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Review

The challenge of PRRS immunology

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) is one of the most challenging subjects of research in veterinary viral immunology, and the immune response against PRRS virus (PRRSV) still is poorly understood. Infected pigs develop a strong and rapid humoral response but these initial antibodies do not confer protection and can even be harmful by mediating an antibody-dependent enhancement of disease. In contrast, development of neutralising antibodies (NAs) is delayed and generation of cell-mediated immune responses, such as PRRSV-specific interferon (IFN)-γ secreting cells, is initially erratic. In spite of this, induction of strong and rapid NAs and IFN-γ responses seem to be required for effective vaccination. PRRSV strongly modulates the host's immune responses. The virus inhibits key cytokines, such as IFN-α, and may induce regulatory cytokines, such as interleukin (IL)-10. Development of NAs seems to be impaired by the existence of a decoy epitope close to the main neutralisation epitope in glycoprotein 5. This ability to modulate the host immune response probably varies among strains or isolates. The genetic diversity of the virus is very high and it has been shown that this diversity can have serious implications for the development of vaccines, since the immunity induced by one strain may be only partial against a different strain, even within the same genotype. With this panorama, the development of newer and universally efficacious PRRSV vaccines is challenging, but the present state of knowledge allows optimism if collaborative efforts are undertaken in the scientific community.

Keywords: Porcine reproductive and respiratory syndrome; PRRS; Virus; Immunology

Introduction

More than 15 years after the emergence of porcine reproductive and respiratory syndrome (PRRS), our understanding of the disease still is far from complete. The clinical features of PRRS are well known, a global picture of the epidemiology has been drawn, although some gaps remain to be filled, management and husbandry procedures have been devised for controlling the disease and vaccines are available. However, infection by PRRS virus (PRRSV) is still widespread and the virus is frequently reintroduced to farms after eradication. What are the reasons for such failures? The answer is not simple, but examination of the scientific literature and problems reported by pig veterinarians indicates that vaccines can be a useful tool, although their efficacy is far from being universal and complete. The approach of "let's take a strain, let's attenuate or inactivate it and, *voilà!* the vaccine will generate sufficient immunity to protect against the disease" is not valid for PRRSV.

The present review discusses what is known and what gaps remain in our understanding of PRRSV immunity and its immunopathogenesis. It concludes with the positive message that currently available information should allow us to identify the crucial points that need to be studied in order to understand the disease and overcome the challenge of PRRSV immunology.

Adaptive immune response to PRRSV

Humoral immunity

The development of adaptive immunity against PRRSV is a tale of unusual features in both the humoral

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and cellular components of the immune response. Circulating antibodies against PRRSV are detectable in some pigs by days 5–7 post-infection (PI) and all animals have seroconverted by day 14 PI (Yoon et al., 1992,1995). PRRSV-specific immunoglobulin M (IgM) reaches a peak at day 14 PI and then declines to undetectable levels by 42 days PI. Concentrations of IgG reach a maximum at 21–49 days PI (Vezina et al., 1996; Loemba et al., 1996). However, this rapid IgM and IgG response does not correspond to neutralising antibodies (NAs) (Yoon et al., 1994).

Nelson et al. (1994) studied the kinetics of the humoral response of pigs against an American strain of PRRSV. The earliest antibodies were directed against the 15 KDa nucleoprotein, followed by the 19 KDa M protein, then the 26 KDa glycoprotein 5 (GP5). Other studies showed that non-structural protein 2 (nsp2) contains a cluster of non-neutralising B-epitopes and probably is the immunodominant protein of PRRSV (Oleksiewicz et al., 2001; de Lima et al., 2006). Most diagnostic tests detect antibodies mainly against the N protein. These antibodies appear around the first week PI and persist for several months, but do not correlate with protection.

NAs are not detected by conventional virus neutralisation tests (VNTs) in the first 4 weeks PI. Addition of fresh complement and prolonged incubation of virus–serum mixtures increases the sensitivity of the VNT and allows detection of NAs earlier (days 9–12 PI) (Takikawa et al., 1996). Other authors showed that complement may increase VNT titres by one dilution (Diaz et al., 2005, 2006). Since the addition of 2-mercaptoethanol significantly reduced the sensitivity of the modified VNT, it is likely that low levels of neutralising IgM appear in the early phases of the infection (Takikawa et al., 1996). However, even with this modified VNT, NA titres were still relatively low (1/32–1/64) by day 42 PI.

