PRRS VIRUS – HOST INTERACTION

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Summary

PRRSV enters pigs through skin breaks or at mucosal surfaces and infects macrophages and dendritic cells. It replicates over a period of hours and exits the cell. Within a day it has infected macrophages throughout the body, especially in the lung and lymph nodes. During this time the pig is largely unaware that it has been infected. The innate immune response is slow and weak, The anti-PRRSV effector adaptive immune response, i.e. antibodies and cytotoxic T cells, are slow to develop. Thus a long viremia of a month or so occurs, followed by a prolonged persistent infection in lymph nodes. The load of virus reaches peak levels in about a week and diminishes steadily. Most of the virus is eliminated before neutralizing antibodies are detected, and in animals lacking an effective T cell response. The mechanism of viral elimination may involve a reduction in the abundance of permissive macrophages. The prolonged period of viremia and extended persistent infection of lymph nodes increases opportunities for transmission and establishment of endemic infection of herds with continuous flow management. Pigs that are infected once are largely resistant to reinfection. There is little or no viremia and little or no change in antibody titers. At the individual pig level, attenuated PRRSV vaccines grow in pigs, induce antibody responses, and substantially or completely prevent infection by field viruses. The great genetic variation in PRRSV allows for situations in which previous exposure to field or vaccine PRRSV does not completely protect against reinfection. Incomplete immunity to rechallenge is common for many pathogens and hosts, such as influenza virus in swine, humans and chickens. For PRRSV in swine, incomplete immunity may be sufficient to block disease spread or it may not be sufficient. The outcome is influenced by herd size, by the specific viruses that are present in the herd, and by management practices. At present it is not possible to predict the level of protection that will be afforded by one PRRSV against another based on genetic similarity or other factors, and experimental comparisons generally show that previous exposure to one virus produces a high level of protection against re-challenge by a variety of unrelated viruses. Improved diagnostics for quantitative assessment of viral load and differentiation of antibody response to vaccines versus field viruses will be helpful determining the effectiveness of control and elimination programs, and the virological and immunological status of herds. Improved diagnostics, combined with a better understanding of PRRSV interactions with its host, will help to control PRRS.

PRRSV invasion and dissemination

Macrophages and other cells of myeloid origin, such as dendritic cells and monocytes, are the only definitive permissive cells for PRRSV growth and replication. PRRSV has direct access to these cells at mucosal surfaces of the lung and reproductive tract, various surfaces of the oral cavity, Peyer's patches of the small intestine, and underneath broken skin, where histiocytes and Langerhans cells are abundant. PRRSV is highly infectious. Jeff Zimmerman and colleagues at

Iowa State University showed that a single viral particle is infectious in the absence of physical barriers, as when injected by needle into muscle tissue. Thus, infection can occur readily in the lungs following inhalation of viral particles, in the reproductive tract by virus in semen from artificial or natural insemination, and presumably in the lymphoid tissues of the head and neck from ingested virus in saliva or on fomites. Infection also can occur through contact with bodily fluids of an infected pig, including saliva and blood, by direct contact of oronasal mucosal surfaces or through broken skin damaged during social interactions, establishment of dominance, and fighting.

Replication of virus in macrophages occurs within 12 hours and infected pigs are viremic at 24 hours after infection. Thus, within a day of infection the virus is disseminated throughout the body. Although alveolar macrophages of the lungs are widely recognized as the site of acute infection, in reality viral replication is widespread, occurring also in lymph nodes, especially those draining the lung and reproductive tract, in spleen, and in tonsil.

Innate cellular immunity

Viral infection of permissive cells is detected by various mechanisms that sense the presence of viral RNA and the presence of molecular features of the virus that are not present in the cells themselves, The sensors trigger a variety of responses designed to destroy the viral RNA, limit viral protein synthesis, degrade viral proteins, and alert nearby cells that danger is present. Interferons and inflammatory cytokines are key mediators of these responses and their production is a sign of viral infection. In the case of PRRSV, this early warning system largely fails. Interferon and inflammatory cytokine responses are weak (Albina et al. 1998; Buddaert et al. 1998; van Reeth et al. 1999). The lack of interferon production facilitates PRRSV replication since it mediates inhibition of PRRSV replication. PRRSV also blocks interferon production after superinfection with TGEV, a strong inducer of interferon. The weak innate response may compromise the subsequent initiation and elaboration of antigen-specific adaptive immunity, since signals from the innate response are important in preparing lymphocytes for development of antigen-specific B cells and T cells. In addition, suppression of innate anti-viral immune mechanisms may increase the risk of secondary infections.

