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Interaction between innate immunity and porcine reproductive and respiratory syndrome virus

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Abstract

Innate immunity provides frontline antiviral protection and bridges adaptive immunity against virus infections. However, viruses can evade innate immune surveillance potentially causing chronic infections that may lead to pandemic diseases. Porcine reproductive and respiratory syndrome virus (PRRSV) is an example of an animal virus that has developed diverse mechanisms to evade porcine antiviral immune responses. Two decades after its discovery, PRRSV is still one of the most globally devastating viruses threatening the swine industry. In this review, we discuss the molecular and cellular composition of the mammalian innate antiviral immune system with emphasis on the porcine system. In particular, we focus on the interaction between PRRSV and porcine innate immunity at cellular and molecular levels. Strategies for targeting innate immune components and other host metabolic factors to induce ideal anti-PRRSV protection are also discussed.

Keywords: innate antiviral immunity, PRRSV, interferon, vaccine

Introduction

The immune system in higher vertebrates involves both innate and adaptive immune responses (Pancer and Cooper, 2006). Innate immune mechanisms, which exist in all organisms from bacteria to humans, provide immediate frontline protection against infections (Beutler, 2004; Pancer and Cooper, 2006). Evidence from both the host and virus indicates that invoking early and appropriate innate immune responses is critical for the outcome of most viral diseases, determining whether an infection is controlled or whether a persistent infection develops (Beutler, 2004; Pancer and Cooper, 2006). The critical role of innate immune cells and their components not only dominates antiviral activity in the early phase of infection, but also potentiates the adaptive immune system for viral clearance (Hoebe *et al.*, 2004; Kabelitz and Medzhitov, 2007). In this review, we provide a brief discussion of the major components of the innate antiviral immune system with particular emphasis on porcine-specific features. Our emphasis is on the response to and subversion of porcine innate antiviral immunity during infection caused by porcine reproductive and respiratory syndrome virus (PRRSV). For more general discussions about the challenges of PRRSV immunology and vaccinology, the reader is referred to several recent reviews (Kimman *et al.*, 2009; Darwich *et al.*, 2010; Yoo *et al.*, 2010).

Overview of mammalian innate antiviral immune system

Innate immune cells

A highly differentiated immune system appears to offer an evolutionary advantage to higher vertebrates. Nonetheless, all nucleated cells are readily capable of mounting

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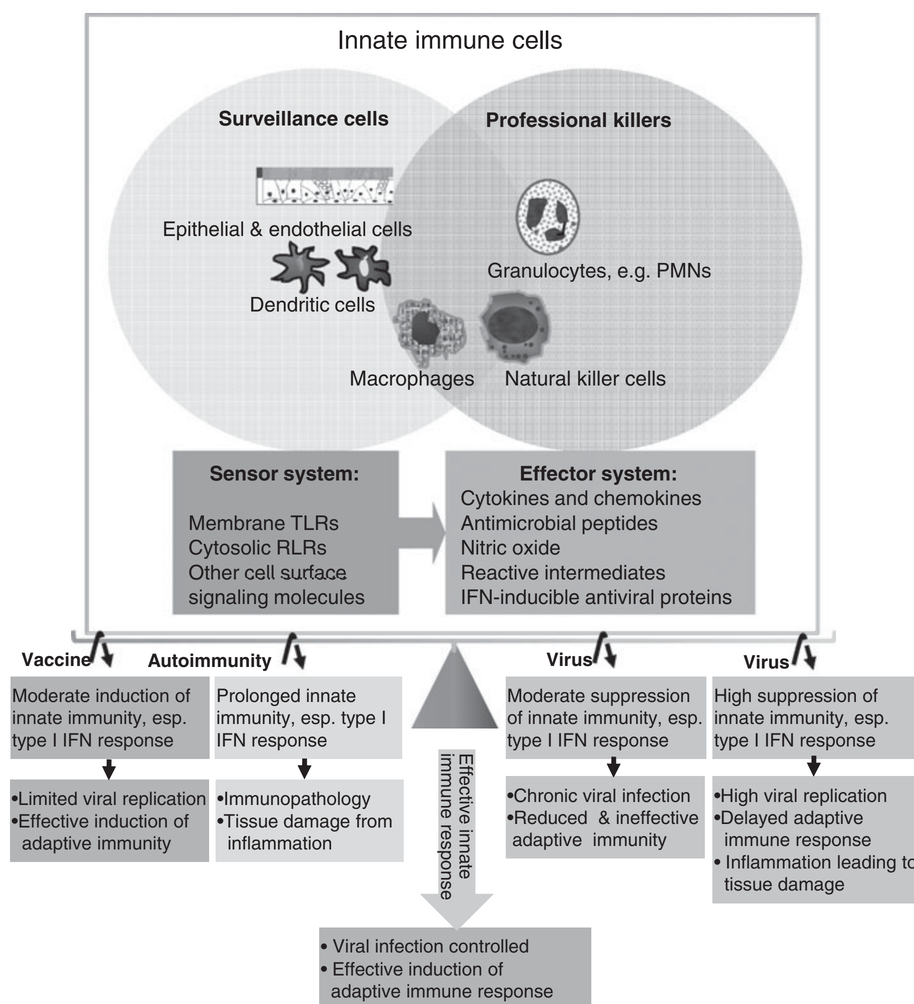

Fig. 1. Innate immune homeostasis and interaction with viral infection. Innate immune cells exert antiviral activities of immune surveillance and direct inactivation using various virus-sensing and effector molecules. Whereas most viral attacks are controlled through innate immunity and synergistic induction of adaptive immunity (including vaccine-induced), virus isolates or species that have the capability to divert innate immunity, especially type I IFN responses, alter innate immune balance causing pandemic diseases (Katze *et al.*, 2008).

innate immune responses upon exposure to a virus (Beutler, 2004). Mammalian innate immune cells, which are specialized for various functions such as pathogen recognition and killing, immune surveillance and antigen presentation, include granulocytes, natural killer (NK) cells, macrophages and dendritic cells (DCs), as well as epithelial and endothelial cells. Granulocytes, macrophages and NK cells are well documented for their role as effector cells in engulfing and digesting microorganisms or promoting active death of infected cells (Ludwig *et al.*, 2006; Appelberg, 2007; Takeuchi and Akira, 2007; Vivier *et al.*, 2008). Depending on their anatomical locations, epithelial and endothelial cells, as well as DCs and macrophages, are among the first groups of cells initially exposed to viruses (Barchet *et al.*, 2005; Opitz *et al.*, 2007; Wen *et al.*, 2008; Hammad and Lambrecht, 2008). Their function in viral recognition and immune surveillance facilitates coordination of subsequent immune responses by linking to adaptive immunity.

Innate immune cells are dually functional as 'sensors' and 'effectors'. Even cells that are primarily associated with killing, e.g., neutrophils, facilitate immune surveillance via Toll-like receptors (TLRs) (Haselmayer *et al.*, 2006; Borregaard *et al.*, 2007). Furthermore, professional antigen presenting cells, such as conventional DCs, potently destroy engulfed pathogens through autophagy (Schmid *et al.*, 2006; Lee and Iwasaki, 2008). This functional plasticity is best known in macrophages, which are composed of diverse subgroups of professional phagocytes as well as immunoregulatory cells (Figure 1; Hashimoto *et al.*, 2007; Kumagai *et al.*, 2007; Randolph *et al.*, 2008). Because innate immune cells such as macrophages, epithelial cells and endothelial cells, often serve as the initial foothold for viral infection, these cells provide an excellent platform for examining virus–host interaction, viral recognition, signaling transduction and antiviral effector function (Opitz *et al.*, 2007; Hammad and Lambrecht, 2008; Wen *et al.*, 2008).

Accumulated evidence indicates that upon viral infection the overall immune response is dependent on the coordination of innate immune cells to exert immune surveillance and to produce immune effectors. The balance resulting from immune regulation or vaccine-induced innate-immune activation will elicit both innate and adaptive immunity to control virus replication. In contrast, if viruses divert the induction of innate immunity, especially a type I interferon (IFN) response, it is likely that disease will result from uncontrolled viral infections (Figure 1; Katze *et al.*, 2008). DCs and activated macrophages are antigen-presenting cells, which directly bridge innate and adaptive immunity. In addition, some newly defined groups of innate immune cells including innate lymphoid cells (ILCs), natural helper cells (NHCs) and innate type 2 helper cells (ih2), which are crucial for the development of lymphoid structures and secrete cytokines similar to those from T helper cells, are also important players that bridge innate and adaptive immunity. However, their role in antiviral responses remains obscure (Sawa *et al.*, 2010; Saenz *et al.*, 2010; Veldhoen and Withers, 2010).

