSV emerged almost simultaneously in North America (genotype 2) and Western Europe (genotype 1) in the late 1980s and early 1990s, respectively (22, 23). The virus strains that originated from the two continents are strikingly different, with only 55–70% nucleotide identity (24, 25). The evolutionary distance between the two lineages has led to the hypothesis that these two lineages have evolved separately from a very distant common ancestor (26).

Initially, PRRS was referred to as mystery swine disease and mystery reproductive syndrome and was characterized as blue-ear pig disease (22, 23). Typical clinical symptoms of PRRS are mild to severe respiratory disease in infected newborn and growing pigs and reproductive failure

in pregnant sows. There are considerable genetic and virulence differences among PRRSV isolates. Depending on viral strain and immune status of the host, some swine farms may have pigs subclinically infected with PRRSV, whereas others experience severe reproductive and/or respiratory disease. Since its emergence, several highly pathogenic PRRSV strains have evolved to cause numerous acute disease outbreaks in different countries. In the late 1990s, the emergence of an atypical PRRSV caused high mortality and abortion storms in the United States (27). Subsequently, a highly virulent 1-8-4 strain was reported in the north-central United States (28).



In China and Southeast Asia, highly pathogenic PRRSV (HP PRRSV) strains were reported to associate with porcine high fever disease, resulting in high mortality (20%) in both young and old pigs along with severe respiratory pathology (29). In Eastern Europe, a highly pathogenic European subtype 3 PRRSV, Lena strain, was isolated from a Belarusian farm with swine reproductive and respiratory failure (30). Infection with these HP PRRSV strains is associated with severe clinical signs, pulmonary lesions, and aberrant host immune responses (30, 31).

PRRSV can cause more complicated disease when functioning as a primary respiratory infectious agent or as a cofactor in porcine respiratory disease complex (PRDC). In clinical cases of PRDC, PRRSV is the most common virus isolated (32). Experimentally, in *Mycoplasma hyopneumoniae* and PRRSV coinfected pigs, more severe and longer duration of lung pneumonia was observed (33); clinical disease was exacerbated in pigs coinfected with PRRSV and *Bordetella bronchiseptica* (34). PRRSV also plays a major role in porcine circovirus–associated diseases (PCVAD); infection of pigs with both PRRSV and PCV-2 induced more severe clinical symptoms and lung lesions than those associated with infection by either agent alone (35). There is evidence of PRRSV interaction with other swine pathogens, including porcine respiratory coronavirus, swine influenza virus, and *Haemophilus parasuis*, by altering the typical host response to the infection of a single pathogen (36, 37). These results demonstrate the complicated nature of swine respiratory disease caused by mixed pathogens. Remarkably, PRRSV is the most common virus associated with PRDC. Because PRRSV can suppress the host immune defense system, this may allow secondary/opportunistic pathogens to establish infections, resulting in more serious and chronic disease. The mechanisms that enable PRRSV infection to potentiate severity of disease owing to secondary infections must be elucidated.

ANTIBODY RESPONSES

Infection with PRRSV stimulates an antibody response by 7–9 days pi (dpi) but with no evidence of protection against PRRSV infection; serum neutralizing antibodies (NAbs) appear only later, typically >28 dpi (**Figure 4**) (38; reviewed in 39). Commercial serum assays typically measure anti-N protein antibodies; these appear early but are nonneutralizing and do not correlate with protection (40, 41). More recent efforts have shown that anti-nsp antibodies also are found early after infection (42, 43). Serum transfer experiments showed that NAb could transfer passive protection (44). They prevented transplacental PRRSV infection of piglets and provided sterilizing immunity against PRRSV challenge to both the dam and her piglets in utero. However, this required transfer of high-titered NAbs (40). Importantly, while NAb can be protective, PRRSV viremia can be controlled even in the absence of detectable NAbs.

Figure 3

Porcine reproductive and respiratory syndrome virus (PRRSV) replication cycle. Following entry by receptor-mediated endocytosis and disassembly, genome translation yields replicase polyproteins pp1a-nsp2TF, pp1a-nsp2N, pp1a, and pp1ab. These polyproteins are cleaved by viral internal proteinases to generate at least 14 nonstructural proteins, which are assembled into a replication and transcription complex (RTC). The RTC first engages in minus-strand RNA synthesis to produce both single-strand full-length and subgenomic (sg)-length minus-strand RNAs. Subsequently, the sg mRNAs serve as templates for the synthesis of plus-strand sg mRNAs required to express the structural protein genes that reside in the 3′-proximal quarter of the genome. Newly generated RNA genomes are packaged into nucleocapsids that become enveloped by budding from smooth intracellular membranes. The new virions are released from the cell using the exocytic pathway.

