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# Immunity to PRRSV: Double-edged sword

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## Abstract

The immune system is a double-edged sword for porcine reproductive and respiratory syndrome virus (PRRSV) infection. On one edge PRRSV has a predilection for immune cells and the disease manifestations can be linked directly to changes in the immune system. PRRSV appears to replicate extensively, if not exclusively, in cells of the immune lineage, notably macrophages; the direct replication of which may lead to immunosuppression, precipitate secondary infection and/or mediate disease. On the other edge, the virus stimulates immunity post-infection that protects an animal from re-infection. A vast array of structural and functionally distinct antibody specific to PRRSV are generated following infection or vaccination. Discrete populations of functional antibodies appear at different times and possibly reflect reactivity to different PRRSV polypeptides. Cell-mediated immune responses specific to PRRSV can be detected in various exposed pigs as well. Thus, the immune system appears to be intimately involved in both the disease process and protection from disease. It is unclear at this state of understanding what immune compartment provides protective immunity. Is it humoral (i.e. antibodies), selective functionally distinct populations of antibodies specific for selected PRRSV polypeptides or is cellular immunity essential for protection, or both. This review will attempt to summarize the current state of knowledge of the complex interaction of the immune system and PRRSV. © 1997 Elsevier Science B.V.

*Keywords:* Porcine reproductive and respiratory syndrome virus; Immunity

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## 1. Introduction

The immune system is a double-edged sword for porcine reproductive and respiratory syndrome virus (PRRSV) infection. On one edge PRRSV has a predilection for immune cells and the disease manifestations can be linked directly to changes in the immune

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system. PRRSV appears to replicate extensively, if not exclusively, in cells of the immune lineage, notably macrophages; the direct replication of which may lead to immunosuppression, precipitate secondary infection and/or mediate disease. On the other edge, the virus stimulates immunity post-infection that protects an animal from re-infection. Thus, the immune system appears to be intimately involved in both the disease process and protection from disease. It is unclear at this state of understanding what immune compartment provides protective immunity. Is it humoral (i.e. antibodies), even selective antibodies of functionally distinct populations or antibodies specific for selected PRRSV polypeptides or is cellular immunity essential for protection. This review will attempt to summarize the current state of knowledge of the complex interaction of the immune system and PRRSV.

## 2. Replication of PRRSV in cells of the immune system

PRRS virus was first isolated on swine alveolar macrophages (AM) (Wensvoort et al., 1991; Pol et al., 1991). AM represent a highly permissive cell for infection yielding progeny virus titers of greater than  $10^7$  TCID<sub>50</sub>/ml. AM serve as sensitive cells for isolation of virus from diagnostic samples and antibody detection using indirect FA or IPT methods. AM are primary targets for virus replication in the infected animal, yet PRRSV is a multisystem disease characterized by profound viremia and virus distribution and replication in multiple organs causing interstitial pneumonia, vasculitis, lymphadenopathy, myocarditis and encephalitis (Rossow et al., 1995). Thus, replication solely in AM does not fully explain the pathogenesis of PRRSV in the infected animal. Furthermore, in vitro studies with AM and collections from PRRSV-infected animals reveal that not all AM become infected. Also, in vitro studies with AM show that not 100% of cells are affected and that there is great variability in susceptibility of AM from various donor pigs. In an attempt to elucidate the susceptibility of various cell types and variability within cell types, we examined the susceptibility of various macrophage populations (i.e. microglia, monocytes) and the heterogeneity of alveolar macrophages to infection by PRRSV. To examine the heterogeneity of AM, a procedure was devised to separate AM into subpopulations by subjecting AM lavaged from lungs to a discontinued gradient centrifugation and each fraction was evaluated for morphology, expression of cell surface markers, macrophage functions and susceptibility to infection by PRRSV. All fractions consisted of greater than 95% macrophages using a panel of monoclonal antibodies against porcine macrophages. The largest cells were found in the lowest density fraction (I) and the smallest cells were in the highest density fraction (V), showing an inverse relationship between cell size and density. Fractions in the lower densities (I and II) appeared to have more cytoplasmic vacuoles and lower nuclear to cytoplasmic ratios relative to fractions III, IV and V and appear to represent mature, differentiated macrophages. Functional differences existed between the fractions of PAM. PAM from the higher density fractions (III, IV and V) showed increased phagocytosis of *Pasteurella multocida*, superoxide anion production and TNF- $\alpha$  production relative to PAM from lower density fractions (I and II). In contrast, binding of opsonized sheep red blood cells through Fc receptors was greatest in the lowest density