NAs are consistently detected by day 28 PI or later for both European and American-type strains of PRRSV (Yoon et al., 1994; Meier et al., 2003; Diaz et al., 2005). These NAs are mainly directed against GP5, which contains the major neutralisation epitope (Nelson et al., 1994; Pirzadeh and Dea, 1997, 1998; Gonin et al., 1999). It has been claimed that GP4 and M proteins also contain neutralising epitopes (Meulenberg et al., 1997; Gonin et al., 1999; Weiland et al., 1999; Yang et al., 2000; Cancel-Tirado et al., 2004) and one report suggested that GP3 also contains a neutralising epitope (Cancel-Tirado et al., 2004). However, these proteins seem to be of minor biological significance compared to GP5.

The early development of non-NAs may have a significant effect on the development of PRRS. It has been shown that non-NAs enhance viral replication in alveolar macrophages, a phenomenon known as antibody-dependent enhancement (ADE) (Yoon et al., 1996, 1997). Targets for these antibodies are GP5 and N proteins (Yoon et al., 1996; Cancel-Tirado et al., 2004). The non-neutralising humoral response may act as a Trojan horse for PRRSV by coating the virus and enhancing the internalisation of viral particles into macrophages. The question is: why NAs do not develop as early as non-NAs?

NAs may play an important role, although their importance may be different in natural infection compared to vaccination. Vezina et al. (1996) reported the isolation of PRRSV from the blood of pigs with NAs. Following experimental infection, viraemia may be resolved without detectable levels of neutralising antibodies (Diaz et al., 2006). Similar to the related virus lactate dehydrogenase virus (LDV), the dynamics of PRRSV-susceptible macrophages may govern the levels of viraemia (Diaz et al., 2006). If cytolysis or apoptosis exhaust most susceptible macrophages, infection will be confined to macrophage-rich organs, such as lymph nodes. In this hypothesis, NAs may be required for resolution of viraemia, if not infection.

A different picture arises when protection before infection is considered. NAs block PRRSV infectivity for macrophages in vitro (Delputte et al., 2004). Transfer of NAs to pregnant sows (NA titres 1/16) protects them against reproductive failure and blocks transplacental infection (Osorio et al., 2002). Using the same antibody transfer system, a titre of 1/8 or higher protected piglets against the development of viraemia, whereas sterilising immunity was attained at NA titres of 1/32 (Lopez et al., 2007). These results suggest that a vaccine capable of inducing NA titres of 1/32 should prevent clinical disease and be a key tool in eradication of PRRSV.

Cell-mediated immunity

Cell-mediated immunity (CMI) is also extremely important in PRRS. Early studies showed that pigs recovering from experimental PRRSV infection had strong lymphocyte proliferative responses, although these responses were not detected until four weeks PI and paralleled the NA response (Bautista and Molitor, 1997; Lopez Fuertes et al., 1999). Cytokine responses were mainly interferon (IFN)-γ and, to a lesser extent, IL-2 (Lopez Fuertes et al., 1999).

After vaccination with a modified live vaccine using an American strain of PRRSV, virus-specific IFN-γ secreting cells first appeared in the third week post-vaccination, fluctuated erratically from 50–100 per million peripheral blood mononuclear cells (PBMCs) for the next ten weeks, then increased to 400–500 per million PBMCs at 48 weeks post-vaccination (Meier et al., 2003). IFN-γ secreting cells were mainly $CD4^{+}CD8^{+}$ cells, with a small proportion of $CD4^{-}/CD8\alpha\beta^{+}$ cytotoxic T cells. A similar delayed development of PRRSV-specific IFN-γ secreting cells was evident after infection or vaccination with European strains of PRRSV (Diaz et al., 2005,2006). In contrast, 200–300 IFN-γ secreting cells per million PBMCs were evident by 3 weeks after vaccination against Aujeszky's disease virus (Meier et al., 2003).