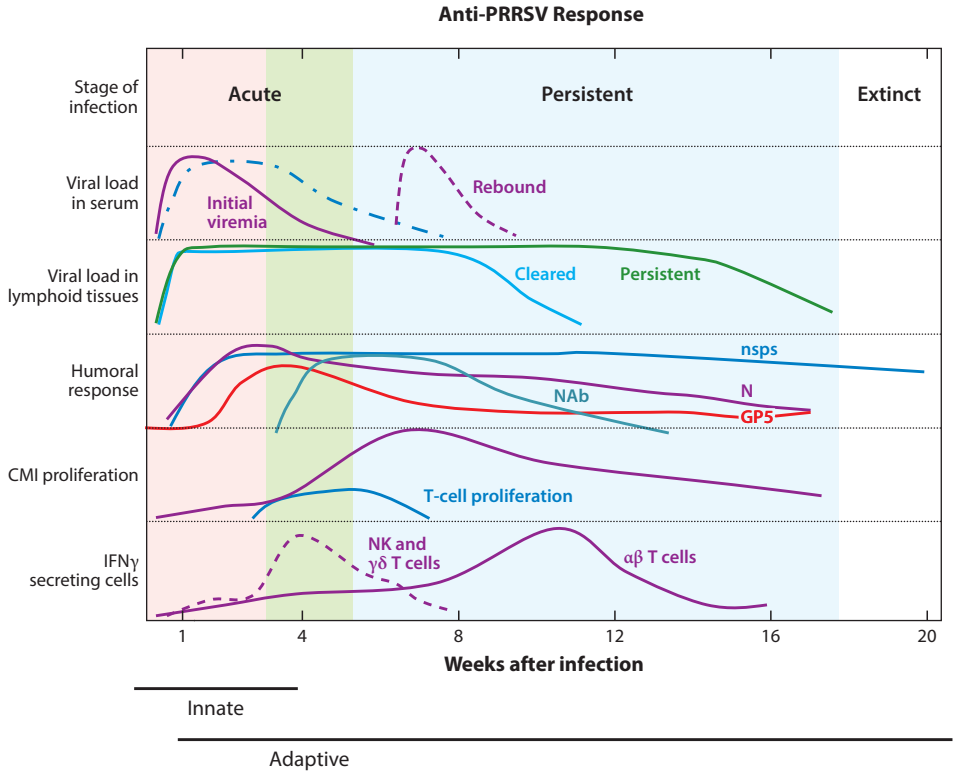


Figure 4

Immune response to porcine reproductive and respiratory syndrome virus (PRRSV) infection. Time points are approximate and could be changed depending on the virus isolate. Viral load in serum is indicated by a range of responses (solid purple for fast serum clearance and dashed blue for slower clearance, dashed purple for rebound virus). The timing of the humoral response is shown as neutralizing antibodies (NAb) and antibodies to PRRS viral proteins: N, GP5, and nonstructural proteins (nsps). Antibodies against nsps are predominantly to nsp1 (α/β), nsp2, and nsp7 (α/β); the other nsps induce low level or undetectable antibody responses. Cell-mediated immune (CMI) responses are weak early and peak later after PRRSV infection, with interferon-γ (IFNγ) secretion from natural killer (NK) and $\gamma\delta$T cells early, followed later from $\alpha\beta$ T cells.

The potential mechanisms responsible for delayed NAbs include (*a*) glycan shielding effects of N-linked glycosylation in GPs (45); (*b*) presence of an immunodominant decoy epitope in GP5 upstream of the neutralizing epitope (46); (*c*) antibody-dependent enhancement of viral entry into target cells (47); (*d*) suppression of innate immune responses, as discussed below (48); and (*e*) prevention of normal B cell repertoire development (49).

Priming of immunity through either natural exposure or vaccination provides only limited protection against secondary challenge. Production of protective levels of NAbs usually requires multiple vaccinations or repeated infections. Moreover, NAbs are usually specific for the vaccine strain (homologous), with lower/no titers of cross-neutralizing (heterologous) antibodies (50, 51). Robinson et al. (52) found that sera from previously infected commercial sows had high levels of NAb against diverse (heterologous) PRRSV strains. Identification of NAb-specific epitopes is ongoing; Lee et al. (53) explored immunization using an inactivated double hypoglycosylated form of GP5 with an adjuvant. This potential improved vaccine resulted in reduced lung lesions and viral

RNA load and induced higher NAb titers in homologous virus-challenged pigs. Trible et al. (54) have identified a unique amino acid in the M protein associated with broad neutralization activity.

INNATE IMMUNE RESPONSES TO PRRSV INFECTION

The innate immune system is the first line of host defense against viral infections. It includes physical barriers, such as skin and mucous membranes; chemical barriers, like antimicrobial peptides, pH, lipids, and enzymes; and immune cells, such as monocytes, macrophages, eosinophils, neutrophils, and natural killer (NK) cells. Following any viral infection, adequate activation of the host innate immune system is critical to prevent viral replication and invasion into mucosal tissues and, importantly, in initiation of the strong adaptive immune response to fight against intracellular pathogens (55). The NK cell is the innate lymphocyte subset that helps in nonspecific clearance of any virus-infected cell from the body. In younger pigs, the NK cell is small to medium sized and lacks adequate intracellular granules (56); thus, in spite of having higher frequency of NK cells, nursery pigs have reduced NK cell cytotoxic activity.