Adaptive immunity and viral dynamics

The adaptive immune response is stimulated by the presentation of viral proteins to B lymphocytes, which respond by making antibodies, and to T lymphocytes, which respond by making virus-specific helper and cytotoxic T cells. Helper T cells play important roles in development of humoral and cell-mediated immunity, whereas cytotoxic T cells recognize and kill cells infected by virus. The general features of porcine B cell and antibody responses to PRRSV infection are similar to other viral infections such as influenza, transmissible gastroenteritis (TGE), and foot and mouth disease (FMD); namely, IgM antibodies first appear at 5-7 days, a switch to IgG occurs within 2-3 days, and total antibodies rapidly increase to a high level. The kinetics of anti-PRRSV antibody isotypes in bronchoalveolar lavage (BAL) fluid are similar to those in serum, indicating that they extravasate from the vasculature. Antibodies present in the first 3 weeks of a PRRSV infection are predominately directed against the nucleocapsid (N) protein and nonstructural proteins, nsp1 and nsp2. These antibodies are not

neutralizing. Neutralizing antibodies first appear at about 21 days of infection at low levels. Neutralization specificities have been reported against envelope glycoprotein GP5, GP4, and the membrane protein (M). A linear epitope on the ectodomain region of GP5 has been identified as the target of neutralizing antibodies by four laboratories, although the characteristics of the specific amino acid sequence involved in neutralization are not fully resolved (Pirzadeh and Dea 1997; Ostrowski et al. 2002; Plagemann et al. 2002; Wissink et al. 2003). Substantial variation is present in the neutralizing antibody responses of individual pigs, including lack of response, the kinetics of appearance, and titer values (Nelson et al. 1994; Loemba et al. 1996).

The T cell response to PRRSV is measured primarily by the production of interferon (IFN) γ in mononuclear cell cultures of blood or tissues following exposure to virus. Alternative classical methods for measuring T cell responses to viral infection, including proliferative and cytotoxic activity assays in response to viral stimulation, have been difficult to establish. The numbers of mononuclear cells that are secreting IFNγ in the blood of pigs infected with PRRSV is highly variable and transient. During acute infection, which commonly lasts from 28-42 days, PRRSV-specific IFNγ-secreting T cell frequencies in blood range from insignificant to a brief, high response that occurs after the peak or in the absence of viremia (Bautista and Molitor 1997; Lopez Fuertes et al. 1999; Xiao et al. 2004). The circulating T cell phenotype is reported as PRRSV-specific CD4+CD8+ memory or CD8+ γδ+, and constitutive CD4+ natural killer (Lopez Fuertes et al. 1999). Similar to the findings in blood, the abundance of virus-specific T cells in tissues of both acutely and persistently infected pigs is highly variable and shows no correlation to the amount of virus that is present in the tissue (Xiao et al. 2004).. Meier et al. (2003) also observed that the initial T cell response to PRRSV is weak and transient, but increases steadily for one to two years after infection.

The amount of PRRSV in an infected pig reaches a maximum in lung and blood at 5-9 days after infection (Labarque et al. 2000; Greiner et al. 2000; Samson et al. 2000; Johnson et al. 2004) and declines thereafter. In the lung, foci of infection decline to near zero by day 20, although virus still may be isolated from lung fluids for extended periods. In peripheral blood, virus levels are reduced 100-1000–fold by day 21 and usually become undectectable within 35 days, though viremia may continue longer (Johnson et al. 2004). Thus, the major decline in viral load during acute infection occurs within 3 weeks of infection, a period in which neutralizing antibodies are not present and the PRRSV-specific T cell response is highly unpredictable. Since viral loads are substantially reduced within 3 weeks of infection in the absence of a consistent and significant adaptive immune response, it appears that other mechanisms of resistance to PRRSV are present. Interestingly, a similar observation of viral control in the absence of adaptive immunity was reported for lactate dehydrogenase-elevating virus (LDV) infection of mice (Onyekaba et al. 1989). These investigators proposed that loss of permissive macrophages due to depletion by cytolytic infection was primarily responsible for control of infection.

