Review article

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Porcine reproductive and respiratory syndrome virus vaccine does not fit in classical vaccinology

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All vaccines are developed to elicit an effective immune response in vaccinated animals such as innate, humoral and cell mediated response to protect animal health. Quality and intensity of the immune responses are differing by characteristics of the vaccine formulation and nature of the infectious agent. Modified live virus vaccines showed advantages over killed vaccines in terms of rapid immune response, duration of the immunity and better cell mediated protection mechanism. The porcine reproductive and respiratory syndrome virus (PRRSV) is relatively newly emerging (1986 in United States, 1990 in Europe) viral pathogen in pigs and tremendous effort has been made to protect pigs from this economically devastating disease such as developing killed, modified live, recombinant protein based and DNA vaccines. However, only cell culture attenuated virus vaccine is practiced with arguably limited efficacy. The PRRSV vaccine did not clear virus from infected pigs nor prevent re-infection of the virus. The vaccine showed very limited innate immune response, low anamnestic immune response and negligible cell mediated immune response. Despite of the current developed scientific technology, there still remain many questions to solve a most important pig disease worldwide.

Keywords: Immunity, Porcine respiratory and reproductive syndrome virus, Vaccines

Introduction

Porcine reproductive and respiratory syndrome (PRRS) was first described in 1987 in United States and subsequently discovered in 1990 in Europe. Despite ofchronicle proximity of the discovery, presumably derived from common ancestor and structural similarity of the viruses, these isolates from two different continents showed very distinct genotypes. The PRRS virus (PRRSV) has spread worldwide and became a most economically important disease in swine industry. PRRSV is a single-stranded, positive-sense RNA virus belongs to family *Arteriviridae* of the order of *Nidovirales*. The virus has genome of ~15 kb which encode around 10 open reading frames, ORF1a, ORF1b, ORF2a, ORF2b, ORF3, ORF4, ORF5, ORF6, and ORF7. Viral protease process translated polyproteins of ORF1a and ORF1b into 14 different non-structural proteins (NSPs). These NSPs have been identified to have function in viral replication and transcription machinery, and some of them involved in regulation of the viral pathogenesis through their involvement in host innate immune response modulation [1].

The major clinical features of the disease are late term reproductive failure in preg-

nant sows and respiratory problems in piglets and growing pigs resulting in enormous economic loses. A tremendous effort has been made to protect pigs from this economically devastating disease by developing such as killed, modified live, recombinant protein based, and DNA vaccines [2-6]. But, only cell culture attenuated virus vaccine is practiced in the field with arguably limited efficacy. The PRRSV vaccine did not clear virus from infected pigs nor prevent re-infection of the virus (Fig. 1) [7]. The vaccine showed no adequate innate immune reaction, low anamnestic immune response and negligible cell mediated immunity [8]. Despite of the current developed scientific technology, there still remain many questions to solve a most important pig disease worldwide. Therefore, no vaccines providing solid protective immunity with excellent safety for the PRRSV have been developed yet.

Infectious diseases in modern animal production exert an enormous economic burden in the industry, instability of the meat market, animal welfare and may threaten human health depending on the infectious agents.To protect animals from infectious disease, vaccinology is considered a most effective solution in animal health. Series of good animals vaccines such as modified live vaccine (MLV) of classical swine fever and canine distemper known to be very effective in vaccinated animals to protect respective virus infection after proper vaccination. Influenza vaccines in animals and human have difficulties to formulate precise antigen make up to expect seasonal epidemics of the serotype and/or subtypes within the serotypes. Genetic heterogeneity of the field virus dampens efficacy of the vaccine and protection of the disease in vaccinated herd but still antigens in the vaccine formulation elicit good immune response.

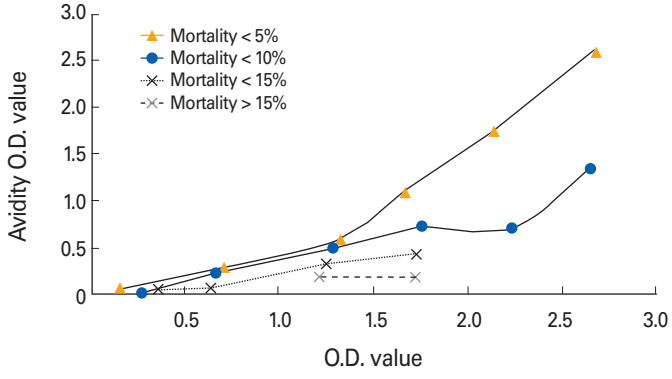

Fig. 1. Relationship between pig mortality and antibody avidity to porcine reproductive and respiratory syndrome virus (PRRSV) was investigated in pig farms. Mortality of over 15%, 10%-15%, 5%-10%, and less than 5% were grouped and avidity of the antibody to PRRSV was determined. Reproduced from Seo and Sunwoo (2012), Proceeding of the 22nd International Pig Veterinary Society Congress [7].