Following acute swine influenza virus infection in pigs, both infected and activated cells in the lungs secrete high concentrations of bioactive interferon-α (IFNα), tumor necrosis factor-α (TNFα), and interleukin-1 (IL-1), coinciding with clinical symptoms and effective clearance of the virus (57). In contrast, infection with PRRSV elicits poor innate and adaptive immune responses associated with immune modulation and incomplete viral clearance in most of the pigs, depending on their age and immune status (57–59; reviewed in 39). Nursery pigs suffer from PRRSV infection more than adult animals (60; reviewed in 49), owing to their poorly developed innate immune system as well as limited response to counter viral immune evasion strategies. Activated innate immune response at mucosal sites plays a major role in induction of protective mucosal immunity against enteric and respiratory infections (61). But many porcine viruses modulate host innate immunity, and in turn adaptive immunity, resulting in chronic persistent infections. Indeed, the PRRSV-modulated immune state favors secondary microbial infections and PRDC, leading to severe morbidity (59, 62).

Infection with certain PRRSV strains induces significant suppression of NK cell cytotoxic activity (59, 63). Surprisingly, this was noticed as early as day two pi (64) and continued for three to four weeks (59). Reduced NK cell activity was found with field isolates (MN184 and MN 1-18-2), the lab-adapted modified-live vaccine (MLV) strain (VR2332), and MLV-PRRS administered either parenterally or intranasally; those pigs also had low levels of IFNα secretion (58, 59, 65).

Reduction in PRRSV-induced NK cell cytotoxicity is independent of NK cell frequency (59, 65) because, despite the fact that NK cell frequency returned to normal levels after a few weeks of PRRSV infection, that did not change their suppressed cytotoxic function, suggesting that PRRSV modulated NK cell cytotoxic activity (**Figure 4**). The regulation of NK cell function during viral infections is coordinated by multiple cytokines, IFNα/β, IL-12, and IL-15 (66). Impaired basal NK cell cytolytic activity, despite the presence of normal NK cell numbers, is mediated through the STAT1 pathway (67).

The quantity of innate cytokines secreted in PRRSV-infected pigs is significantly lower than with other viral infections (57) and is strain dependent. Thus, activation of adaptive immunity is delayed and dampened. Indeed, secretion of several important serum cytokines (e.g., IL-8, IL-1β, IFNγ) is correlated with virus level, accounting for approximately 84% of the variations observed (68). PRRSV infection is a poor inducer of IFNα, and its level remains low throughout the course of infection, as noted in pigs infected with many field isolates (58, 62; reviewed in 39). Stimulation of IFNα has been shown in vitro to be downregulated mainly by viral nonstructural proteins (nsp1, 2, 4, 11) (69). Genetic studies indicate that all PRRSV-infected pigs have detectable IFNα in serum

by 4 dpi. In fact, quick resolution at 11–14 dpi of serum IFNα upregulation was found in pigs with lower viral load over the first 21 dpi in PRRS Host Genetics Consortium (PHGC) trial pigs (J. Lunney, personal communication). In vitro stimulation of porcine monocytes and macrophages with low levels of IFNα stimulates the expression of sialoadhesin (Sn/CD169), a putative PRRSV receptor in macrophages. Interestingly, such a subtle stimulation of macrophages during the first 2 dpi is sufficient to enhance the efficiency of PRRSV infection by nearly 20-fold (70). In a study involving 50 PRRSV-infected pigs maintained under field conditions, secretion of low levels of IFNα early pi coincided with detection of viremia from day 2 pi in most pigs (64). Thus, to establish clinical disease in pigs, PRRSV modulates the host innate immunity through dysregulation of NK cell function and IFNα production.

At 2 days post PRRSV infection, the secretion of cytokine IL-4 was significantly upregulated in greater than 90% of pigs (64). In both mice and humans, IL-4 is essential for antibody production and is a soluble diagnostic marker of Th2 immune response. In contrast, IL-4 is thought not to be a stimulatory factor for porcine B cells; in fact, it blocks antibody and IL-6 secretion and suppresses antigen-stimulated proliferation of B cells (71). In respiratory diseases, IL-4 suppresses the transcriptional activity of many inflammatory cytokines and plays an important role in regulation of inflammatory activity in pig alveolar macrophages (72). Thus, the role of IL-4 in pigs is different when compared with mice and humans, but it also appears to be involved in modulation of the host innate immunity early post PRRSV infection.

ADAPTIVE CELL-MEDIATED ANTI-PRRS IMMUNITY

Recent efforts have compared immunity to type 1 PRRSV isolates varying in virulence. Infection with virulent type 1 (Lena) PRRSV resulted in a more severe disease than with other type 1 (Belgium A or Lelystad = LV) strains (73). Lena caused more severe pathology, with increased IL-1α production in the lungs and lymph nodes and a leukocyte influx (neutrophils, monocytes) into the bronchoalveolar lavage (BAL) fluid. By 5 weeks pi, BAL from all infected pigs had a higher percentage of $CD8^+$ T cells and higher levels of IFNγ-producing cells compared with controls. Infection with Lena PRRSV resulted in increased levels of IL-1β, IFNα, IL-10, IL-12, TNFα, and IFNγ mRNA during the first week of infection (74). Lena PRRSV infection induced a stronger early inflammatory response with associated pathology; there was faster clearance of virus in tissues compared with other type 1 strains, possibly contributing to viral virulence (75). This difference might lead to lower bactericidal activity of macrophages, leading to increased susceptibility to secondary bacterial infections and PRDC (76). Cross-reactivity against divergent PRRSV can show a different intensity and be differently associated with cytotoxic $CD8^+IFN\gamma$ as well as $CD8^-IFN\gamma^+$ cells. Especially after infection, a different immune reactivity was evident upon stimulation with various virus isolates in terms of frequency and CD8 phenotype of PRRSV-specific IFNγ-producing cells. The modulation of cytokines in vaccinated pigs appeared to be more dependent on vaccination or infection condition than on stimulation by different isolates; changes in production of IL-10 appear to be more relevant than those of TNFα at gene and protein levels.