Monocytes represent 10–30% of all peripheral blood mononuclear cells (PBMC) and are progenitors of most tissue macrophages (Randolph *et al.*, 2008; Cassol *et al.*, 2010). These myeloid monocytes circulate for about 1–2 days before migrating into peripheral tissues and differentiating into resident tissue macrophages (Martinez *et al.*, 2009). Macrophages are anatomically and functionally heterogeneous. Tissue-specific macrophages are specific to anatomic locations including blood monocytes, peritoneal macrophages, pulmonary macrophages, Kupffer cells in the liver and microglia in the brain (Taylor *et al.*, 2005; Naito, 2008; Randolph *et al.*, 2008). Even within an organ, macrophages are further categorized based on their micro-anatomical location. The lung provides an example of this macrophage categorization. For example, pulmonary macrophages can be divided into three subgroups based on their microenvironment within the lung: pulmonary alveolar macrophages (PAMs), pulmonary intravascular macrophages (PIMs) and interstitial macrophages (ISMs) (Naito, 2008; Randolph *et al.*, 2008).

PAMs are the most abundant pulmonary immune cells in the alveolus located at the interface between air and lung tissues. Substantial numbers of ISMs are detected within the lung stroma and PIMs are mature phagocytes adhered to capillary endothelial cells within the lung that cover approximately 16% of the lung capillary surface in species such as pigs and ruminants (Chitko-McKown and Blecha, 1992). With respect to airway viral infections, PAMs are first to express early activation for scavenging and killing mucus-trapped viral particles through phagocytosis. To fulfill this task, activated PAMs are equipped with a variety of surface and internal receptors to detect antibody/complement-engaged viral particles (by surface Fc or complement receptors) and to recognize viral components by membrane-associated or cytosolic receptors (Fantuzzi *et al.*, 2003; Beisswenger and Bals, 2005; Daffis *et al.*, 2007; Kumagai *et al.*, 2007). PAMs are very active killer cells known to inactivate trapped viruses with both oxidative and non-oxidative mechanisms and to lower the chance of airborne viruses initializing infections on pneumocytes as well as pulmonary endothelial cells (Taylor *et al.*, 2005; Naito, 2008; Randolph *et al.*, 2008). PAMs are activated through phagocytosis or receptor recognition of viral components. They are active producers of type I IFNs and other pro-inflammatory cytokines, which lead to antiviral responses including the regulatory loop by type I IFNs and recruitment of other immune cells to infection sites (Kumagai *et al.*, 2007; Takeuchi and Akira, 2007). Normally, PAMs represent >90% of immune cells in bronchoalveolar lavage (BAL) fluid prior to the increase of granulocytes in BAL fluid after an infection (White *et al.*, 2007). PAMs have been identified as the primary IFN-α producer in murine models upon respiratory viral infections (Kumagai *et al.*, 2007; Takeuchi and Akira, 2007).

Compared to PAMs, ISMs are less phagocytic and potentially more active in repairing tissue damage. PIMs are highly phagocytic and mainly exist in ruminants, pigs and horses (Longworth, 1997). Compared to PAMs, porcine PIMs are almost equally permissive to PRRSV infection and their bactericidal and phagocytic activities are significantly suppressed by PRRSV infection much like PAMs (Thanawongnuwech *et al.*, 2000).

The importance of macrophages in an antiviral immune response is also reflected by the virus' ability to surpass this first line of surveillance allowing for a much higher chance of escaping innate immune defenses in turn causing persistent infections. Several viruses possess the ability to directly infect and undermine immune responses (such as production and signaling of type I IFNs) of macrophages (Table 1). For example, macrophages infected by human immunodeficiency virus-1 (HIV-1) and PRRSV are functionally compromised in many ways including cytokine production, receptor expression, phagocytosis and antigen presentation (Ieong *et al.*, 2000; Martinelli *et al.*, 2007; Darwich *et al.*, 2010; Thanawongnuwech and Suradhat, 2010). In this regard, direct infection of macrophages may divert overall homeostasis of cell activation status causing exacerbating co-infections and complicated syndromes.

Macrophages have been classified into two major types according to their activation status and functional difference: the classical type 1 macrophages (M1) and alternative type 2 macrophages (M2). M1 cells are conventionally activated by Th1 cytokines such as IFN-γ and IL-12. Alternatively, M2 macrophages are more heterogeneous than M1 cells depending on the stimuli. M2a macrophages are induced by Th2 cytokines such as IL-4/IL-13, while M2b macrophages are activated by immune complexes, TLR stimulation, or by IL-1Ra. M2c macrophages are generated by stimulation with the

Table 1. Innate immune evasion mechanisms of various monocytotropic viruses

Virus: [genome, family]	PRRs*	Mechanism and effect on type I IFN and other cytokine production*	References
HRSV: [(−)ssRNA, Paramyxoviridae]	Multiple TLRs, RIG-I, PKR	Viral NS1 and NS2 proteins inhibit IFN production and signaling; stimulates the production of Th2 cytokines and IL-10 to skew alternative activation of macrophages	Schlender *et al.* (2005), Munir *et al.* (2008), Shirey *et al.* (2010)
IVA: [Segmented (−)RNA, Orthomyxoviridae]	Multiple TLRs and RLRs	NS1 gene product inhibits IFN secretion and activity; suppresses IL-8 production; pandemic strains may skew M2b and Th2 response through immune complexes	Ehrhardt (2010), Koyama *et al.* (2007), Calzada-Nova *et al.* (2010a, b), Monsalvo *et al.* (2011)
SARS-CoV: [(+)ssRNA, Coronaviridae]	MyD88-dependent TLRs	A papain-like protease inhibits IFN secretion via IRF3 interference; elevates pro-inflammatory cytokines and chemokines in lung and blood	Chen and Subbarao (2007), Devaraj *et al.* (2007), Sheahan *et al.* (2008)
HIV1: [(+)ssRNA, Retroviridae]	Multiple TLRs	Skews M1 activation at acute phase for establishing viral reservoirs and M2 macrophages transmission and leads to immune failure at late stages	Thibault *et al.* (2009), Sanders *et al.* (2008), Herbein and Varin (2010)
SeV: [(−)ssRNA, Paramyxoviridae]	Multiple TLRs and RLRs	Sendai virus C protein blocks signal transduction of IFN	Kato *et al.* (2007), Bousse *et al.* (2006)
CSFV: [(+)ssRNA, Flaviviridae]	Multiple TLRs	Viral protein N mediates IRF3 degradation and suppresses IFN production, but no modulation of IL-10 and pro-inflammatory cytokines	Carrasco *et al.* (2004), Bauhofer *et al.* (2007)
PrV: [dsDNA, Herpesviridae]	Viral DNA sensors?	Viral EP0 protein targets STAT1 to prevent IFN-induced antiviral state; may suppress IL-12 production	Brukman and Enquist (2006a, b), Calzada-Nova *et al.* (2010a, b)
ASFV: [dsDNA, Asfarviridae]	TLR3	Strain-dependent suppression of TLR3 signaling and IFN response; highly virulent strains are more potent suppressors of IFN and pro-inflammatory cytokine production	Afonso *et al.* (2004), Zhang *et al.* (2006), Gil *et al.* (2008), de Oliveira *et al.* (2011)
PCV2: [ssDNA, Circoviridae]	Possible TLR7/9	Impairs TLR9 ligand-induced IFN and pro-inflammatory cytokine production; stimulates IL-10 production of bystander T cells but not infected macrophages	Chang *et al.* (2006), Vincent *et al.* (2007), Wikström *et al.* (2011)
PFMDV: [(+)ssRNA, Picornaviridae]	TLR7/8	Viral L protein mediates suppression of IFN synthesis and ISG induction; induces IL-10 production of pDCs during acute phase for immune suppression	de Los Santos et al. (2006, 2007, 2009), Díaz-San Segundo et al. (2009, 2010)
PRRSV: [(+)ssRNA, Arteriviridae]	TLR3/7/8/9 and RLRs	Viral NSPs target IRF3, IRF7 and ISG3 to suppress IFN production and signaling; induction of IL-10 and suppression of IL-12 has been observed	Fang and Snijder (2010), Yoo *et al.* (2010), Calzada-Nova *et al.* (2010a, b)