Despite the current scientific achievement in immunology and vaccinology, vaccines against human immunodeficiency virus (HIV) in human and African swine fever in pigs are not available at the moment. Efficacy of the animal vaccines currently available varies depending on immunogens in vaccine formula such as modified live, killed or recombinant proteins.

PPRSV persist long term in the infected host due to the delayed and lack of both innate and adaptive immunity. As well huge discrepancies were observed between experimentally infected groups in isolated facilities and observations made in the farm animals.

Innate Immunity

The early stage of a virus infection in animals is a critical race between the infected virus and host's immune system, in which host try to halt virus replication and defend invaders. There are various physico-chemical barriers to defend against virus infection such as acidic pH, proteolytic enzymes, and layers of mucus. The innate immune system plays very important role in control of the early stage of virus replication and delay spread of the virus to other parts of the body. Especially, the innate immunity differs from specific immunity, which elicit broad-spectrum of the anti-viral effect. Innate immunity is triggered by recognition of the microbial patterns characteristic of virus but not host cellular components. Type I interferons (IFNs) exert direct anti-viral activity as well activate other innate and adaptive immune responses. Measuring type I IFN response in virus infected animals is often used as a reliable good tool to evaluate innate immunity of the host. The type I IFN is a major player of the host innate immune response in viral infection. During a virus infection and replication process, viral intermediates trigger the expression of the IFN gene in cells and infected host. In this events, the RIG-1 like helicase and TLR-3 trigger signaling cascade and activate IRF3, nuclear factor kB and ATF-2 that drives IFN gene expression. Nsp1 (nsp1 subunits) of the PRRSV partly blocks type I IFN production in the virus infected cells [1,9]. The PRRSV infected host either does not produce IFN or showed significantly reduced production of the IFN, which is an important host defense mechanism in virus infected cells that eventually provide virus spread to the adjacent cells. Not only nsp1 but other PRRSV nsp are also modulate innate immune signaling pathways (Table 1).

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Table 1. Arterivirus proteins modulating innate immune signaling

Virus	Protein	Modulatory function for innate immunity
PRRSV	nsp1*a*	Inhibits production of type I IFNs and impairs IFN promoter activity
		Suppresses NF-κB activation
		Induces CBP degradation
		Suppresses TNF-α promoter activity
	nsp1*13*	Inhibits production of type I IFNs and impairs IFN promoter activity
		Impairs IRF3 phosphorylation and IRF3 nuclear localization
		Interferes with IFN-α induction and ISG expression
	nsp2 (PLP2)	Blocks nuclear translocation of ISGF3 by inducing KPNA1 degradation
		Suppresses TNF-α promoter activity
		Antagonizes type I interferon induction
		Interferes with NF-κB signaling pathway
		Prevents IκBα degradation by OTU domain
		Inhibits ISG15 production and ISGylation
		Activates NF-κB
	nsp4	Inhibits IFN-13 promoter activity
		Suppresses NF-κB mediated signaling pathway in the nucleus
	nsp11	Impair IFN promoter activity
		Participates in suppression of RIG-I signaling
		Degrades mRNA of IPS-1
		Inhibits production of type I IFNs and impairs IFN promoter activity
	N	Impairs IRF3 phosphorylation and IRF3 nuclear localization
		Upregulates IL-10 gene expression
		Activates NF-κB
EAV	nsp1	Inhibits production of type I IFNs and impairs IFN promoter activity
	nsp2 (PLP2)	Inhibits RIG-I-mediated innate immune signaling
		Inhibits RIG-I ubiquitination by its DUB activity
LDV	nsp1*a*	Inhibits production of type I IFNs and impairs IFN promoter activity
		Induces CBP degradation
	nsp1*13*	Inhibits production of type I IFNs and impairs IFN promoter activity
SHFV	nsp1*a13*	Inhibits production of type I IFNs and impairs IFN promoter activity