Using IFNγ ELISPOT assays, Xiao et al. (77) demonstrated that PRRSV-specific T cells were observed as early as 2 weeks pi, with no significant difference in these T cells in lymphoid tissues during or post PRRSV infection. Viral loads were shown to be decreased by 3–4 logs in persistent infection primarily in tonsils and sternal and inguinal lymph nodes. However, there was no apparent correlation of tissue viral levels and PRRSV-specific T-cell frequencies (77). When the IFNγ-secreting $CD8^+$ T-cell response was evaluated, a late and low virus-specific response was observed (78). Overall, the effect of PRRSV infection on specific $CD8^+$ T-cell frequencies in lymphoid tissues has not been established. There are limited indications of effective $CD8^+$

cytotoxic T cells (CTLs) controlling primary PRRSV infection, as only after clearance of viremia were anti-PRRSV-targeted CTLs detected (79). As Loving et al. (39) pointed out, more basic immune reagents (pig-specific monoclonal antibodies, major histocompatibility complex antigen tetramers, and well-characterized cell lines) are required to address these important issues.

Viruses evade host immunity by promoting the secretion of immunosuppressive cytokines IL-10 and transforming growth factor-β (TGF-β), which antagonize induction of strong cell-mediated immune response. PRRSV infection induced a strong immunosuppressive response, resulting in delayed onset of a Th1 immune response (59, 80–82). Immunomodulatory properties of PRRSV N protein resulted in upregulation of the frequency of $Foxp3^+$ T-regulatory cells (Tregs) and IL-10 production (83). Both live and inactivated PRRSV significantly increased IL-10 gene expression (82); an increased concentration of IL-10 was found in pig lungs even after clearance of viremia (59, 80). The role of Tregs in establishment of chronic persistent HIV, hepatitis C and B viruses, cytomegalovirus, and Epstein–Barr virus infections has been reported (84). Similarly, a coordinated immunosuppressive function of PRRSV was shown to likely be mediated by the cytokines IL-10 and TGF-β and Tregs (59, 63, 65, 80–83). All these studies pointed to the contribution of dysregulated expression of immune molecules following PRRSV infection, resulting in weakened adaptive immunity.

Induced Tregs could suppress antiviral immunity and thus facilitate establishment of PRRSV infection, although the data are inconsistent. $FoxP3^+$ T cells may also be involved (85–87). Apoptosis in B- and T-cell areas may also be a factor but must be affirmed (76); with HP PRRS, apoptosis may be an even greater factor (88). Moreover, there is a major need to evaluate the different roles of effector versus memory T-cell populations in anti-PRRSV responses, and in turn to stimulate protective versus pathologic responses. As more cell and immune reagents become available, more detailed research will be possible to address these complex immune regulatory issues.

EFFECT OF PRRSV INFECTION ON GENE AND PROTEIN EXPRESSION

International efforts are under way to assess resistance and susceptibility to PRRSV infections using tools such as transcriptome analyses (RNA-seq) and gene arrays, single-nucleotide polymorphism (SNP) chips, genome-wide association studies, proteomics, metabolomics, and advanced bioinformatics (reviewed in 89). Important pathways and mechanisms, QTLs, and candidate genes influencing anti-PRRSV responses have been identified and are in the process of being fully characterized. Sun et al. (90) reviewed data that affirmed that nsp1, nsp2, and nsp11 are early proteins, and N a late protein, involved in controlling gene expression pathways for IFNα suppression and NF-κB regulation of adaptive immunity. Localized gene expression, using laser capture microdissection, revealed significant downregulation of TNFα and IFNα in follicular and interfollicular areas of the mediastinal lymph nodes from 3 dpi in all PRRSV-infected pigs, with delayed upregulation of IFNγ and IL-23p19 mainly in the follicles (91). Using cultured pig microglia, Chen et al. (92) proved that microglia could support HP PRRSV infection, resulting in upregulation of expression of cytokine genes and reactive oxygen intermediates, crucial for proinflammatory cytokine production, and likely contributing to the neurotoxicity seen with HP PRRS.

A meta-analysis of porcine transcriptomic data showed activation of well-defined pathways (TREM1, Toll-like receptor and hypercytokinemia signaling), the central role of the cross talk between innate and adaptive immune responses, and roles for transcription factors (HMGB1, IRF1, IRF3, IRF5, and IRF8) (93). Several studies have pointed out the complex roles of microRNAs: miR-181 downregulates CD163 expression, miR-23 induces type I interferon expression through IRF3/IRF7 activation, miR-125b regulates the NF-κB pathway, and miR-24-3p suppresses heme

oxygenase-1 expression (94–97). Others suggested that miRNAs contribute to the pathogenesis of PRRSV infection (98). Numerous studies are under way, using samples generated in vivo and in vitro and RNA-seq analyses, to pinpoint novel pathways and genes involved in regulating PRRSV infection processes and subsequent effects on PRRS control, pathology, and persistence (99, 100). Overall, understanding the molecular bases for virus-mediated modulation of host immunity will help us to design new vaccines and biotherapeutics to help control PRRS.