Modulation and immune evasion by PRRSV

Interferons

The unusual characteristics of the adaptive immune response to PRRSV suggest that the virus strongly modulates the immune response. Early studies showed that PRRSV is highly susceptible to the action of type I IFNs and suggested that the virus was able to inhibit IFN-α responses, since this cytokine could not be detected in the lungs of pigs in which PRRSV was actively replicating (Albina et al., 1998; Buddaert et al., 1998). IFN-α levels in the lungs of PRRSV-infected pigs were much lower than in the lungs of pigs infected with porcine coronavirus or swine influenza virus (van Reeth et al., 1999). Frequencies of virus-specific IFN-γ secreting cells were correlated with the frequencies of IFN-α secreting cells in pigs infected with PRRSV (Royaee et al., 2004). Although the exact mechanism by which PRRSV inhibits IFN-α is unknown, it does not involve inhibition of the nuclear factor (NF)-κ B pathway (Lee and Kleiboeker, 2005).

Different PRRSV isolates and different plaque clones of the same strain have different abilities to induce or inhibit IFN-α (Lee et al., 2004). Preliminary results indicate that different European PRRSV isolates have different abilities to induce not only IFN-α, but also TNF-α, IL-10 and IL-12, in alveolar macrophages and dendritic cells (unpublished observations). Impairment of IFN-α secretion would be expected to affect the development of an effective T helper type 1 (Th1) immune response.

Cytokines

IL-10 may have an important role in the regulation of the immune response to PRRSV. After infection with either European or American strains of PRRSV, levels of IL-10 mRNA were increased in porcine PBMCs (Suradhat and Thanawongnuwech, 2003; Suradhat et al., 2003) and concentrations of IL-10 were increased in bronchoalveolar lavage (BAL) fluid (Thanawongnuwech et al., 2004). Some European strains of PRRSV induce strong IL-10 responses in PBMCs from naïve pigs, suggesting that this is a not a memory feature (Diaz et al., 2006). Pigs vaccinated with IL-10-inducing strains had lower frequencies of PRRSV-specific IFN-γ secreting cells than animals vaccinated with a non-IL-10 inducing strain (Diaz et al., 2006). Monocytes appear to be the major source of IL-10 in PRRSV infection (Charerntantanakul et al., 2006). PRRSV also appears to induce IL-6 (Asai et al., 1999; Sipos et al., 2003), whereas the role of transforming growth factor-β in PRRSV infection is unclear (Royaee et al., 2004).

Antigen presentation

PRRSV may interfere with correct antigen presentation and activation of T lymphocytes. PRRSV down-regulated expression of major histocompatibility complex (MHC)-I in dendritic cells (DCs), although this was not correlated with impaired proliferative responses in the mixed leucocyte reaction (Loving et al., 2007). Expression of MHC-I and MHC-II, as well as CD14, was down-regulated in monocyte-derived DCs stimulated by infectious but not inactivated PRRSV (Wang et al., 2007). In this study, proliferative responses were decreased when infected DCs were used with syngeneic or allogeneic lymphocytes, suggesting that infected DCs present antigens less efficiently (Wang et al., 2007). PRRSV may down-regulate the innate immune response by altering the cytokine patterns of macrophages and dendritic cells, as well as by modifying the expression of molecules involved in antigen presentation.

Humoral immunity

As discussed previously, one of the intriguing features of PRRSV infection is the delayed development of NAs. The main neutralisation epitope of PRRSV, designated epitope B, is located in the N-terminal ectodomain of GP5 (amino acids 37–44) in both American and European strains (Ostrowski et al., 2002; Plagemann et al., 2002; Wissink et al., 2003; Plagemann, 2004). This neutralisation epitope is flanked by glycosylation sites.

An additional immunodominant epitope, designated epitope A, is located in the N-terminal ectodomain of GP5 (amino acids 27 and 31) and has the characteristics of a decoy epitope, similar to that in human immunodeficiency virus type 1 (Ostrowski et al., 2002). The decoy epitope may interfere with the immune response to the main neutralisation epitope B, resulting in a delay in the NA response. Insertion of a pan-DR helper T cell epitope between the epitope B and the decoy epitope increased the immunogenicity of epitope B in mice (Fang et al., 2006), suggesting that the proximity of epitopes A and B is important in delaying the NA response.