Table 1  
PRRSV infection of immune cells for PRRSV replication

	Permissive	Virus titers TCID <sub>50</sub> /ml
Macrophages		
(a) Alveolar macrophages subpopulations (No. 1–5)	++++ (– to 4+)	$> 10^7$ ( $10^1$ – $10^7$ )
(b) Spleen macrophage	+++	$10^6$ – $10^7$
(c) Brain microglia	++++	$> 10^7$
(d) Monocytes		
• alone	+ / –	$< 10^1$ , $10^4$
• M-CSF	++	$10^4$
• adherence to endothelial	++	$10^4$
Lymphocytes		
B-cells	–	$< 10^1$
T-cells	–	$< 10^1$

Pol et al., 1991 (AM splenic macrophages).

Voicu et al., 1994 (monocytes).

Choi et al., 1995 (subpopulation of AM, monocytes, microglia).

cells (I). Increased PRRS virus replication was observed in PAM from the higher density fractions (III, IV and V) representing more immature cells (Choi et al., 1995). Further studies examined the susceptibility of populations of macrophages including brain microglia and peripheral blood monocytes. Swine microglia were highly permissive to infection with PRRSV yielding progeny titers of greater than  $10^7$  TCID<sub>50</sub>/ml. In contrast, monocytes directly collected and exposed to PRRSV yielded low progeny titers of less than  $10^2$  TCID<sub>50</sub>/ml. Yet when monocytes were induced to mature through treatment with M-CSF or activated via the adherence to porcine endothelial cells, monocytes became permissive to PRRSV infection. Voicu et al. (1994) demonstrated that peripheral blood monocytes collected via adherence to plastic were permissive to PRRSV infection yielding virus titers of  $10^4$  TCID<sub>50</sub>/ml, a 1,000-fold reduction compared to AM yet nonetheless significant titers. Although there is an apparent paradox in the two findings, it appears that maturation and activation of monocytes is necessary for productive infection. The adherence to plastic could in fact activate the cells. Collectively, these results indicate that heterogeneous PAM populations exist and that these cells showed different morphological and functional properties. Furthermore, the activation/maturation stage of macrophages modulate the susceptibility to virus infection. The susceptibility of various immune cells to PRRSV is summarized in Table 1.

### 3. Immunosuppression

Clinical and experimental studies suggest that PRRSV modulates host responses based on two observations: (1) secondary infections, e.g. pneumonia, arthritis, eye infections, meningitis and infections with PRV, PPV and SIV are common following PRRS virus infection (Collins and Rossow, 1993; Zeman et al., 1993; Done and Paton,

1995) and (2) experimental infection with PRRSV precipitates clinical disease in piglets challenged with *Streptococcus suis* (Galina et al., 1994) or with either of two respiratory viruses, porcine respiratory coronavirus (PRCV) or swine influenza (SI, Van Reeth et al., 1994). PRRSV infections are commonly followed by severe, secondary bacterial diseases which greatly reduce the performance of growing pigs (Collins and Rossow, 1993). In a few selected herds, a thorough pathological and microbiological work-up has been performed. A variety of infectious agents have been isolated in concert with PRRSV: *Actinobacillus pleuropneumonia*, *Mycoplasma hyopneumoniae*, *Haemophilus parasuis*, *Actinomyces pyogenes*, *Streptococcus suis* and swine influenza (Zeman et al., 1993). The conclusion from this field investigation was that PRRSV can increase the incidence of other common diseases. One of the agents identified, *H. parasuis*, has been diagnosed increasingly in the upper midwest, since the appearance of PRRSV. Iowa State Diagnostic Laboratory identified 142 isolations in 1992, compared to 79 in 1990. South Dakota State Laboratory identified 144 cases in 1992, compared to 54 in 1990. Minnesota State Laboratory identified 318 in 1992, compared to 35 in 1988. Indirect evidence of the role of PRRSV in precipitating secondary infections comes from successfully improved growth and decreased mortality on farms that have eliminated PRRSV through nursery depopulation (Dee and Joo, 1994). With the elimination of PRRSV on farms, production improved and the isolation and association of disease with secondary agents dramatically decreased. Clinically, there is little doubt that concurrent PRRSV frequently results in devastating secondary infectious diseases in pigs; however, although clinical and diagnostic laboratory data support the hypothesis of an interaction between PRRSV and other agents, yet attempts to demonstrate PRRSV as a predisposing factor in respiratory disease under experimental conditions, with the exceptions of *Streptococcus suis* and two viruses, porcine respiratory coronavirus and swine influenza, have been unsuccessful.