MAPPING GENES REGULATING PRRS RESISTANCE

Numerous groups have probed for genes and genetic variants and identified QTL involved in swine health, immune response, and disease resistance traits (reviewed in 89). Genetic variation in PRRS resistance/susceptibility very likely will be polygenic, regulating aspects of both innate resistance and acquired immunity. With the swine genome completed and immunome annotated (101, 102), studies have accelerated. The goal is to identify genomic regions and DNA markers useful for selecting pigs with improved PRRS resistance while retaining desired production traits. Research on genetic resistance to PRRS is multifactorial; it is aimed at identifying and understanding the host allelic variation associated with virus replication, which is dependent on the isolate, its virulence, tissue tropism, persistence, and route of infection, as well as on the host immune response and the speed and regulation of innate and adaptive antiviral immunity. This is also influenced by the pig's health status and its microbiome, concomitant infections, and nutritional plane. From a genetic selection standpoint, it would be desirable to select on a trait in uninfected pigs (i.e., before PRRSV infection) that is correlated with a response after infection. Vincent et al. (103) found that macrophage antiviral responses are only partially predictive of breed and line associations with PRRSV resistance.

Breed differences clearly play a role in determining resistance/susceptibility to PRRS; numerous studies have affirmed that lines or breeds with improved reproductive traits, e.g., Meishan or Large White, are more resistant to the effects of PRRS (reviewed in 89). Useful candidate genes will likely assist in improving our understanding not just of resistance to PRRS but also of protective immune mechanisms and thus vaccine development (reviewed in 39). Already, gene knockout technology has affirmed that intact sialoadhesin (Sn/SIGLEC1/CD169) is not required for attachment/internalization of PRRSV (13). Similar studies using gene knockout and editing (e.g., CRISPR, Talen) techniques are under way to assess the role of CD163 in PRRS infections (104).

The PRRS Host Genetics Consortium (PHGC) conducted detailed studies of genetic resistance to PRRSV infection using a nursery-pig model and commercial crossbred pigs. The studies involved deep sampling and analyses of phenotypic traits, extensive genotyping (60K SNPchip), and a shared database (**http://www.animalgenome.org/lunney/**) (105, 106). Results from 15 PHGC trials of ~200 pigs with each of 2 different PRRSV isolates (NVSL-97 and KS-06) have confirmed that all pigs become PRRSV infected. Boddicker et al. (107–109) identified a genomic region on *Sus scrofa* chromosome (SSC)4, in high linkage disequilibrium with SNP WUR10000125 or rs80800372, which has a significant impact on variation in viral load (15%) and growth (11%) response. Further work has revealed a strong candidate causal mutation owing to allele-specific expression in resistant (AB) individuals; guanylate binding protein 5 (GBP5) is differentially expressed ($p < 0.05$) in blood from susceptible AA versus resistant AB pigs at 7, 11, and 14 dpi (110). For pig health, inheritance of specific alleles within the swine major histocompatibility or swine leukocyte antigen (SLA) complex (SLA on SSC7) positively influences disease and vaccine responses (reviewed in 111); analyses of NAbs have indicated SLA-associated control (A.S. Hess, Z. Islam, M.K. Hess, R.R.R. Rowland, J.K. Lunney, A. Doeschl-Wilson, S. Bishop, G.S. Plastow, and J.C.M. Dekkers, manuscript in preparation).

Genomic studies should have substantial impact on the pig industry because it is now possible to include the use of biomarkers for basic health traits alongside a broader set of genomic markers used for improved performance and reproductive traits, as well as pork quality. These molecular studies may reveal alternate PRRS control mechanisms that can be exploited for novel drugs, biotherapeutics, and vaccine designs. Identifying genes that prevent viral persistence and those that influence host tolerance (good growth despite high viral burden) are two additional targets for overall PRRS control.

Because of the progress in genomics, genetic prediction can now be based on allele sharing rather than traditional pedigree relationships. For enhanced genetic resistance to disease to be useful for marker-assisted selection or genome-wide selection, careful planning is required, as Mellencamp et al. (112) pointed out. The stage is now set for deeper probing of the role of alleles and haplotypes involved in controlling specific antiviral responses, and for determining specific genes and their SNPs that are associated with antiviral immune and vaccine responses or that stimulate critical immune cell subsets and cellular interactions. Moreover, selection using genomic markers that can be measured in uninfected pigs is an advantage. This change has opened opportunities to expand genetic selection to a larger number of traits, simultaneously monitoring numerous phenotypes and integrating health information with growth traits. One factor to consider about selecting for disease resistance is the possibility that the pressure put on PRRSV by the presence of genetic resistance in pigs will mean that the virus will evolve to overcome resistance. A similar scenario already exists for PRRS vaccines. Studies in the next decade will verify whether marker-assisted selection for improved viral disease resistance will be effective in commercial settings.