*Based on information from PAMs and other cells. ASFV, African swine fever virus; CSFV, classical swine fever virus; HIV1, human immune-deficiency virus-1; HRSV, human respiratory syncytial virus; IVA: human (swine) Influenza A; SAR, severe acute respiratory syndrome coronavirus; SeV, mouse Sendai virus; PCV2, porcine circovirus-2; PFMDV, porcine foot-and-mouth disease virus; PRRSV, porcine respiratory and reproductive syndrome virus; PrV, porcine pseudorabies virus. See figure legends for other abbreviations.

immune suppressive cytokine IL-10 and glucocorticoids (Martinez *et al*., 2009; Herbein and Varin, 2010; Odegaard and Chawla, 2011). In general, M1 macrophages are pro-inflammatory and induce cellular immunity with higher microbicidal as well as tissue-destructive activities. M2a/b cells are anti-inflammatory and induce humoral immunity with higher anti-parasite, tissue repairing as well as allergic activities. In contrast, deactivated M2c macrophages are primarily immunosuppressive along with secretion of anti-inflammatory cytokines (Martinez *et al*., 2009; Herbein and Varin, 2010; Odegaard and Chawla, 2011). Accumulated evidence indicates that macrophages are functionally plastic cells with the potential to alter their activities progressively and reversibly in response to changes in the tissue environment (Stout *et al*., 2009). For example, mouse peripheral monocytes or peritoneal macrophages shift from M2a to M2c or from M1 to M2 after sequential treatment with corresponding cytokines (Stout *et al*., 2005). Thus, hypothetically, macrophages at different activation statuses vary in their antimicrobial activity or capacity to resolve tissue damage from infections.

The link between macrophage polarization and virus infection has recently been studied in HIV-1 and respiratory syncytial virus (RSV)-infected human and murine cells (Cassol *et al*., 2009; Shirey *et al*., 2010). *In vitro* polarization of human blood monocytes into M1 cells prevents HIV-1 infection and M2a polarization inhibits viral replication at a post-integration level but facilitates macrophage-mediated transmission of HIV-1 to $CD4^+$ T cells (Cassol *et al*., 2009). In mice, RSV infection induces acute inflammatory responses at the early phase, but macrophages are skewed to the M2 phenotype progressively during the later phase of infection (Shirey *et al*., 2010). This alternative transition of macrophages counteracts the early inflammatory responses for resolution of tissue damage, but decreases the higher antiviral ability associated with the M1 state, which may help prevent virus escape from innate immune surveillance. In addition, if the M2 phenotype persists, it may lead to a 'Th2-skewed' adaptive immune response resulting in hypersensitivity to allergies and autoimmune diseases (Shirey *et al*., 2010). The increase of deactivated M2c macrophages in response to IL-10 and glucocorticoids at a later phase of viral infection decreases aggressive immune responses thus limiting pathological damage. However, some viruses, such as RSV and possibly PRRSV, may escape this mechanism facilitating the development of persistent/chronic infection. Therefore, the macrophage polarization scheme provides a valuable and straightforward framework for investigating the complexity of host–virus interaction in macrophages (Herbein and Varin, 2010).

There is broad divergence regarding permissiveness of porcine macrophages to PRRSV infection and although the activation status of macrophage subsets is critical in supporting virus infection and replication, this scheme has yet to be studied in pigs. In this respect, the differential expression of virus receptors [including heparin, sialoadhesin (CD169) and the scavenger receptor CD163] and immunomodulating cytokines should be scrutinized in different subsets of macrophages due to determinants correlating with macrophage activation and PRRSV permissiveness. Interestingly, in addition to membrane-bound CD163, a large amount of soluble CD163 is present in the circulation. It will be informative to determine how circulating soluble CD163 interacts with PRRSV (Van Gorp *et al*., 2008; Patton *et al*., 2009; Welch and Calvert, 2010). In addition, an increase of IL-10 and glucocorticoids as well as suppression of IFNs has been associated with PRRSV infection. It remains to be determined whether these changes contribute to deactivation of macrophages and thereby to PRRSV pathogenesis (Borghetti *et al*., 2011).

Macrophages are abundant in mucosal membranes and, unlike DCs, do not migrate to distal tissues. PRRSV exists in tonsils and lymphoid nodes of infected piglets and a recent study of vertical transmission has shown that the targets of PRRSV replication are in the fetal thymus and presumably are monocytic (Rowland, 2010). Therefore, macrophages are likely contributors to PRRSV pathogenesis leading to infected DCs and blood monocytes, which may be more responsible for viral transmission in lymphatic tissues.

DCs represent a primary group of innate immune cells that bridge innate and adaptive immune responses (Iwasaki, 2007; Hammad and Lambrecht, 2008; Wen *et al*., 2008). The two major types of DCs are classified as conventional and plasmacytoid DCs (cDCs and pDCs, respectively). The cDCs are prominent antigen-presenting cells with high autophagy activity (a process to uptake an antigen). Although cDCs are very important in antigen presentation for T cells in secondary lymphoid tissues, no direct antiviral role of peripheral cDCs has been defined in a primary viral infection (Iwasaki, 2007; Hammad and Lambrecht, 2008). The pDCs are known as natural IFN-producing cells for their high-level production of IFN-α after activation, which is essential for inducing a series of antiviral IFN-stimulated genes (ISGs) and establishing an antiviral state in surrounding cells (Barchet *et al*., 2005; Sen and Sarkar, 2007; Zuniga *et al*., 2007). To a lesser extent, pDC also produce TNF-α, IL-6 and IL-12, which are thought to influence T cells towards a Th1 response (Barchet *et al*., 2005). When porcine pDCs are exposed to viral mimics (such as ligands for TLR7/8 and TLR9) and viruses [such as pseudorabies (PrV), swine influenza virus (SIV) and transmissible gastroenteritis coronavirus (TEGV)], the expression of IFN-α, TNF-α, IL-2, IL-6, and IFN-γ is significantly stimulated; however, production of IL-12 is only stimulated by the viral mimic and not viruses themselves. Significantly, the production of all of the aforementioned cytokines was not observed when pDCs were exposed to PRRSV, and IL-8 production was not responsive to both PRRSV and SIV (Calzada-Nova *et al*.,

2010a). A small number of viruses, which include measles virus (MV), human RSV A2 strain and classical swine fever virus (CSFV), are able to infect DCs and subvert type I IFN synthesis and/or signaling to some extent (Table 1). In this regard, PRRSV is capable of infecting DCs, primarily monocyte-derived DCs (mDCs, representing mostly cDCs) but not lung DCs. Infection of mDCs by PRRSV significantly suppresses IFN-α but not IFN-β production (Loving *et al.*, 2007). PRRSV does not infect pDCs and the presence of either live or inactivated PRRSV did not induce IFN-α (Calzada-Nova *et al.*, 2010b). In addition, PRRSV also inhibits the production of some pro-inflammatory cytokines including TNF-α and IL-6 but not IL-8 stimulated by a TLR9 agonist (Calzada-Nova *et al.*, 2010a). However, CSFV-infected porcine pDCs had suppressed IFN-α production without inhibition of other cytokines (Carrasco *et al.*, 2004). This suggests that the production and action of type I IFNs are primary innate antiviral responses targeted by multiple viruses for successful infection, whereas modulation of other innate immune responses are complementary for disease development (Table 1).

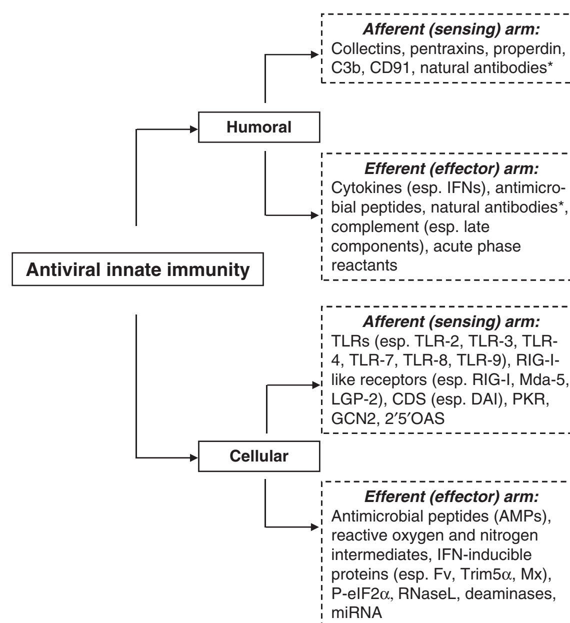

Fig. 2. Afferent and efferent arms of the innate antiviral immune response. Examples of humoral and cellular components of innate antiviral immune responses are indicated. *Natural antibodies belong to the 'innate' aspect of specific immunity. Abbreviations: CDS, cytosolic DNA sensor; DAI, DNA-dependent activator of IFN-regulatory factors; GCN2, general control nonderepressible-2; LGP-2, laboratory of genetics and physiology 2; Mda-5, melanoma differentiation-associated antigen 5; 2′5′OAS, 2′,5′-oligoadenylate synthetase; PKR, protein kinase R; RIG-I, retinoic acid-inducible gene I; TLR, Toll-like receptor; TRIM5α, tripartite motif protein.