*Streptococcus suis* (*S. suis*) serotype 2 infection causes septicemia, meningitis, arthritis and death, usually in young weaned pigs. Interactions of *S. suis* serotype 2 with other microorganisms such as *Bordetella bronchiseptica* (Vecht et al., 1992) and pseudorabies virus (PRV) (Iglesias et al., 1992) have been demonstrated experimentally. However, it has been difficult to study clinical disease caused by *S. suis* infection because clinical disease is difficult to reproduce using natural routes of exposure when challenging with *S. suis* alone. The objectives of experimental studies performed by Galina et al. (1994) were to characterize the interaction of PRRSV and *S. suis* under controlled conditions and to determine if PRRSV pre-disposes pigs to *S. suis* disease. Pigs inoculated with PRRSV (VR-2332) followed by challenge with a virulent strain (87555) of *S. suis* serotype 2 developed clinical signs, suppurative meningitis and abundant growth of *S. suis* from tissues, including brain-meninges. Pigs inoculated with PRRSV alone, *S. suis* alone, PRRSV and the DH5 strain of *S. suis* serotype 2 (lacking a protein associated with virulence) or control piglets did not have clinical signs, lesions or large amounts of bacteria in their tissues. Results of this study suggest that PRRSV pre-disposes SPF pigs to infection and disease caused by virulent *S. suis* serotype 2. In a study examining the effect of concurrent infection of PRRSV with porcine respiratory coronavirus (PRCV) and swine influenza (SI) Van Reeth et al. (1994) found that PRRSV by itself was clinically inapparent in feeder pigs while severe respiratory disease

and production losses occur when PRCV and SI were superimposed on PRRSV infection. Studies with sequential infection of PRRSV followed by *H. parasuis*, *P. multocida* or *A. pleuropneumoniae* have failed to demonstrate increased severity of disease (Solano et al., 1997).

If indeed pigs are more susceptible to secondary infections following PRRSV, a presumed mechanism underlying the increased susceptibility could be suppression of the immune system. Two studies have attempted to define putative immunosuppression, following experimental infections with PRRSV in pigs and measuring immune response parameters including immune cell profiles and antibody-mediated and cellular immunity to foreign antigens (Molitor et al., 1995; Albina et al., 1995). In the study of Molitor et al. (1995), cell distribution in the lung of infected animals changed dramatically by day 7 post-infection, evidenced by a marked decrease in the percentage of alveolar macrophages and an increase in the percentage of lymphocytes and neutrophils. Associated with the altered lung cell dynamics was a decrease in the functional ability of alveolar macrophages to release superoxide anion. In sharp contrast was the finding of profound enhancement of humoral and cell-mediated function in the systemic circulation of PRRSV infected swine. Pseudorabies virus (PRV) and *Brucella abortus* antibody titers and delayed type hypersensitivity responses to the antigen DNFB were significantly enhanced in infected pigs of all age groups. A separate study by Albina et al. (1995) demonstrated that PRRSV did not impair the immune response following PRV vaccination. On the contrary, immune response and disease resistance of pigs previously infected with PRRSV and then challenged with virulent PRV were increased. In addition, immune parameters such as killer-cell activity and lymphocyte response to antigenic stimulation were not affected following PRRSV infection, leaving to question the role of PRRSV in causing systemic immunosuppression. The effects of PRRSV on non-specific and antigen-specific immune responses are summarized in Table 2. Local virus-mediated destruction of alveolar macrophages may account for the extensive respiratory infection in PRRSV infected pigs. Enhanced systemic responses to exogenous antigens may be due, in part, to polyclonal activation of immune components, which has been described in LDV infection.

## 4. Immunity to the virus

### 4.1. Humoral immunity

Upon exposure of pigs to PRRSV, pigs immunologically respond with a heterogeneous array of responses specific to PRRSV virus. Included are structural (i.e. class of immunoglobulins) and functional distinct antibodies and cell-mediated responses. Experimental data showing that previous infection prevented animals from developing clinical signs after re-exposure (Gorcyca et al., 1993) suggest that protective immune mechanisms are developed in swine upon infection. At present, it is unclear which immune mechanisms are involved in protection against PRRSV in swine. Serological assays, including immunoperoxidase monolayer assay (IPMA, Wensvoort et al., 1991), indirect fluorescent antibodies (IFA, Yoon et al., 1992), serum neutralization (SN, Hill et al.,