IMMUNE RESPONSE TO REPRODUCTIVE PRRSV INFECTION

Reproductive disease associated with PRRSV contributes to over $300 million in losses annually in the United States alone (1). Nevertheless, a relatively small amount of research has focused on the reproductive form of the disease, and the underlying mechanisms of PRRSV-induced reproductive failure are still poorly understood. Clinical presentation of PRRS varies greatly between herds and can range from asymptomatic to devastating disease. Besides reproductive failure, clinical signs in pregnant sows and gilts are often mild or absent. Reproductive signs largely depend on the stage of gestation. In early gestation, PRRSV can cause embryonic death, inducing low conception and increased return to estrus rates (113, 114). Although in midgestation the virus does not readily cross the placenta and does not induce reproductive failure (115, 116), PRRSV infection in late gestation consistently results in transplacental infection of fetuses and clinical manifestations. The transplacental transmission of PRRSV was shown to be independent of the virulence of the isolate (117, 118). Clinical signs include abortions, early farrowings, fetal death, and the birth of weak, congenitally infected piglets, resulting in elevated preweaning mortality (116, 119–121). The mechanisms of how transplacental infection from dam to fetuses occurs, and why viral transmission is restricted to late gestation leading to fetal death, are still unclear.

IMMUNE RESPONSES TO PRRSV INFECTION IN PREGNANT FEMALES

Investigations on immune responses against PRRSV in vivo, like the investigation of cellular and humoral immune responses or the measurement of cytokine production, were performed mainly in nursery or growing pigs by using respiratory models of PRRS, whereas reports on immune responses of pregnant females are sparse. Peripheral blood leukocytes were investigated in sows experimentally infected with PRRSV in midgestation, and a significant decrease was shown in total

leukocyte counts at 3 and 7 dpi (115). Absolute numbers of CD172a$^+$ and CD1$^+$cells, and CD4$^+$ and CD8α^+ T cells, were decreased significantly compared with noninfected controls at 3 to 7 dpi; cell counts returned to normal levels by 14 dpi. Similarly, a massive, acute decrease in total leukocyte counts was confirmed early after infection of late-term pregnant gilts, which affected all peripheral blood mononuclear cell (PBMC) populations, most severely NK cells and CTLs (122). Inactive and naïve B cells, T-helper cells, and CTLs showed a stronger initial recruitment from the systemic circulation than did their respective effector or memory counterparts, which might indicate a higher retention of these naïve cells in PRRSV-infected lymphatic tissues. The acuteness of the drop and subsequent rebound suggested that cells of various PBMC subsets traffic to sites of infection, but the relevance of this massive leukopenia in regard to PRRSV-related host-pathogen interactions is currently unknown.

So far, little is known of which cellular mechanisms could be important to prevent reproductive disease owing to PRRSV infection. Lowe et al. (123) reported that the number of IFNγ-producing cells measured by ELISPOT was correlated with protection against reproductive disease in three of four commercial herds experiencing outbreaks of PRRS. They also found that sows within farms varied considerably in their immune responsiveness and degree of clinical protection. In contrast, levels of IFNγ in serum and supernatants of PRRSV-stimulated PBMC from experimentally infected late-term pregnant gilts were not associated with fetal death (124). Results of the two reports cannot be compared directly because experimental conditions and timing of exposure, as well as laboratory methods, differed radically. The experimental study performed in late-term pregnant gilts also found that IFNα, a potent antiviral molecule, was one of the most important cytokines in reproductive PRRS (124). Interestingly, levels of IFNα were positively associated with fetal mortality. Negative effects of IFNα could be confirmed in a different experiment comparing the pathogenicity of three type 2 PRRSV strains in a reproductive model (117). The negative effects of IFNα in reproductive PRRS might be explained by the knowledge that IFNα upregulated the expression of sialoadhesin, which enhanced PRRSV infection of monocytes (70).

Humoral immune responses might also be crucial for preventing reproductive disease owing to PRRSV; as discussed above, NAbs can fully prevent the transplacental transmission of PRRSV and extinguish the infection in pregnant females (44). The role of NAbs in controlling PRRSV infection is controversial, but for effective PRRSV vaccines it might be essential to induce high levels of NAbs. The high genetic diversity of PRRSV, however, is complicating the design of protective vaccines, because NAbs would have to be cross-protecting against heterologous virus isolates. In this context, host genomics should be considered in future research. As Rowland et al. (125) showed, a certain number of animals within a population are capable of producing NAbs, which are able to cross-neutralize heterologous strains of PRRSV. In contrast to nursery pigs, for which a SNP on SSC4 (WUR10000125) was found to be associated with lower PRRS viral load and higher average daily gain (107–109), little is known regarding genetic susceptibility to reproductive PRRS. Recently, the genetic basis of antibody response and reproductive traits in a commercial multiplier sow herd before and after a PRRS outbreak was investigated (126). A significant genomic component associated with PRRSV antibody response measured by ELISA and the number of stillborn piglets was found. So far, the WUR10000125 SNP on SSC4, associated with PRRS resilience/tolerance in nursery pigs, could not be associated with reproductive outcome after PRRSV infection (126, 127).