Cell-based viral recognition mechanisms

As illustrated in Figure 2, the innate immune system, similar to its adaptive counterpart, comprises both afferent and efferent arms to discriminate and kill pathogens (Beutler, 2004). In this context, animal cells use various receptors to perceive viral infections by recognizing pathogen-associated molecular patterns (PAMPs) culminating in the induction of antiviral responses (Pichlmair and Reis e Sousa, 2007). Prominent among these receptors are TLRs, which are vertebrate homologues revealed and named after *Drosophila* Toll receptors (West *et al.*, 2006; Takeuchi and Akira, 2007). TLRs are critical for innate immune recognition and for inducing immune responses to most microbial infections (Beutler, 2004; West *et al.*, 2006). Mammalian genome projects reveal that each mammalian species has approximately 10 TLRs, which are functional for detection of a multitude of molecular ligands derived from various microorganisms (West *et al.*, 2006; Gay and Gangloff, 2007). Six of these TLRs have been instrumental in the response to viral infection through sensing viral components (Akira *et al.*, 2006; Pichlmair and Reis e Sousa, 2007; Takeuchi and Akira, 2007). Among them, TLR2 and TLR4, hinged on the cell cytoplasmic membrane, recognize several viral proteins (Akira *et al.*, 2006; Pichlmair and Reis e Sousa, 2007; Takeuchi and Akira, 2007). The functional group of TLR3, TLR7, TLR8 and TLR9 sense viral nucleic acid, either virus-derived RNA or DNA molecules (Gay and Gangloff, 2007; Forsbach *et al.*, 2008). Accordingly, these nucleic acid-sensing TLRs are responsive mainly in acidified intracellular compartments including late endosomes and lysosomes, where most viruses undergo a de-coating process in infection routes (Pichlmair and Reis e Sousa, 2007). Besides TLR-mediated viral recognition, mainly in endosomal or lysosomal compartments, animal cells also bear viral recognition and signaling mechanisms in the cytosol, where most viruses are obligated to carry out their entire or part of their infectious cycles (Pichlmair and Reis e Sousa, 2007). Four cytosol pattern recognition receptors (PRRs), including retinotic acid inducible gene I protein (RIG-I), melanoma differentiation factor-5 (Mda5) and laboratory of genetics and physiology-2 (LGP2), recognize virus derived RNA, as well as a cytosol dsDNA sensor named DNA-dependent activator of IFN regulatory factors (IRFs) (DAI), have been collectively termed RIG-I-like receptors (RLRs) (Lee and Kim, 2007; Pichlmair and Reis e Sousa, 2007; Takaoka *et al.*, 2007). These cytosol receptors recognize distinct molecular patterns of virus-derived nucleic acids and signal the production of innate immune IFNs including types I and III IFNs (Takaoka and Yani, 2006, 2007; Onoguchi *et al.*, 2007). Detailed information about the receptor ligand specificity and antiviral signaling transduction has been reviewed elsewhere (Akira *et al.*, 2006; Onoguchi *et al.*, 2007; Pichlmair and Reis e Sousa, 2007; Takeuchi and Akira, 2007; Takaoka *et al.*, 2007).

TLRs are conserved in pigs as indicated from gene sequences of porcine TLR1–11 (Shinkai *et al.*, 2006a, 2006b; Sang *et al.*, 2008a and unpublished data). In addition, porcine RIG-I and Mda5 of RLRs and the related adaptor protein of IFN-β promoter stimulator 1 (IPS-1) have been identified (Wang *et al.*, 2008; Kojima-Shibata *et al.*, 2009). Pertaining to PRRSV infection, RNA helicase RIG-I has been shown to have differential expression in PRRSV infected tissues (Zhang *et al.*, 2000). We have shown that transcripts of TLR3, TLR7 and TLR9 are significantly stimulated in PRRSV infected lungs and macrophages by the North American type 2 PRRSV (Sang *et al.*, 2008a, b and unpublished data). Using RNA library deep sequencing, Xiao *et al.* (2010a, b) recently showed that multiple TLRs, RIG-I and Mda5 are stimulated by an emerged sub-strain of highly virulent Chinese-type PRRSV, but no stimulation of TLR3 was reported. It is not clear whether the highly virulent Chinese-type virus has a mechanism to suppress TLR3 gene transcription. However, we know that suppression of TLR3 expression increases PRRSV replication and infection in porcine cells (Sang *et al.*, 2008b). The antagonism of PRRSV to inactivate the RIG-I-adaptor protein (i.e. IPS-1) and to reduce the activation of TLR3-adaptor protein (TRIF) has been demonstrated in a PRRSV-permissive monkey kidney cell line (MARC-145) (Luo *et al.*, 2008). Thus, accumulated evidence shows that PRRSV has evolved to deviate porcine cells from perceiving and transmitting antiviral signaling before the production of antiviral effectors (Figure 3) (Luo *et al.*, 2008; Sang *et al.*, 2008b; Xiao *et al.*, 2010a, b).

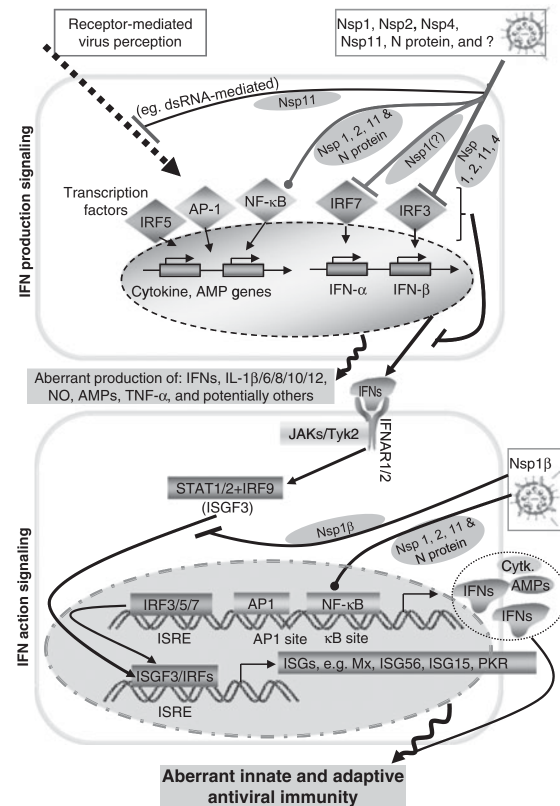

Fig. 3. Interactions between PRRSV and porcine innate immunity. PRRSV, via non-structural proteins (Nsp) or nucleocapsid (N) protein, alter antiviral signaling through suppression of upstream virus perception of type I IFN production and action. Major virus-targeting factors include transcription factors that mediate the production/action of type I IFN and other innate immune antiviral effectors such as inflammatory cytokines and antimicrobials. Lines with blunt heads indicate suppression and lines with ball heads indicate suppression or activation. Abbreviations: AMP, antimicrobial peptide; AP-1, activator protein 1; Cytk, cytokine; IFN, interferon; IFNAR, IFN-α receptor; IRF, IFN-regulatory factor; ISG, IFN-stimulated gene; ISRE, IFN-stimulated response element; JAK/Tyk2, Janus kinase/tyrosine-protein kinase 2; NF-κB, nuclear factor κB.

Type I IFNs and other antiviral effectors in innate immunity

Major classes of innate immune effectors are listed in Figure 2 and extensively reviewed elsewhere (Beutler, 2004; Klotman and Chang, 2006; Takaoka and Yanai, 2006; Lehrer, 2007; Umbach and Cullen, 2009; Hartshorn, 2010). Two major groups of innate immune effectors, type I IFNs and antimicrobial peptides (AMPs), and their role in antiviral responses will be discussed here. Type I IFNs are prominent in eliciting antiviral responses, and comprise several subtypes in mammals: IFN-α, IFN-β, IFN-ε, IFN-ω and IFN-κ (Pestka, 2007). Humans have multiple IFN-αs, and single members of IFN-β, IFN-ε, IFN-κ and IFN-ω (Takaoka and Yanai, 2006). Additional type I IFNs include IFN-δ, -τ and -ζ (limitin), which are only detected in pigs and cattle (IFN-δ), ruminants (IFN-τ) and mice (IFN-ζ) (Takaoka and Yanai, 2006).