Table 2

Immunomodulating effects of PRRS virus

	Effect	Ref.
(A) Cellular changes		
(1) Cell distribution: lung		
• macrophage	↓ ↓	Molitor et al., 1995
• lymphocytes	↑ ↑	
• neutrophils	↑ ↑	
(2) Cell distribution: systemic		
• leukocyte counts	↓ ↓	Molitor et al., 1995
(B) Functional effects		
(1) Lung macrophage function		
• superoxide anion	↓ ↓ d7	Molitor et al., 1995
(2) Systemic function		
• NK activity	–	Albina et al., 1995
(C) Antigen-specific		
(1) Antibody response		
• <i>Brucella abortus</i>	↑ ↑	Molitor et al., 1995
• Pseudorabies vaccine	↑ ↑	Molitor et al., 1995; Albina et al., 1995
(2) Cell-mediated immunity		
• DTH to DNFB	↑ ↑	Molitor et al., 1995
(D) Secondary infections (experimental)		
(1) <i>Streptococcus suis</i>	↑ ↑	Galina et al., 1994
(2) PRCV and SI	↑ ↑	Van Reeth et al., 1994
(3) PRV	–	Albina et al., 1994
<i>Mycoplasma hyopneumonia</i>	–	Albina et al., 1994

↓ ↓ depression in response compared to noninfected control.

↑ ↑ stimulation over that of noninfected control.

–: no effect.

1993), western blot (Nelson et al., 1993; Nelson et al., 1994), indirect enzyme-linked immunosorbent assay (ELISA, Albina et al., 1992) and blocking ELISA (Houben et al., 1995) have been used to identify antibody responses to PRRSV (Table 3).

Following exposure to virus, swine synthesize PRRSV-specific antibodies detected by IFA and IPMA which appear at one to two weeks post-infection and can persist for up to one year. Antibodies detected by serum neutralization test SNT appear later (as much as six weeks post-infection) and disappear sooner (Morrison et al., 1992). A modified SN test has been reported (Yoon et al., 1994; Yoon et al., 1996) that detects SN antibody responses at 9–11 days post-inoculation. The antigenic specificity of the SNT is more definitive than that of the IPMA as well. Antibodies can also be shown that recognize virus-specific polypeptides by Western blot (Nelson et al., 1993) and immunoprecipitation. An indirect ELISA was first described for detecting antibodies to PRRSV in sera (Albina et al., 1992). Subsequently, a blocking ELISA technique was developed by Houben et al. (1995). The blocking ELISA appears to have the advantage of detecting low antibody titers. Thus, there exist discrete populations of functional

Table 3  
Immunity to PRRSV

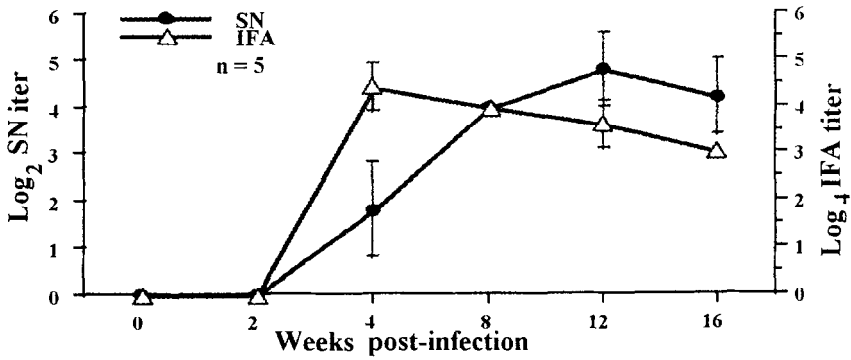
Type of response	Principles	Ref.
(A) Humoral		
(1) Immunofluorescence (IFA)	react with virus-infected cells	Yoon et al., 1992; Nelson et al., 1994
Immunoperoxidase (IPT)	react with virus-infected cells	Wensvoort et al., 1991
(2) Serum-neutralization (SN)	neutralize live virus to infect monkey cells	Nelson et al., 1994; Yoon et al., 1994; Yoon et al., 1996
Modified SN complement	neutralize live virus to infect monkey cells	Nelson et al., 1994; Yoon et al., 1994; Yoon et al., 1996
(3) Enzyme-linked immunosorbent assay	virus	Albina et al., 1992; Houben et al., 1995
(4) Western blot	denatured viral polypeptides	Nelson et al., 1994
(5) Antibody-dependent enhancement	Antibodies enhance infections of alveolar macrophages	Choi et al., 1992; Yoon et al., 1994; Yoon et al., 1996
(B) Cell-mediated		
(1) Lymphocyte blastogenesis	T-cell response to live virus	Bautista et al., 1995
(2) Delayed-type hypersensitivity	in vivo T-cell response to virus	Bautista et al., 1995