Han and Yoo. Virus Res 2014;194:100-9 [1].
PRRSV, porcine reproductive and respiratory syndrome virus; nsp, nonstructural protein; IFN, interferon; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; CBP, CREB-binding protein; TNF, tumor necrosis factor; IRF, interferon regulatory transcription factor; ISG, interferon-stimulated gene; PLP, papain-like cysteine protease; ISGF, interferon-stimulated gene factor; KPNA, karyopherin alpha 1; IκBα, NF-κB1; OTU, ovarian tumor; IL, interleukin.

Humoral Immunity

Specific antibodies to the antigen provide major barrier against virus spread between susceptible cells and tissues are particularly important in limiting virus spread through vascular system. Classical immunology claims that neutralizing antibody is the first line of the defense against free virus particles. Virus neutralization test provides an important indicator for the humoral protection index in virus-infected host. PRRSV is different other common virus infection. Pigs either infected or vaccinated pigs respond to the PRRSV proteins and produce virus specific antibodies but early antibodies did not show virus-neutralizing activities [10]. As shown in Fig. 2, viremia last long time in the infected pigs and gradually diminished and total antibody response was similar to that of the other viral infection but appearance of the serum neutralizing antibody was independent to viral clearance (Fig. 2) [11]. This indicates that virus clearance is not directly responsible for the protection even neutralization antibody is an important factor for the humoral protection mechanism. Research data obtained in late 90s thought PRRSV is persistent in the infected animals, which is reliable reason that memory responses would be difficult to demonstrate *in vivo* due to the continual presence of antigen that could simply be eliciting

active immunity [12]. During the course of acute with prolonged viremia, lymphoid tissue infection, PRRSV-specific memory B-cells peak in number at 40-100 days of infection then declined [13]. But, re-challenge with genetically similar or unrelated viruses at about 200 days of infection did not show any noticeable anemnestic response (Murtaugh, unpublished data). Rather, the parameters of infection and host immunity need to be determined and immunological effector responses evaluated for usefulness as surrogate measures of protection against future infectious challenge. Another key feature of the adaptive immunity are the establishment of the immunological memory, which is very common in animals vaccinated and/or naturally infected. However, the PRRSV vaccinated and/or infected pigs did not show booster effect by the second exposures of the antigen [14]. Measuring total antibodies by an enzyme-linked immunosorbent assay or neutralization antibodies by virus neutralization test did not provide similar result that of other animal vaccines (Fig. 1). The research finding from the previous study that antibody avidity correlates with virus clearance from the circulating

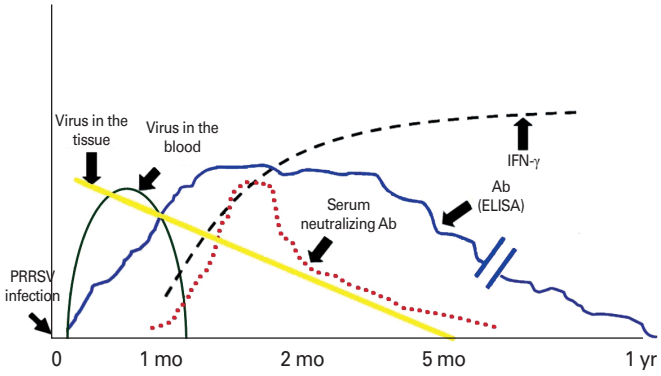

Fig. 2. The porcine reproductive and respiratory syndrome virus (PRRSV) infection and repertoire of the immune response in the virus infected pigs. Reproduced from Lopez and Osorio (2004), Elsevier, Veterinary Immunology and Immunopathology [11]. ELISA, enzyme-linked immunosorbent assay; IFN-γ, interferon γ.