The decoy epitope is not the only way by which PRRSV can evade the humoral immune response. GP5, the main target for NAs, contains up to four glycosylation sites, located in or close to the neutralising epitope. American field strains of PRRSV lacking glycosylation sites in the upstream hypervariable region induced NAs more rapidly and more strongly in infected pigs than strains lacking the downstream glycosylation site at position 44 (N-44), even though all strains were equally susceptible to NAs (Faaberg et al., 2006). As Spanish PRRSV strains have evolved from 1991–2005, there has been a trend to lose the glycosylation site at N-46 (equivalent to N-44 of American strains) and to maintain or gain glycosylations in the flanking regions (N-37 and N-53), consistent with selection of strains inducing weaker NA responses (Mateu et al., 2006).

Genetic diversity of PRRSV and implications for vaccine development

PRRSV is divided into European (type I) and American (type II) genotypes; four subtypes have been identified

within the European genotype (Stadejek et al., 2006). Diversity within a genotype or subtype can be high (Forsberg et al., 2002; Stadejek et al., 2002; Larochelle et al., 2003; Mateu et al., 2003; Stadejek et al., 2006). Furthermore, PRRSV also demonstrates the phenomenon of quasispecies generation (Rowland et al., 1999; Goldberg et al., 2003). What is the impact of this genetic diversity of PRRSV upon the immune response and protection afforded by vaccination?

PRRS emerged in Europe and America almost simultaneously. The first vaccine against PRRSV marketed internationally was a modified live vaccine derived from the prototypic American strain VR-2332. At that time, it was obvious that the genetic diversity of PRRSV could pose problems regarding the efficacy of the vaccine, particularly with infections by the European genotype.

Pregnant gilts infected with the American isolate NADC-8 and challenged with the European isolate Lelystad virus (LV) late in gestation had only partial protection against transplacental infection (virus crossed the placenta in 1/7 gilts), whereas all gilts challenged with the homologous virus were fully protected (Lager et al., 1999). These results showed that heterologous protection existed, but was only partial, and suggested that common epitopes are likely to be involved in protection in both European and American-type strains.

Furthermore, monoclonal antibodies against the neutralising epitope of the related virus LDV are able to neutralise both VR-2332 and LV, indicating that the neutralising epitope in GP5 is shared to some extent by diverse arteriviruses (Plagemann et al., 2002) Although partial heterologous protection might be beneficial under some circumstances, a genotype-based vaccine is insufficient to produce immunising sterility.

Given the genetic diversity of the virus within one genotype, the question then was whether this phenomenon might influence the efficacy of a homologous vaccine. Piglets vaccinated with attenuated versions of American strains NADC-8, 9 or 14 were challenged 21 days later with a mixture of the virulent versions of the same strains (Mengeling et al., 2003). A given virulent strain was not present after challenge if the piglets had previously received the attenuated version of that strain, whereas infections with the other virulent strains were established, indicating that immunity to PRRSV may be strain-related. After vaccination with a European-type modified live vaccine, pigs were mostly negative for virus in serum or BAL fluid after challenge with LV (Labarque et al., 2004) In contrast, vaccinated pigs developed viraemia over 15 days and were positive for virus in BAL fluid when challenged with an Italian variant strain that had 84% similarity in ORF5 to the vaccine strain.

Protection against PRRSV infection by a strain different to the one used as a vaccine is somewhat more complex than a matter of genetic similarity. Pigs were vaccinated with two different European-type vaccines (v1 and v3), then challenged with a strain similar to one of the vaccines and slightly different to the other (92–96% similarity) (Diaz et al., 2006). Surprisingly, the "heterologous" v3 vaccine afforded sterilising immunity, while the homologous v1 vaccine did not. The v3 vaccine induced higher levels of IFN-γ secreting cells, whereas v1 induced IL-10 release by PMBCs. The ability of each strain to induce a strong cell-mediated immune response was more important that the genetic similarity inducing protection.

Prospects for the development of universal PRRSV vaccines

The complexity of the immune response to PRRSV and the ability of the virus to escape or modulate the host's immune system make it difficult to develop a vaccine that can be used to eradicate the disease. Such a vaccine should accomplish at least four requirements: efficacy, universality, safety and ability to differentiate vaccinated from infected animals. The first line of investigation is the detailed investigation of B and T cell epitopes involved in the development of protective immunity. Neutralising epitopes have been established definitively. Little is known regarding T cell epitopes, although T cell responses to individual PRRSV polypeptides have been reported in virus-infected animals (Bautista et al., 1999). Common critical epitopes in both European and American strains of PRRSV have to be clearly identified to support the development of universal vaccines.