antibodies that display different kinetics post-infection and possibly reflect reactivity to different PRRS virus polypeptides. Pigs vaccinated with modified-live virus similar to infected pig vaccine immunologically respond and synthesize antibodies of various structure and functional heterogeneity. Pigs born from PRRSV-infected dams maintain maternal antibody titers to PRRSV until 4 weeks of age, as determined by IFA (Dee et al., 1993), 4–8 weeks of age in a study using the indirect ELISA (Albina et al., 1994) and up to 10 weeks of age using the blocking ELISA (Houben et al., 1995). These three studies were performed on pigs from infected herds, representing potential differences in pigs, source, virus strain and farm management factors.

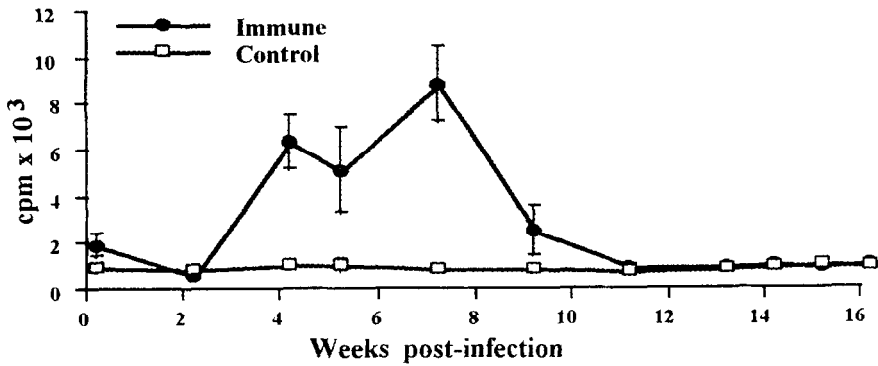
The role of the humoral immune response in protection from challenge remains questionable, based on the observations that viremia is detected albeit in the presence of antibodies (Rossow et al., 1995) and that a population of antibodies enhance virus replication in alveolar macrophages in vitro (Choi et al., 1992; Yoon et al., 1996) and in vivo in infected fetuses (Christianson et al., 1993) and feeder pigs (Yoon et al., 1994; Yoon et al., 1996). It is postulated that the mechanisms of enhancement of replication is through Fc receptors. Protection from re-infection has been documented in field conditions and experimentally described in pregnant sows (Morrison et al., 1992). Sows that were infected with the prototype USA isolate of PRRSV, VR-2332 and that recovered from infection were protected from manifestations of clinical signs and showed no viremia upon re-infection with the homologous strain. This protection could be transferred through colostrum to susceptible newborn pigs (Morrison et al., 1992). To determine whether this protection was provided by antibodies an experiment was performed by passively transferring high titered anti-PRRSV antibodies to 1-week-old pigs from non-immune dams and challenging the pigs with PRRS virus 24 h later. Pigs from immune dams were protected from experimental challenge, but pigs that received



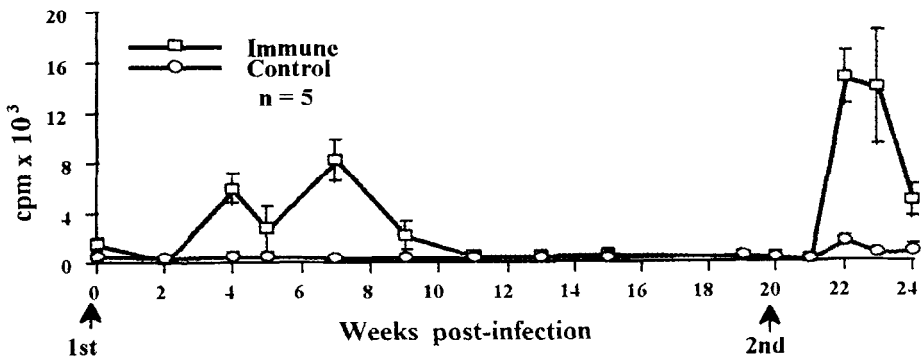
### A. Antibody response



### B. T-cell proliferation response



### C. Primary and secondary response



concentrated anti-PRRSV sera failed to be protected from challenge as were pigs receiving non-immune sera or PBS as controls. These results document that immunity can be transferred via colostrum, but antibodies by themselves fail to totally protect, thereby suggesting a role of cellular immunity in protection from disease.