In pigs, type I IFNs consist of multiple IFN-α, IFN-δ and IFN-ω like molecules, such as porcine IFN-α, which are encoded by as many as 17 functional genes (Sang *et al.*, 2010a) (Table 2). In addition, pigs have single gene loci encoding each of IFN-β, IFN-ε and IFN-κ (Artursson *et al.*, 1992; Sang *et al.*, 2010a). In most mammalian species, ubiquitously expressed IFN-α/β are among the most studied subtypes in antiviral responses. Although less extensively studied, the tissue-/cell-specific expressed subtypes, such as IFN-ω in various leukocytes, IFN-δ/ε in female reproductive tissues and IFN-κ in epidermal keratinocytes, are potently induced by viral infection in these cell types and confer antiviral states on uninfected cells (Takaoka and Yanai, 2006; Pestka, 2007). Using a real-time RT-PCR array, we have detected significant expression of multiple-type IFN genes in porcine skin, intestine, lymph nodes, spleen and testis. For example, in skin, 15 IFN genes belonging to all subclasses are highly expressed, likely contributing to the skin's antiviral role as protection from repetitive exposure to viral attacks (Table 2). IFNs, except for subclass IFN-α, are highly expressed in intestine (10 genes) and lymph nodes

Table 2. Porcine IFN family members and gene candidates, receptors, number of amino acids, and major expression pattern

Type	Subtype	Gene locus and (number of subtypes)	Receptor	Number of amino acids	Major expression pattern
I	IFN-α	1q22-q27 (17 and 2ψ[d])	IFNAR1	181–189	Ubiquitous expression
	IFN-αω	1q22-q27 (1)	IFNAR2	185	Skin
	IFN-β	1q23-q27(1)		186	Ubiquitous expression
	IFN-δ[a]	SSA1 (11)		153–184	Trophoblast, skin, MLN, Intestine, skin, MLN and uterus
	IFN-ε	SSA1 (1)		193	Multiple tissues, skin and MLN
	IFN-ω	SSA1 (7 and 2ψ)		176–190	Intestine, MLN
	IFN-κ	SSA10 (1)		207	Skin
	IFN-τ[b]	NA			
	IFN-ζ[c]	NA			
II	IFN-γ	5p1.2-q1.1/(1)	IFNGR1/IFNGR2	166	Activated T cells, NK-cells
III	IFN-λ1 (IL-29)	?	IL-28Rα/IL-10R2	191	Ubiquitous expression
	IFN-λ2 (IL-28A)	NA		NA	Intestine and MLN
	IFN-λ3 (IL-28B)	SSA14		195	

Modified from Chang *et al.* (2006), Takaoka and Yanai (2006), and Sang *et al.* (2010a, b). Found only in [a]pigs and cattle, [b]ruminants, or [c]mice. [d]ψ, pseudo genes; MLN, mesenteric lymph nodes; NA, not applicable.

(9 genes). In contrast, bone marrow cells and liver showed a relatively weaker expression pattern of type I IFNs. Relative to subtype differences, IFN-α1/8/12 and IFN-β are detected in all tested tissues; and porcine IFN-δ5/6/8, IFN-ω1/2/3 and single-subtype subclasses IFN-ε and IFN-κ are detectable in most tested tissues. IFN-αω, a unique subtype only found in pigs and cattle, is highly expressed in porcine skin, but detectable in the intestine, lymph nodes and spleen, implying an intensification of IFN response (Sang *et al.*, 2010a) (Table 2).

Type I IFNs are central cytokines in antiviral innate immunity. The local production of type I IFNs around infection sites comprises a major antiviral barrier to inactivate viruses and limit virus spreading. Natural or modified IFN peptides have been well documented for various IFN-based antiviral therapies, which are effective against many viral diseases including viral hepatitis, HIV and SARS infections (Haagmans and Osterhaus, 2006; Deutsch and Hadziyannis, 2008; Sulkowski and Benhamou, 2007). Type I IFNs, produced during the early phase of virus-cell interaction, not only activate antiviral responses via autocrine mechanisms, but also diffuse or transmit systemically to induce an antiviral state in surrounding and distal cells. The induction of an antiviral state, which involves the suppression of cellular metabolic levels of protein synthesis and the profound expression of genes encoding antiviral products (Haller and Weber, 2007; Zuniga *et al.*, 2007), is critical for developing effective immune protection against viral infections. Type I IFNs collectively induce antiviral responses through a common receptor composed of two subunits, IFN-α/β receptor (IFNAR)-1 and IFNAR-2 (Table 2). However, the efficacy for induction of antiviral responses is different among subtypes and even between members belonging to the same subtype. For example, human IFN-αs vary in their ability to activate human NK cells, IFN-β shows more potency than IFN-α2 in inhibition of monocyte proliferation (García-Sastre and Biron, 2006; Takaoka and Yanai, 2006). Functional differences among type I IFNs are related to their diverse affinities and kinetics in interaction with IFNAR subunits. In addition, differential expression of each type I IFN and receptor subunits with regard to tissue/cell types also contributes to distinct regulation of antiviral responses (Uzé *et al.*, 2007).

Porcine IFNs have varying levels of activity against PRRSV and other viruses in cells. In general, most subtypes of the IFN-α subclass are highly active against PRRSV; however, other subclasses including IFN-β, IFN-δ, IFN-ε and IFN-κ, are not (Sang *et al.*, 2010a). Studies indicate that IFN-α subtypes are mainly down-regulated in response to PRRSV infection (Loving *et al.*, 2007; Jung *et al.*, 2009; Sang *et al.*, 2010a). This suggests that IFN-α subtypes, not IFN-β, should receive emphasis in the modulation of porcine anti-PRRSV innate immunity. This viewpoint is supported by findings showing that PRRSV isolates differ in their sensitivity to IFN-α suppression (Lee *et al.*, 2004) and by the recent identification of PRRSV-diminishing IFN-α production through intervening signal transducers and activators of transcription (STAT)1–IRF7 signaling in pDCs (Calzada-Nova *et al.*, 2010b).

The interaction of type I IFNs with their receptors leads to the activation of transcription factors of STATs by two IFNAR-associated kinases. The activated STAT1, STAT2 and IRF9 form an activator complex of IFN-activated trimeric transcription factor, ISGF3, which interacts with the IFN-stimulated response element (ISRE) in promoters of ISGs to prompt transcription. Hundreds of ISGs have direct virus targeting functions (e.g. MxA, RNase L and RNA deaminases), amplifying antiviral resistance (e.g. PKR, 2′5′OAS, and type I IFN themselves), and sequestration of cellular metabolic processes to repress virus replication (e.g. PKR-mediated arrest of protein synthesis) (Sen and Sarkar, 2007). It is notable that most notorious viruses have improved their capability to evade or subvert the IFN system for their own benefit. Extensive reviews

(Iannello *et al.*, 2006; Haller and Weber, 2007; Loo and Gale, 2007) on this topic indicate that a collection of virus-derived factors may interfere with IFN production and/or IFN-action pathways (Table 1).