The second line of research is to determine which components of the virion or viral genome are involved in the down-regulation or modulation of the host's immune system and the mechanisms by which this occurs. This is critical for development of a live attenuated vaccine. The efficacy of a given vaccine is not only related to its immunological properties, but also to the characteristics of the challenging strain. Therefore, studies on the relationship between genetic diversity and the immunopathological properties of different strains are needed. Reverse genetics and characterisation of the modulating properties of an extensive set of strains are necessary; this can only be achieved through a serious international collaborative effort.

Thirdly, the developed vaccine should be safe. This means that any possibility of reversion to virulence should be eliminated and transmission of the vaccine strain between pigs should be minimal or non-existent. One obvious way to gain in safety is by using non-replicating vaccines. However, it is unclear if non-replicating vaccines are able to induce NAs and adequate cell-mediated immune responses (Zuckermann et al., 2007). Research on subunit or vector-based vaccines and adjuvants should be undertaken.

Fourthly, the development of a differential vaccine is highly desirable. Since PRRSV is a virus with a relatively small genome, it is difficult to find targets for deletion, although, to our knowledge, an extensive study of essential and non-essential parts of the viral genome has not been performed. The occurrence of natural variants with small

Table 1
Some of the known, assumed and unknown features of the immune response in PRRSV infection relevant to the development of vaccines

	Known	Assumed/Supposed	Unknown yet
Neutralising antibodies (NA)	GP5, GP4	M, GP3 (?)	
Role of NA in protection	Passive transfer protects against challenge		Exact role of neutralising antibodies in natural infection
Critical T cell epitopes	T cell responses to individual PRRSV polypeptides have been reported	Several viral proteins contain T cell epitopes	Full set of critical epitopes
Cell-mediated immunity	Frequencies of IFN-γ secreting cells correlate with protection against developing viraemia in piglets in a challenge model	IFN-γ may mediate sterilising immunity	Precise requirement of IFN-γ secreting cells for protection
Viral immune modulation	PRRSV contains a decoy epitope in GP5	The decoy epitope delays development of neutralising antibodies	Viral proteins or mechanisms by which IFN-α is inhibited or IL-10 induced
	Certain PRRSV strains may inhibit IFN-α Certain PRRSV strains may induce IL-10 release	Lack of IFN-α and release of IL-10 may delay cell-mediated responses	
Genetic diversity	High genetic diversity within each genotype Genetic diversity affects vaccine efficacy	Genetic diversity might be related to the immunopathological characteristics of PRRSV strains	How genetic diversity correlates with virulence or immunological properties of PRRSV strains
Essential and non-essential proteins	Structural proteins seem to be mainly essential Some of the non-structural proteins may suffer natural deletions	Some non-structural proteins might be non-essential	Exact map of essential and non-essential proteins

deletions in nsp2 indicates that non-structural proteins could be a target for constructing differential vaccines (Fang et al., 2004).

It is necessary to demonstrate that a vaccine protects against infection, not only in challenge experiments against one strain of the same or a different genotype, but also against different strains within a given genotype. Table 1 summarises some of the knowledge already known or required for developing newer vaccines.

Conclusions

The number of important questions that remain to be solved in PRRS immunology is considerable. For example, the development of the adaptive immune response after infection with PRRSV or vaccination is anomalous. IFN-γ secreting cells appear late and evolve erratically during the first weeks after infection; NA responses are also delayed. NAs may protect against disease if present in sufficient quantities before infection, but they do not seem to be essential for clearing virus in blood during the course of the infection. PRRSV is able to modulate innate responses, probably through the regulation of IFN-α and IL-10 responses. Two different PRRSV genotypes exist that have evolved in parallel. Cross protection afforded by each genotype is only partial and genetic diversity within each genotype can be high enough to allow a vaccinated animal to be re-infected by a different strain of the same genotype. These circumstances create difficulty in understanding how the immune system and the virus interact.

It is possible that different PRRSV strains are able to modulate or regulate the immune system in different ways. Therefore, published experiments should be always interpreted with caution, particularly when trying to extract general principles from a particular experiment. Collaboration between researchers is the best way to enhance our understanding of PRRSV immunology.

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