IMMUNE RESPONSES IN THE MATERNAL-FETAL INTERFACE

Mechanisms involved in cellular changes and local immune responses within the maternal-fetal interface, as well as their pathophysiological role in PRRS-related reproductive disorders, are

not well understood. PRRSV reaches the endometrial connective tissue most likely in association with blood monocytes migrating through endometrial vessels; it subsequently replicates in Sn/CD169$^+$ and CD163$^+$ macrophages (128, 129). Virus replication causes death of local infected and surrounding cells through apoptosis and probably secondary necrosis, which could induce focal detachment and degeneration of the fetal placenta (129, 130). This was hypothesized to be the cause of fetal death rather than the direct result of PRRSV replication within fetal tissues (130). Virus replication was shown to be influenced by the number of target cells present in endometrium and fetal placenta. Whereas the number of CD163$^+$ cells was consistently high throughout gestation, the number of Sn/CD169$^+$ cells, particularly within the fetal placenta, was highest in late gestation (131). This might explain why transplacental PRRSV infection is mostly restricted to late gestation. Furthermore, it could be demonstrated that a significantly higher number of Sn/CD169$^+$ macrophages was present in endometrium and placenta of late-term PRRSV inoculated sows compared with noninoculated controls (132).

In humans, innate immune responses to viral infection in the endometrium involve cellular interactions between uterine NK cells and macrophages (133). Porcine uterine NK cells were studied in early pregnancy (134–136), but little is known about their role in combating viral infections later in gestation. Karniychuk et al. (128) found increased numbers of CD8$^+$ cells in the maternal-fetal interface after PRRSV infection and hypothesized that activated endometrial NK cells might contribute to the development of histologic lesions, such as the local separation between uterine epithelium and trophoblast, which leads to complete degradation of the placenta. However, porcine uterine NK cells are not well defined phenotypically and warrant further research to determine their function.

IMMUNE RESPONSES IN FETUSES

After PRRSV reaches the fetus, virus replication takes place in several tissues, including lung, liver, spleen, heart, and kidney. Most consistently, virus can be detected in lymphatic tissues and the fetal thymus; the latter was proposed as the primary site of virus replication (125, 137, 138). Karniychuk & Nauwynck (131) demonstrated that Sn/CD169$^+$ and CD163$^+$ macrophages are abundant in fetal organs, including liver, lung, and spleen; the presence of those cells in thymus was not investigated. In addition to macrophages, there are indications that fetal endothelial and epithelial cells might be susceptible to PRRSV (121, 139). Rossow et al. (139) investigated 11 fetuses from 4 herds with clinical outbreaks of reproductive PRRS and identified PRRSV antigen in endothelial cells of arterioles in fetal lung tissue via immunohistochemical staining. Cheon & Chae (121) demonstrated viral antigen within epithelial cells of fetal thymus and endothelial cells of small capillaries in fetal heart tissue. In contrast, type 1 PRRSV RNA was found exclusively within macrophages in various fetal tissues via in situ hybridization (138). Those differences might be explained by biological differences between different virus isolates. Indeed, type 1 PRRSV strains of varying virulence have different invasion strategies and cell tropism in nasal mucosa (140), thus supporting this theory.

Although not fully developed (141), pig fetuses possess functional B and T cells at the time of in utero PRRSV infection and were shown to initiate an antiviral response measured by upregulated IFNγ and TNFα mRNA levels in fetal tissues and IFNγ and TNFα protein levels in fetal serum (125). Nevertheless, piglets surviving in utero infection can develop persistent infection and can support virus replication within lymphatic tissues for at least 132 days postfarrowing (19). Because congenitally infected pigs were shown to transmit the virus to PRRSV-negative sentinel pigs for at least 112 days after birth (19), they play an important role in maintaining virus circulation within a herd. If piglets survive in utero infection with PRRSV, they can suffer from thymic atrophy

and apoptosis (138, 142), impaired development of lymphocytes (49), and altered immune cell populations (143) and immune cell functions (144).

Mechanisms of fetal survival or death after transplacental infection with PRRSV are not well characterized, and the preservation/infection status of fetuses within a litter can vary greatly (145). Owing to the lack of severe microscopic lesions in PRRSV-infected fetuses (139, 146), it has been suggested that fetal death may not be a direct result of PRRSV replication in fetal tissues, but may rather be due to apoptosis of infected and surrounding cells in the maternal-fetal interface, leading to focal detachment and degeneration of the fetal placenta (129, 130). However, a recent experiment performed on an extraordinarily large number of pregnant gilts showed that fetal infection plays a central role in the pathogenesis of reproductive PRRS because >95% of dead fetuses were infected with PRRSV, and the presence of PRRSV in fetuses, particularly at high levels in thymus, increased the likelihood of fetal death (117, 127, 145). The same experiment indicated that the status of adjacent fetuses and interfetal transmission of PRRSV significantly influenced fetal outcome. This supported previous findings of lateral transmission of porcine parvoviruses and porcine circovirus type 2 between adjacent fetuses (147, 148).