In addition to type I IFNs, three type III IFNs (IFN-λ1–λ3, also known as IL-29, IL-28A and IL-28B, respectively), have been identified. Accumulated evidence indicates that type III IFNs are induced through similar signal transduction pathways as type I IFNs (Ank and Paludan, 2009). For example, activation of signaling pathways mediated by TLR3 and RIG-I, which has been well characterized to induce IFN-α/β production, also induces type III IFNs in murine and human cells (Ank and Paludan, 2009). The antiviral activity of human type III IFNs has been associated with multiple viral infections including those caused by hepatitis C virus, influenza A virus and cytomegalovirus (Ank *et al.*, 2006; Ank and Paludan, 2009). However, type III IFNs are distinct from type I IFNs in their gene and protein structures as well as their receptors and expression patterns. For example, genes of all known mammalian type III IFNs have multiple exons (usually five) in contrast to the single-exon genes for type I IFNs (Fox *et al.*, 2009). The protein structure of human IFN-λ3 more closely resembles IL-22 of the IL-10 cytokine family rather than other IFNs (Gad *et al.*, 2009). Critically, type III IFNs act through cell receptors of the IL-28RA/IL-10R2 complex rather than the IFNAR1/IFNAR2 receptors of the type I IFNs (Kotenko *et al.*, 2003; Sheppard *et al.*, 2003). In addition, type III IFNs and their receptors are prominent in epithelial tissues, suggesting their involvement in epithelial antiviral immunity (Ank and Paludan, 2009). Thus, type III IFNs comprise a group of newly identified antiviral cytokines that are functionally similar to type I IFNs and elicit first-line antiviral responses, especially in epithelial cells. We have shown that pigs have at least two type III IFNs, IFN-λ1 and IFN-λ3, and that both exert similar but lower activity than IFN-α/β against PRRSV in cells (Sang *et al.*, 2010b). Non-IFN cytokines, chemokines and AMPs are other groups of important innate immune effectors (Figure 2) (Beutler, 2004; Klotman and Chang, 2006; Pancer and Cooper, 2006; Takaoka and Yanai, 2006; Lehrer, 2007; Sang and Blecha, 2008; Umbach and Cullen, 2009; Hartshorn, 2010). The innate immune roles of cytokines and chemokines have been emphasized in aspects of pro-/anti-inflammation and attraction/activation of immune cells, which are important for overall immune responses. Although the antiviral activity of AMPs has been known for some time (Daher *et al.*, 1986), research in this area has recently intensified (reviewed in Klotman and Chang, 2006; Lehrer, 2007). We, along with others, have demonstrated that PRRSV infection suppresses the expression of AMPs and general antimicrobial activity especially in the lungs of young pigs (Sang and Blecha, 2009; Sang *et al.*, 2009; Jung *et al.*, 2010). Direct inactivation of PRRSV by porcine β-defensin (pBD) 3 and protegrin 4 has also been demonstrated *in vitro* (Sang *et al.*, 2009). Animal AMPs exert antiviral activity by distortion of virion glycoproteins and lipid membranes in enveloped viruses, and by impeding viral entrance into host cells. Other mechanisms of AMP antiviral activity have also been proposed, including down regulation of viral receptors (e.g. hBD3 for CXCR4 of receptor to HIV-1) (Feng *et al.*, 2006), modulation of cellular antiviral signaling (e.g. HNP-1 for PKC signaling) (Salvatore *et al.*, 2007) and potentiating adaptive immunity (Klotman and Chang, 2006). In addition, other carbohydrate binding proteins such as collectins, including Mannan-binding lectin (MBL), surfactant protein A (SP-A), surfactant protein D (SP-D) and ficolin, have been shown to participate in innate immune responses exerting antiviral activity. Recently, a porcine plasma ficolin was reported to have inhibitory activity against PRRSV replication in a N-acetylated glycan-dependent manner (Keirstead *et al.*, 2008). These findings indicate that intervening in the interaction between sugar moieties of the viral envelope and host cells is a target for innate immune molecules to inhibit PRRSV infection (Klotman and Chang, 2006; Lehrer, 2007).

The interaction of PRRSV and porcine innate immunity

Aberration of porcine innate immunity by PRRSV

Two decades ago, initial reports of PRRSV occurred almost simultaneously in the U.S. and central Europe. These viruses were defined as type 1 European-PRRSV and type 2 North American-PRRSV. PRRS continues to be the most significant worldwide swine disease and a persistent challenge in both immunology and vaccinology (Kimman *et al.*, 2009; Darwich *et al.*, 2010; Huang and Meng, 2010; Murtaugh *et al.*, 2010). PRRSV evolves at a high mutation rate compared with other RNA viruses and has the potential to subvert host innate immune responses by various means. These include intervening in cell recognition of the virus, diverting antiviral cytokine (especially type I IFNs, IL-1, IL-10 and TNF-α) production and action, directing cytolysis, reducing antigen presentation activity and suppressing phagocytic and microbicidal activity (Darwich *et al.*, 2010; Thanawongnuwech and Suradhat, 2010; Yoo *et al.*, 2010). The innate immune aberration could further contribute to inefficiently bridging adaptive immunity, which in cooperation with other diverting mechanisms on adaptive immunity, causes overall immune inefficacy to PRRSV and other co-infections (Darwich *et al.*, 2010).

Collectively, data show that PRRSV infection leads to an increase of most if not all viral sensing TLRs in the lungs or lymphoid organs, including TLR2, TLR3, TLR4, TLR7, TLR8 and TLR9 (Sang *et al.*, 2008b; Liu *et al.*, 2009; Xiao *et al.*, 2010a, b). The increased TLR transcripts of TLR7 and TLR9 may last for at least 1-week post infection

(Xiao *et al.*, 2010a, b) with TLR2 and TLR4 returning toward the basal levels after 3-days post infection. It appears that upregulation of TLR3 is condition-dependent. The *in vitro* infection of PAMs and mDCs (Sang *et al.*, 2008b; Chaung *et al.*, 2010) or *in vivo* infection of fetal lungs (Sang *et al.*, 2008b) fails to increase or even transiently decrease TLR3 expression. Similarly, no significant increase of TLR3 and TLR8 expression was found in lungs of pigs infected with the highly virulent Chinese-type PRRSV (Xiao *et al.*, 2010a, b). Expression of cytosol virus sensing receptors of RIG-I and Mda5 is also significantly stimulated in lungs infected by type 2 PRRSV (Xiao *et al.*, 2010a, b). The increase in these virus-sensing receptors may potentially lead to antiviral responses. Overexpression of TLR3 resulted in enhancement and reduction of TLR3 suppressed anti-PRRSV activity in virus-infected cells (Sang *et al.*, 2008b). Although it is unknown whether PRRSV has a mechanism to reduce the expression of these receptors, the activation of TLR7 and TLR9 signaling in pDCs appears inhibited by the presence of PRRSV (Calzada-Nova *et al.*, 2010a). The implication of data using MARC-145 cells is that PRRSV may interfere with adaptor proteins to suppress both RLR- and TLR3-mediated stimulation of IFN production (Luo *et al.*, 2008; Miller *et al.*, 2009). Notably, the acute activation of these receptor-mediated signaling pathways also leads to increased production of pro-inflammatory cytokines and chemokines as well as activation of the complement systems; this process is thought to be the cause of pneumonia in PRRS cases (Xiao *et al.*, 2010a). In addition, the inflammatory response could be amplified in the presence of bacterial endotoxin (Qiao *et al.*, 2011) or low-avidity immune complex (Monsalvo *et al.*, 2011) to promote macrophages skewing to the M2 status (Stout *et al.*, 2009). Human M2 macrophages show higher expression of CD163, heparin sulfate and IL-10 (Cassol *et al.*, 2010); similar responses in pigs could potentially exacerbate PRRSV infection (Patton *et al.*, 2009; Welch and Calvert, 2010).

The production and action of the type I IFN system are hallmarks of innate antiviral immunity. Earlier reports have shown that pigs infected with PRRSV produce very low levels of type I IFNs. Exogenous application of IFN-α could control the virus infection in porcine PAMs and MARC-145 cells (Albina *et al.*, 1998; Buddaert *et al.*, 1998). Studies by Lee *et al.* (2004) showed that different field isolates and even virus quasi-species rescued from individual plaque clones of the same isolate vary in their ability to induce IFN-α and susceptibility to IFN-α treatment. PRRSV also infects mDCs and significantly suppresses type I IFN production of the IFN-α subtype but not the IFN-β subtype (Loving *et al.* 2007). Extensive analysis of porcine type I IFN profiles indicates that pigs have as many as 39 functional genes including 17 IFN-α, 11 IFN-δ, 7 IFN-ω, as well as a single member of each of the IFN-αω, IFN-β, IFN-ε and IFN-κ subtypes (Sang *et al.*, 2010a). Comparative antiviral analyses in both porcine PAMs and MARC-145 cells indicate that most IFN-α and IFN-αω have higher activity against PRRSV infection than do other subtype members (Sang *et al.*, 2010a).