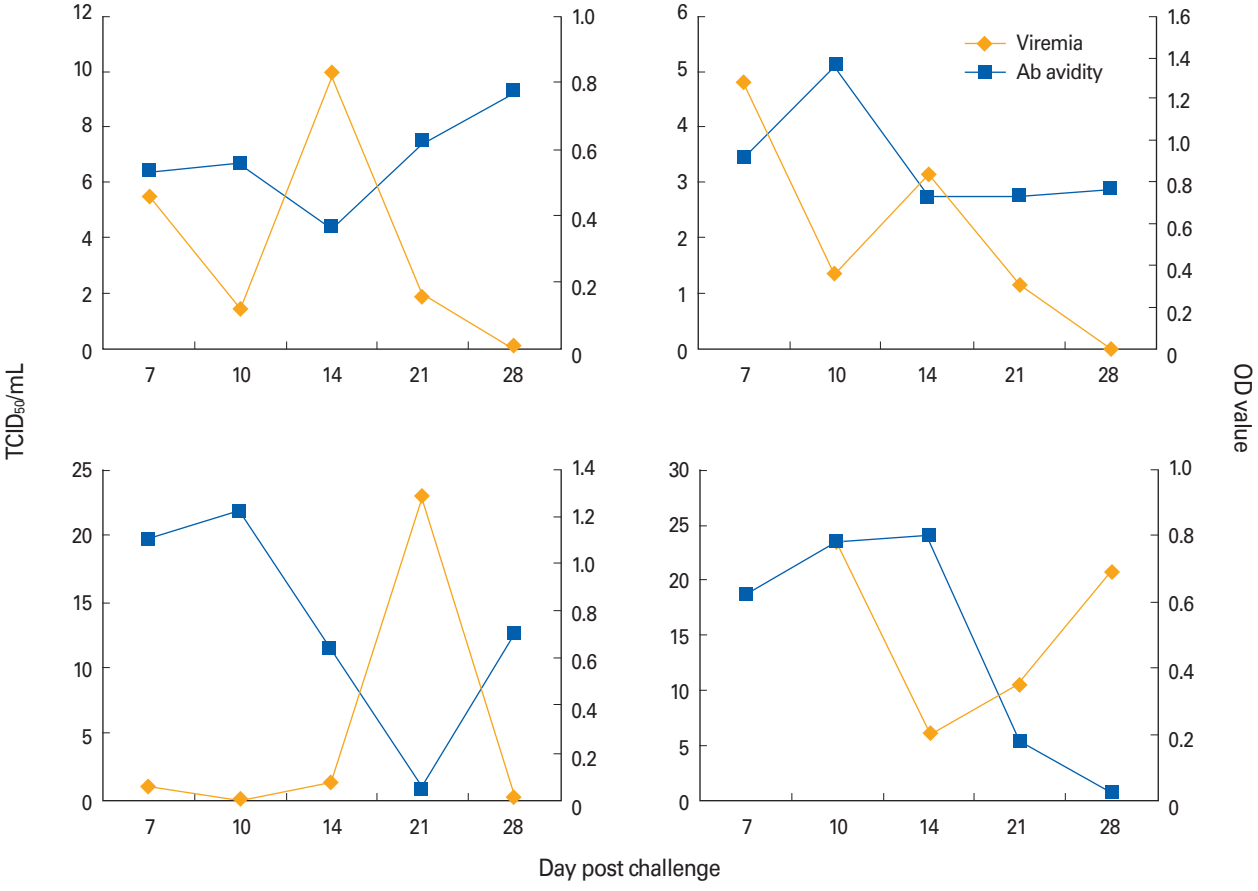

Fig. 3. Antibody maturation leads virus clearance in the vaccinated pigs after challenge. Pigs were vaccinated and virus in the blood and avidity of the antibody specific to porcine reproductive and respiratory syndrome virus (PRRSV) was monitored. Reproduced from Seo and Sunwoo (2012), Proceeding of the 22nd International Pig Veterinary Society Congress [7].

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blood indicates that maturation of the antibody plays an important role in free virus control in the virus infected host animals. Groups of pigs with high avidity to PRRSV showed low mortality and low avidity groups showed relatively higher mortality (Figs. 1, 3).

Cell Mediated Immunity

T cells exhibit multiple functions in antiviral immunity such as restricting establishment of virus infections, immunoglobulin isotype switching and maturation, activation of macrophages and protecting re-infections of the virus to other tissues.