In summary, mechanisms of reproductive failure owing to PRRSV infection are not well understood. Future research should focus on the investigation of local immune responses, including cell recruitment and cellular changes within the maternal-fetal interface, to answer questions of how the virus is able to cross the placental barrier and why transplacental transmission of PRRSV is restricted to late gestation. Understanding those mechanisms will be crucial for the development of improved vaccines that should be able to prevent the transplacental spread of PRRSV. Moreover, host genetics influencing the susceptibility to reproductive PRRS must be investigated further to provide additional tools for combating PRRS via the breeding of less-susceptible animals. Finally, identification of genomic markers that are related to improved responses following vaccination could help select for vaccine-ready pigs.

FUTURE PERSPECTIVES

A major driving force behind these basic pathogenesis and immunological studies is to develop improved vaccines and disease-control strategies. PRRSV is one of the most economically important swine pathogens, and recent HP PRRSV outbreaks in Asia caused enormous economic losses. The inability to completely control PRRSV infection is due to the natural characteristics of RNA viruses, which undergo rapid evolution and present as a genetically and antigenically heterogeneous population. It also highlights our inadequate knowledge of PRRS viral pathogenesis and host immunity.

There have been major breakthroughs in understanding the biology and ecology of PRRSV, but the complexities of virus-host interaction and vaccinology are still not completely understood. There are serious deficits in our knowledge of the key immunological targets for both B- and T-cell-directed protection, and of the viral proteins/elements involved in molecular and cellular mechanisms, to regulate the induction and maturation of the immune response (reviewed in 39, 49, 149). Persistence is another significant factor impeding the successful control of viral infection and transmission. Current diagnostic assay targets (viral antigens) are unable to identify persistently infected animals. Recent enhancements of our knowledge of PRRSV nsps identified innate immune antagonists that present potential novel targets for vaccines and diagnostic assay development.

Currently, there is a growing demand to consider regional elimination of PRRS, but that requires reliable vaccines, i.e., those that cannot revert to virulence and spread to nonvaccinates and persist within the swine herds long term. Achieving this goal requires extensive research into new technologies, including novel adjuvants and immunomodulators, improved inactivation methods

and nanoparticle delivery systems, novel DNA and vector-based subunit vaccines, and strategies for viral protein engineering and immune cell targeting (150–152; reviewed in 149, 153). Ideally, the next generation of PRRS vaccines will incorporate several of these novel approaches and should include markers to both differentiate infected from vaccinated animals (DIVA) and affirm positive vaccination responses (50). Future studies combining basic viral biology and pathogenesis, host genomics, and immunology are required for advancing our understanding of PRRS; these will provide the basis for developing new prevention and control strategies.

Although MLV-PRRS vaccines have been in wide use for the past 25 years, they are not sufficient to provide complete protection against emerging and remerging field viral strains that represent genetically and antigenically diversified populations. Moreover, MLV-PRRS vaccines may have safety issues, reverting to more virulent forms of the virus and subsequently causing transmission of the vaccine, as well as mutated virus, to susceptible pigs. Thus, development of broadly effective, inactivated virus vaccines would lead to superior control and even eradication of PRRS globally. Since the 1990s, several researchers have attempted to develop killed PRRS vaccines and identify immunogenic peptide targets, but most candidate vaccines have failed to induce the required level of immunity for virus clearance (154, 155). Recent efforts have shown some promise, but they must be pursued further to improve their efficacy and address quality-control and cost issues (156; reviewed in 149, 153).

Reverse genetics systems provide a powerful tool for PRRS research. Currently, numerous PRRSV full-length cDNA infectious clones, representing both genotype 1 and 2 viruses, provide a great resource for developing novel genetically engineered MLV vaccines, as well as exploring innovative strategies to improve the safety and cross-protective efficacy of PRRSV vaccines (reviewed in 31). Technical advances from engineering the PRRSV genome using reverse genetics allow introduction of targeted mutations/deletions into viral genomic regions, e.g., those that encode immune antagonists or virulence determinants. Recent studies represent initial steps for uncovering the specific roles of PRRSV proteins in virus–host interactions. Basic knowledge generated from these studies will be applied for designing novel vaccines and diagnostic assays.

Future directions for generation of improved PRRS candidate vaccines should consider the following characteristics: They should be able to (*a*) upregulate the host innate and adaptive immune responses, (*b*) incorporate positive and negative markers, (*c*) include antigens from other porcine pathogens for multivalent vaccines, (*d*) use novel adjuvants, and (*e*) induce broad cross-protection against an array of genetically diversified field isolates. The ongoing challenges facing today's PRRS vaccinology are to find innovative strategies to boost cross-protective immunity beyond that provided by natural infection (the gold standard for protection) and to attenuate new MLVs to the point where they are considered as safe as inactivated vaccines and appropriate for use in naïve herds. Evaluation and commercialization of such improved live PRRSV vaccines is an ultimate goal of PRRS researchers for combating this devastating pig disease.

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literature; recent US National Pork Board–funded literature reviews provide excellent additional background data and analyses (31, 39, 149). Thus, we have cited recent reviews and the most important references and developments in PRRS virology, pathogenesis, and immunology. Our literature review was completed in May 2015. The authors acknowledge funding support for their research from the US Department of Agriculture Agricultural Research Service and National Institute of Food and Agriculture, the US National Pork Board, and Genome Canada.

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