Determined by the stability of the ternary IFN-receptor complex, type I IFNs biological activities proceed in two directions, antiviral activity and immunomodulatory activity (Uzé *et al.*, 2007; Kalie *et al.*, 2008). Whereas IFN-α subtypes generally have more antiviral potency, IFN-β displays more immunomodulatory activity such as promotion of cell proliferation (Kalie *et al.*, 2008). Recent studies have shown that IFN-β has anti-inflammatory properties inducing IL-10 production in human mDCs (Wang *et al.*, 2011) and regulating alternative activation of macrophages through induction of IL-4, IL-13 and IL-10 in RSV infected murine lungs (Shirey *et al.*, 2010). Interestingly, PRRSV suppression of IFN-β production through targeting IRF3 has been mostly observed in MARC-145 cells or experimental human cell lines (Miller *et al.*, 2004; Luo *et al.*, 2008; Shi *et al.*, 2010; Chen *et al.*, 2010a; Kim *et al.*, 2010; Li *et al.*, 2010; Beura *et al.*, 2010; Song *et al.*, 2010). These observations are not always consistent with the data from infected pigs or porcine cells (Loving *et al.*, 2007; Genini *et al.*, 2008). In contrast, *in vivo* or *ex vivo* tests in porcine lungs strongly support PRRSV-mediated IFN-α suppression in porcine lungs (Jung *et al.*, 2009), PAMs (Albina *et al.*, 1998; Genini *et al.*, 2008; Patel *et al.*, 2010), mDC (Loving *et al.*, 2007) and pDCs (Calzada-Nova *et al.*, 2010a, b).

Cell type difference in respect to response to PRRSV mediated IFN-suppression has recently been noted (Loving *et al.*, 2008; Sang *et al.*, 2010a; He *et al.*, 2011). In addition, the suppression of IFN-δ or IFN-ω (termed SPI IFN in the reference) has also been observed (Xiao *et al.*, 2010b; Sang, unpublished data); therefore, whether all type I IFNs are generally suppressed or differentially regulated by PRRSV *in vivo* to facilitate virus infection should be determined. PRRSV suppresses type I IFN signaling primarily in infected cells, as indicated by suppression of ISG15 and ISG56 in PRRSV infected MARC-145 and PAMs (Patel *et al.*, 2010). However, exogenous application of IFNs does prevent PRRSV-suppression of IFN signaling due to the treatment of PAMs with either porcine type I, II or III IFNs, and especially IFN-α, which induce significant anti-PRRSV activity (Rowland *et al.*, 2001; Sang *et al.*, 2010a, b).

PRRSV infection also diverts the production of other cytokines and antimicrobial molecules, such as AMPs and nitric oxide (NO). However, reports regarding PRRSV regulation of cytokines are quite controversial. Both increases and decreases of pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6, have been shown in PRRSV infected pigs and PAMs (Darwich *et al.*, 2010). PRRSV does prevent the production of IL-8 (Calzada-Nova *et al.*, 2010a) in pDCs but other studies have found that PRRSV induces IL-8 (Darwich *et al.*, 2010). More extensive cytokine analyses have linked IL-1β, IL-8 and IFN-γ but not IL-12 with PRRSV clearance (Lawson *et al.*, 2010;

Lunney *et al.*, 2010). IL-10 is an immunosuppressive cytokine, which is up-regulated by PRRSV infection (Thanawongnuwech and Suradhat, 2010) and induces PAM permissiveness to PRRSV (Patton *et al.*, 2009). Similar to the regulation of other cytokines, PRRSV infected pigs or cells have been shown to have either increased or decreased IL-10 production (Klinge *et al.*, 2009; Darwich *et al.*, 2010; Subramaniam *et al.*, 2011). Therefore, as suggested by studies of Díaz *et al.* (2006) and Silva-Camp *et al.* (2010), PRRSV-regulation of IL-10 may be both pig- and virus-strain dependent. Studies evaluating PRRSV-suppression of antimicrobial and phagocytic activities are consistent in virus-infected lungs and PAMs, showing that, in general, PRRSV decreases the production of AMPs and NO, and suppresses the microbicidal activity of both PAMs and NK cells (Thanawongnuwech *et al.*, 2000; Jung *et al.*, 2009; Sang *et al.*, 2009). PRRSV also directs cytolysis in infected PAMs and mDCs and cell death is prominent in activated M2 macrophages (unpublished data). The suppression of microbicidal activity and direction of cell death of innate immune cells at the cellular and molecular levels may be linked to co-infections with PRRSV.

Viral mechanisms responsible for innate immune aberration

PRRSV is an enveloped virus, which has a ~15 kb (+)ssRNA genome containing nine open reading frames (ORF). The 5′ end ORF1a and ORF1b encode two replicase polyproteins, pp1a and pp1b. The proteolytic pp1a products self cleave into nine non-structural proteins (Nsp) (Nsp1α, Nsp1β and Nsp2–8), and cleave pp1b into four Nsp (Nsp9–12). The 3′ end seven ORFs encode four minor (GP2a, GP3, GP4 and E proteins) and three major (GP5, M and N proteins) structural proteins. Besides their essential role in viral replication, recent studies highlight the roles of the Nsp in immune modulation of innate immune effectors (Fang and Snijder, 2010; Yoo *et al.*, 2010). Four viral Nsp have strong to moderate inhibitory effects (Nsp1>Nsp2>Nsp11>Nsp4) on IFN-β production through inactivating IRF3, which is a key transcription factor responsible for activation of the IFN-β promoter (Beura *et al.*, 2010).

Nsp1 and its two autocleaved products, Nsp1α and Nsp1β, have been shown to have the highest activity inhibiting IRF3 activation (Beura *et al.*, 2010; Yoo *et al.*, 2010). Beura *et al.* (2010) indicated that Nsp1α and Nsp1β block IRF3 nuclear translocation; however, Yoo *et al.* (2010) observed no blocking of Nsp1α and Nsp1β in IRF3 nuclear translocation, and proposed a mechanism based on Nsp1 promoting degradation of the CREB (cyclic AMP response element binding)-binding protein (CBP). CBP has histone acetyltransferase activity functioning in dissociation of histones from the DNA promoter region, and the CBP/p300 co-activators function in concert with a variety of transcription factors including STATs, NF-κB, PIAS1 and the IRF family (Yoo *et al.*, 2010). Nsp1-mediated CBP-degradation may provide a general explanation of PRRSV immune suppression via interaction with IRF3 as well as STAT2, because the decrease in STAT2 at the protein level has been reported in PRRSV-infected cells (Patel *et al.*, 2010). In another report, both Nsp1α and Nsp1β were shown to dramatically inhibit IFN-β expression and Nsp1β also suppressed IFN signaling via inhibiting STAT1 nuclear translocation (Chen *et al.*, 2010a). Patel *et al.* (2010) further demonstrated that STAT1 is not the only factor inhibited as nuclear translocation of ISGF3 (ISG factor 3, composed by STAT1/STAT2/IRF9) was also inhibited by Nsp1β to suppress IFN-α signaling in the virus-infected cells. PRRSV does not infect pDCs, but the presence of some uncharacterized viral component blocks STAT1 nuclear localization thereby reducing the availability of IRF7, which has been thought to be a mechanism for PRRSV inhibition of IFN-α production in pDCs (Calzada-Nova *et al.*, 2010b). Nsp1α and Nsp1β also block NF-κB activation (Song *et al.*, 2010), a critical transcription factor in innate immune signaling not only for antiviral responses; therefore, Nsp1 proteins may also be the viral components responsible for suppressing other cytokines and AMPs in addition to IFNs (Figure 3).

PRRSV Nsp2 represents another major immunomodulatory protein (Fang and Snijder, 2010). The region of 691–722 residues in Nsp2 has been shown to be important for virus mediation of production of pro-inflammatory cytokines including IL-1β and TNF-α (Chen *et al.*, 2010b). Biochemically, Nsp2 belongs to the deubiquitinase superfamily; through this activity, it can interfere with the polyubiquitination process of ISG15 and IKKα (inhibitor of nuclear factor kappa-B kinase (NF-κB) subunit α), thereby targeting the IFN response and NF-κB signaling pathways respectively (Sun *et al.*, 2010).

PRRSV Nsp11 also has dual roles in suppression of IFN responses (Yoo *et al.*, 2010) and reduction of NF-κB activation (Beura *et al.*, 2010). Nsp11 has endo-RNase activity; its suppression of IFN response may partially be due to cleavage of viral RNA patterns to dampen the binding by multiple cellular antiviral receptors including TLR3/7/8/9, RIG-I, and Mda5, thus reducing the upstream viral sensing in antiviral signaling (Yoo *et al.*, 2010). In addition, Nsp11 has been shown to block IRF3 phosphorylation and nuclear translocation (Yoo *et al.*, 2010), but it is unclear whether this resulted from weakened upstream signaling (i.e., diminishing dsRNA binding) or Nsp11 targeting IRF3 directly through other mechanisms.