#### 4.2. Cell-mediated immunity

It is well recognized that in viral diseases an important role of cellular immunity is the clearance of virus and protection against disease. The data available suggest that cellular immune mechanisms might have an important role (Choi et al., 1992; Molitor et al., 1995; Rossow et al., 1995) and that antigenic diversity among PRRSV isolates may originate strain-specific immune responses (Wensvoort et al., 1992; Nelson et al., 1993; Bautista et al., 1993). In an attempt to determine whether PRRSV induced a cell-mediated immune response, studies were undertaken to establish methods for detecting antigen-specific cell-mediated immune responses to PRRS virus.

The purpose was to develop methods to detect CMI responses to PRRSV, both ex-vivo, in the form of T-cell proliferation, and in vivo as delayed-type hypersensitivity (DTH) responses. To demonstrate proliferation responses, peripheral blood mononuclear cells from PRRSV-infected and control pigs were stimulated in vitro with virus antigens for various incubation periods and T-cell proliferation determined by the uptake of  $^3\text{H}$ -thymidine. T-cell proliferation to PRRSV virus was robustly detected in virus-exposed animals. The lymphocyte proliferation was PRRSV specific and virus concentration dependent. The proliferation was mediated primarily by CD4+ T-cells since antibodies to CD4 blocked the response. The kinetics of T-cell proliferation response were evaluated in virus-infected pigs before and every other week post-infection (PI) and compared to viremia and antibody response (SN and IFA). The secondary response was analyzed in the same pigs after re-exposure at 20 weeks PI. Virus-infected, but not control pigs, developed viremia detected at 1 and 2 weeks post-infection and antibody titers (Fig. 1a). The IFA titers developed rapidly and were detected in all infected animals with the highest response at 4 weeks post-infection. The SN titers developed slowly with lower titers detected first at 4 weeks post-infection and the highest response at 12 weeks post-infection. Antigen-specific lymphocyte proliferation response to PRRSV was first detected in virus-infected animals at 4 weeks post-infection, peaked at 7 weeks post-infection and appeared to decline after 11 weeks post-infection (Fig. 1b). The response and decrease in lag time of the same animals to a secondary exposure to virus

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Fig. 1. Kinetics of humoral and cell-mediated immune responses to PRRSV. The primary immune responses were determined in infected pigs as compared to control animals. Data for the first 16 weeks are shown for antibody response (panel A) and lymphocyte proliferation (panel B). The secondary response is shown in panel C. Antibodies were detected in infected pigs by SN and IFA tests at 4 weeks post-infection (PI) and persisted through the 16 week period. Proliferation responses were also detected at 4 weeks PI, peaked at 7 weeks PI and declined after 11 weeks PI. No antibody or proliferation responses were detected in control pigs. The proliferation response increased after secondary exposure. The results showed that pigs develop both humoral and cell-mediated primary immune responses specific to PRRSV infection. Furthermore a memory T-cell response was evident upon secondary exposure.

resulted in a T-cell proliferation response which increased in magnitude (Fig. 1c). Although there was some variability in the response among the infected animals, the proliferative response was significantly different from the control non-infected pigs ( $p < 0.05$ ) as determined statistically by the method of repeated measure analysis of variance and orthogonal polynomial contrast to test the difference in time effect.

In an in vivo measure of a cellular immune response in delayed-type hypersensitivity (DTH), pigs either infected or vaccinated with Resp PRRSV™ responded with a DTH response, specific to virus antigen. Thus, it is clear that cellular immune response in addition to humoral immunal responses are induced following exposure to virus vaccination. Yet, the question left unresolved is the role of CMI in protection.

## 5. Conclusion

It is abundantly clear that a vast array of antibody populations are generated following exposure to virus. Functions of antibody populations differ in their onset and duration. In addition, cell-mediated responses are generated following infection with PRRSV and following vaccination. Notably, pigs previously exposed to virus are protected from re-exposure to at least the homologous virus challenge. The immune response can be both beneficial and detrimental to the host following infection. These are probably critical components of immunological defenses, critical for protection, yet our knowledge to date is restricted to the documentation of the heterologous response. There remains a lengthy list of questions relating to immune mechanism of protection. The application of a vaccine enhances an awareness of the immunological mechanism of protection. Attempts to answer these questions are currently underway.

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