To promote virus survival in the infected host, viruses have evolved various strategies for evasion from the host immune system. Evasion strategies of the virus can be categorized according to mechanisms such as impairing host response, avoiding recognition by the host immune system and restricting control by immune effector mechanism. In the course of the either European or North American type virus infection showed very little if any PRRSV-specific IFN-γ releases response from whole blood, whereas non-specific responses were consistently observed [15-17]. As shown in Fig. 2 viral clearance in the blood and viral load in the lymphoid tissue does not co-relate with cell-mediated immunity. IFN-γ assay is neither absolute nor only indicator for the evaluation of the cell-mediated immunity but this assay is common method for the cell-mediated immune (CMI) response. Highly variable number of the T cell in either acutely or persistently animals were detected and showed no close correlation to the level of the virus in the lymphoid tissues. There are no significant changes in CD4+ and CD8+ T-cell frequencies after PRRSV infection were observed, though a decrease number of gamma and delta T cells were recorded [18]. This result support that there is no or very little contribution of the CMI to the PRRSV infection and suggest PRRSV suppresses T-cell recognition of the infected macrophages. Even weak CMI response in PRRSV infection, 9 antigenic regions on the 5 of the viral proteins determined by IFN-γ ELISpot assay indicate that there are T-cell specific epitopes on the viral proteins [19].

Conclusion

Despite the current scientific achievement in immunology and vaccinology, vaccines against HIV in human and African swine fever in pigs are not available at the moment. Efficacy of the animal vaccines currently available varies depending on immunogens in vaccine formula such as modified live, killed or recombinant proteins. To protect animals from infectious diseases, vaccination has been considered a most effective solution in animal health. Series of good animal vaccines has been developed and practiced such as MLV of classical swine fever and canine distemper. These vaccines are known to be very effective in protection against the respective disease agents for the properly vaccinated animals. Despite the good success of various types of vaccines, influenza vaccines in animals and human have been experienced difficulties in formulating precise antigen make-up through expecting in-coming seasonal epidemics of the serotype and/or subtypes within the serotypes. Often genetic heterogeneity of the field virus dampens efficacy of the vaccine and protection of the disease in vaccinated herd but still antigens in the vaccine formulation elicit good immune response. Several viral agents efficiently evade host immune system and survive prolonged time in the host tissues.

Infectious diseases in modern animal production exert an enormous economic burden in the industry, instability of the meat market, animal welfare and may threaten human health depending on the infectious agents.

PPRSV persist long term in the infected host due to the delayed and lack of both innate and adaptive immunity. As well huge discrepancies were observed between experimentally infected groups in isolated facilities and observations made in the farm animals [20]. Viruses evolved and earned various evasion strategies to host immune system. PRRSV have at least several evasion tools to survive prolonged time in the infected host [21,22]. Non-structural proteins of the PRRSV plays very important role to inhibit innate immunity and several mechanisms were investigated. Since there is no close correlation between viral clearance and neutralization antibody, we need to find a mechanism behind viral elimination from the blood and tissues. Viruses evolved and earned various evasion strategies to host immune system. PRRSV have at least several evasion tools to survive prolonged time in the infected host based on the known fact such as poor innate immunity, low anamnestic humoral immune response and negligible cellular immunity in early infection. PPRSV persist long term in the infected host due to the delayed and lack of both innate and adaptive immunity. As well huge discrepancies were observed between experimentally infected groups in isolated facilities and observations made in the farm animals. Current understandings on the PRRSV vaccinology is a

tip of icebergs and further studies to develop measures against PRRSV infection in the pigs are seemed to be needed.