As previously mentioned, PRRSV has been reported to strongly induce IL-10 production during the early phase of infection in pigs (Thanawongnuwech and Suradhat, 2010) and in cultured porcine PBMCs and PAMs (Yoo *et al.*, 2010). The viral mechanism to induce IL-10 is reported to be the nucleocapsid (N) protein (Yoo *et al.*, 2010). The N protein is a small basic protein of 123 (or 128

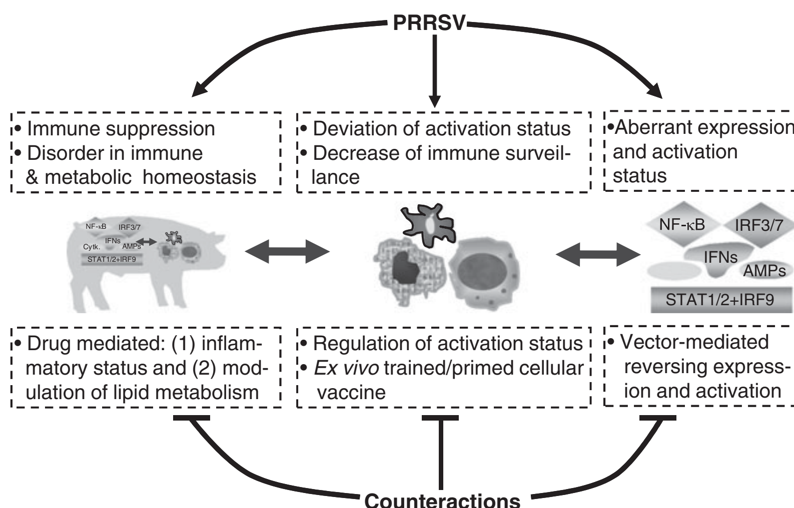

Fig. 4. Tactics to counteract PRRSV deviation of porcine innate immunity at the molecular, cellular and animal levels. Viral aberration is indicated in the top portion of the figure. Vector-based expression or suppression of the virus-aberrant antiviral effectors (or signaling molecules) may work alone or in concert with a subunit vaccine to stimulate effective anti-PRRSV protection. Innate immune cells, especially PRRSV-infected monocytic cells, are platforms to manipulate virus-aberrant cell activation status and innate immune response. In pigs, drug-based approaches to modulate inflammatory and lipid metabolic status may be feasible to alter host response to vaccination or infection.

of type I PRRSV) residues and it is the most abundant virion component and the most immunogenic protein in virus-infected pigs and cells (Music and Gagnon, 2010). The mechanism of N protein induction of IL-10 is unknown. Given that nucleolus-localized N protein interacts with several cell transfactors (Yoo *et al.*, 2010), there is potential of the N protein directly targeting the IL-10 gene promoter. In addition, recent evidence shows that IFN-β, produced from LPS activation of macrophages or DCs, induces IL-10 in these cells (Chang *et al.*, 2007; Wang *et al.*, 2011). The N protein may activate the NF-κB pathway to induce IFN-β production through TLR4 activation of NF-κB pathways, therefore, inducing IL-10 stimulation and NF-κB activation with LPS-TLR4 signaling (Chang *et al.*, 2007; Wang *et al.*, 2011). This assumption is supported by the understanding that N protein does activate NF-κB in MARC-145 cells and the region between residue 30–73 of N protein is essential for this function (Luo *et al.*, 2011), and TLR4 is induced in PRRSV infected pigs (Xiao *et al.*, 2010a). Integrating the points of N protein activation of NF-κB, LPS-TLR4 mediated IFN-β production and IL-10 induction by IFN-β explains several controversial observations such as the induction/no-induction of IFN-β and IL-10 as well as the activation/suppression of NF-κB in PRRSV infected pigs or cells. Therefore, the consequent levels of IFN-β and IL-10 (and probably other innate immune effectors too) are not only dependent on PRRSV infection but also the activation of LPS-TLR4 signaling by some bacterial endotoxins; and the suppression or activation of NF-κB signaling should be variable according to the intensity and tempo between the N protein's positive and Nsp's negative effects. In summary, innate immune deficiency caused by PRRSV infection complicates the viral disease creating a complex syndrome. The challenge faced is not only from the small virus *per se*, but mostly, if not always, from the interaction of other opportunistic infections in a host with a disruption in homeostasis.

Concluding remarks

In Figure 4, we briefly summarize our understanding of PRRSV diversion of innate immunity at the levels of the animal, cell and molecule. We propose that through viral parasitization and interaction to deviate the expression and/or activation states of innate immune molecules, PRRSV alters the environment for development/activation of innate immune cells, and particularly dominates the functional status of monocytic cells including tissue macrophages and circulating DCs by direct infection or standby suppression in pDCs. The viral aberration of innate immunity has been shown at the cellular level in two aspects. Firstly, the skewing or adapting of innate immune cells to the status of anti-inflammation and immunosuppression at the early phase of infection, such as the M2 status of macrophages, which are observed to be more permissive to PRRSV infection than M1 cells (Patton *et al.*, 2009; Sang, unpublished data). Secondly, there is suppression of immune surveillance including dampened microbicidal and antigen processing activity in DCs and macrophages (Sang *et al.*, 2009; Jung *et al.*, 2009; Thanawongnuwech and Suradhat, 2010).

Pertaining to the characteristics of tissue- or cell-tropism, the primary sites of PRRSV infection, including reproductive and pulmonary tissues, contain monocytic cells that are naturally inclined to a M2 or immunosuppressive

status, especially those in the reproductive tissues with immune privilege. This viewpoint suggests that PRRSV has evolved to adapt rather than skew innate immune cells to establish an infection at the animal level, and the high-virulence PRRSV strains, which cause significant host mortality because of the dramatic inflammatory response (Xiao *et al.*, 2010a), are unique cases of unsuccessful parasitization. Therefore, the suppression of M1 activation especially antiviral status induced by type I IFNs in innate immune cells during the early phase of infection might be a key for designing counteractions to target PRRSV infection at both cell and animal levels. However, unlike in *ex vivo* cells, time and intensity are more critical for the success of inflammatory and immune regulation *in vivo* (Figure 4). In this line, some herbal therapies, which have been shown effective in treatment of inflammatory symptoms in SARS (Leung, 2007) and pandemic influenza (Ge *et al.*, 2010), are worth evaluating in PRRSV pandemics. In addition, lipid metabolites such as sphingosine-1-phosphate (S1P) have been known for critical regulation of inflammation and immune cell recruitment/activation (Spiegel and Milstien, 2011). Several recent studies elegantly linked lipid metabolism to immune status of macrophages (Im *et al.*, 2011), T cells and DCs (Herber *et al.*, 2010), as well as cytokine storms in influenza-infected lungs (Teijaro *et al.*, 2011). It will be informative to examine the role of modulators/metabolites of lipid metabolism in regulation of inflammatory and immune status during PRRSV infection and vaccine development.

Finally, the development of vaccines to induce effective protection against heterologous PRRSV isolates should be focused at the cellular and molecular levels. To this end, the adjuvant mechanisms underlying innate immunity (Coffman *et al.*, 2010) should be considered in respect to PRRSV-diverted innate immune components as reviewed above. Positive effects have been obtained in studies with expression of IFN-α alone (Brockmeier *et al.*, 2009) or conjugated expression of innate immune effectors including CD40L (Cao *et al.*, 2010), GM-CSF (Wang *et al.*, 2009) and HSP70 (Li *et al.*, 2009b) with the viral epitopes using adenovirus vectors. In addition, several studies using RNA interference techniques have shown promise in suppression of PRRSV infection in both cells and pigs (Lu *et al.*, 2006; Li G *et al.*, 2009a), implying that miRNA-mediated antiviral mechanisms, which have been found in regulation of HCV and HIV infection (Skalsky and Cullen, 2010), could be also functional in PRRSV-host interaction. Viruses are dependent on cell metabolism and genome-wide screening of host factors critical for host–viral interaction has revealed a large number of candidates belonging to metabolic pathways besides immune genes (Karlas *et al.*, 2010; König *et al.*, 2010). To this end, it is likely that aberration of lipid metabolism is a major consequence in PRRSV-host interaction (Xiao *et al.*, 2010b) and the regulation of lipid signaling has potential to prime activation status and immune surveillance of innate immune cells. In summary, to induce ideal anti-PRRSV protection, strategies should be considered for targeting innate immune components to counteract viral replication/spreading and subversion of immunity as well as to mitigate immune pathology from excessive/persistent activated innate immune responses (Figure 4).

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