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References

1. Han M, Yoo D. Modulation of innate immune signaling by nonstructural protein 1 (nsp1) in the family Arteriviridae. Virus Res 2014;194:100-9.
2. Carter QL, Curiel RE. Interleukin-12 (IL-12) ameliorates the effects of porcine respiratory and reproductive syndrome virus (PRRSV) infection. Vet Immunol Immunopathol 2005;107:105-18.
3. Charerntantanakul W, Yamkanchoo S, Kasinrerk W. Plasmids expressing porcine interferon gamma up-regulate pro-inflammatory cytokine and co-stimulatory molecule expression which are suppressed by porcine reproductive and respiratory syndrome virus. Vet Immunol Immunopathol 2013;153:107-17.
4. Meier WA, Husmann RJ, Schnitzlein WM, Osorio FA, Lunney JK, Zuckermann FA. Cytokines and synthetic double-stranded RNA augment the T helper 1 immune response of swine to porcine reproductive and respiratory syndrome virus. Vet Immunol Immunopathol 2004;102:299-314.
5. Rompato G, Ling E, Chen Z, Van Kruiningen H, Garmendia AE. Positive inductive effect of IL-2 on virus-specific cellular responses elicited by a PRRSV-ORF7 DNA vaccine in swine. Vet Immunol Immunopathol 2006;109:151-60.
6. Xue Q, Zhao YG, Zhou YJ, et al. Immune responses of swine following DNA immunization with plasmids encoding porcine reproductive and respiratory syndrome virus ORFs 5 and 7, and porcine IL-2 and IFNgamma. Vet Immunol Immunopathol 2004;102:291-8.
7. Seo SW, Sunwoo SY. PRRSV antibody maturation is time dependent in the infected pigs. In: Proceeding of the 22nd International Pig Veterinary Society Congress; 2012 Jun 10-13; Jeju, Korea. IPVS Press; 2012.
8. Meier WA, Galeota J, Osorio FA, Husmann RJ, Schnitzlein WM, Zuckermann FA. Gradual development of the interferon-gamma response of swine to porcine reproductive and respiratory syndrome virus infection or vaccination. Virology 2003;309:18-31.
9. Beura LK, Subramaniam S, Vu HL, Kwon B, Pattnaik AK, Osorio FA. Identification of amino acid residues important for anti-IFN activity of porcine reproductive and respiratory syndrome virus non-structural protein 1. Virology 2012;433:431-9.
10. Labarque GG, Nauwynck HJ, Van Reeth K, Pensaert MB. Effect of cellular changes and onset of humoral immunity on the replication of porcine reproductive and respiratory syndrome virus in the lungs of pigs. J Gen Virol 2000;81:1327-34.
11. Lopez OJ, Osorio FA. Role of neutralizing antibodies in PRRSV protective immunity. Vet Immunol Immunopathol 2004;102:155-63.
12. Wills RW, Zimmerman JJ, Yoon KJ, et al. Porcine reproductive and respiratory syndrome virus: a persistent infection. Vet Microbiol 1997;55:231-40.
13. Mulupuri P, Zimmerman JJ, Hermann J, et al. Antigen-specific B-cell responses to porcine reproductive and respiratory syndrome virus infection. J Virol 2008;82:358-70.
14. Murtaugh MP. Advances in swine immunology help move vaccine technology forward. Vet Immunol Immunopathol 2014;159:202-7.
15. Barranco I, Gomez-Laguna J, Rodriguez-Gomez IM, et al. Immunohistochemical expression of IL-12, IL-10, IFN-alpha and IFN-gamma in lymphoid organs of porcine reproductive and respiratory syndrome virus-infected pigs. Vet Immunol Immunopathol 2012;149:262-71.
16. Lopez Fuertes L, Domenech N, Alvarez B, et al. Analysis of cellular immune response in pigs recovered from porcine respiratory and reproductive syndrome infection. Virus Res 1999;64:33-42.
17. Wesley RD, Lager KM, Kehrli ME Jr. Infection with Porcine reproductive and respiratory syndrome virus stimulates an early gamma interferon response in the serum of pigs. Can J Vet Res 2006;70:176-82.
18. Xiao Z, Batista L, Dee S, Halbur P, Murtaugh MP. The level of virus-specific T-cell and macrophage recruitment in porcine reproductive and respiratory syndrome virus infection in pigs is independent of virus load. J Virol 2004;78:5923-33.
19. Mokhtar H, Eck M, Morgan SB, et al. Proteome-wide screening of the European porcine reproductive and respiratory syndrome virus reveals a broad range of T cell antigen reactivity. Vaccine 2014;32:6828-37.
20. Dotti S, Guadagnini G, Salvini F, et al. Time-course of antibody and cell-mediated immune responses to porcine reproductive and respiratory syndrome virus under field

conditions. Res Vet Sci 2013;94:510-7.
21. Chung HK, Chae C. Expression of interleukin-10 and interleukin-12 in piglets experimentally infected with porcine reproductive and respiratory syndrome virus (PRRSV). J Comp Pathol 2003;129:205-12.
22. Wongyanin P, Buranapraditkun S, Chokeshai-Usaha K, Thanawonguwech R, Suradhat S. Induction of inducible CD4+CD25+Foxp3+ regulatory T lymphocytes by porcine reproductive and respiratory syndrome virus (PRRSV). Vet Immunol Immunopathol 2010;133:170-82.