PRRSV continues to circulate in blood after neutralizing antibodies are present (Loemba et al. 1996; Molitor et al. 1997). Viremia in the presence of neutralizing antibodies suggests that the levels of neutralizing antibodies normally attained against PRRSV may not be sufficient to eliminate circulating virus. Indeed, levels of neutralizing antibodies in acute infection may be quite low (Nilubol et al. 2004). Moreover, low levels of neutralizing antibodies may exacerbate PRRSV infection. Since macrophages express receptors for the Fc fragment of

immunoglobulins, PRRSV infection may be maintained in part by antibody-mediated uptake of virus into permissive macrophages (Yoon et al. 1996). Thus it appears that antibody neutralization plays a secondary role in adaptive immune responses to PRRSV or possibly prolongs the infection.

The overall picture of anti-PRRSV adaptive immunity from these studies is that anti-PRRSV immunity is relatively weak and sporadic, and is not directly linked to the control of primary, acute PRRSV infection. The adaptive immune response to PRRSV is different from the response to other important swine viral pathogens. In these cases, a strong and timely adaptive immune response rapidly reduces viral loads and achieves viral elimination within 7-20 days. Examples include FMDV (Salt 1993; Saiz et al. 2002; Alexandersen et al. 2003), influenza virus (van Reeth and Nauwynck 2000), and Aujesky's disease virus (Wittmann et al. 1980). Thus, PRRSV interacts with swine differently than do other viral pathogens. The immune response is incomplete, inconsistent, and ineffective. Neutralizing antibodies appear late in acute infection and only at low levels, and antigen-specific T cell responses are not correlated with viral loads. The inability of the adaptive immune response to control and eliminate the virus provides the opportunity for persistent infection of lymph nodes, a hallmark of PRRS. Interestingly, lymph node macrophages or dendritic cells in the persistent phase of infection stain positively for PRRSV antigen, even in tissues that are negative for PRRSV RNA (Xiao et al. 2004). This observation suggests that viral antigen is not being presented effectively to cytotoxic T cells or to helper T cells, or both.

Persistence of PRRSV in pigs

After viremia is resolved, PRRSV continues to persist for extended periods in lymphoid tissue in a process that involves replication (Wills et al., 1997; Allende et al. 2000; Chang et al. 2002; Horter et al. 2002). Primary sites of persistence appear to be tonsil and lymph nodes draining the lung, including sternal and tracheobronchial lymph nodes. Persistence in lymph nodes despite the presence of neutralizing antibodies and cell-mediated immunity argues that other factors, such as alteration in macrophage permissiveness to infection and innate immunity, may be important in control of PRRSV infection. The broad genetic and antigenic variation in PRRS virus and the presence of multiple viral genotypes circulating on farms or within production systems simultaneously also has an unknown effect on the efficacy of humoral and cell-mediated immune responses.

Protection against rechallenge

Pigs infected or vaccinated with live PRRSV are resistant to re-infection for more than 600 days (Lager et al. 1997b). Exposure to PRRSV therefore establishes some form of immunological memory that restricts or prevents a second infection. The level of protection may be profound; Foss et al. (2002) observed the complete absence of PRRSV following challenge of vaccinated pigs even though there was no change in specific antibody by two separate measures. Partial to high levels of protection also are achieved to re-infection of immune pigs with heterologous PRRSV strains (Lager et al. 2003; Mengeling et al. 1999, 2003a, 2003b).

The mechanisms of protection against PRRSV are assumed to operate according to accepted immunological principles. Viral infections induce innate antiviral responses, triggering adaptive immune responses through T_H1 and T_H2 pathways to elicit cytotoxic T cell and neutralizing antibody functions, respectively (Mosmann et al. 1986; Heinzel et al. 1989). The hallmark of T_H1 and T_H2 responses is the presence of antigen-specific T_H cells that secrete IFNγ or IL-4, respectively. However, in pigs methods have not been developed to culture antigen-specific T_H cells to assess cytokine secretion patterns, other cell types in addition to T_H cells secrete IFNγ, and the activities of IL-4 in pigs are not yet characterized. For these reasons, inferences about protection against PRRSV infection based on direct challenge studies are more reliable than inferences based on measurements of immune responsiveness.

The role of antibodies in protection against PRRS has focused on neutralization. Neutralizing antibodies are presumed to play an important role in resistance to reinfection and in prevention or reduction of viral spread from animal to animal, since they have the potential to clear free virus from the circulation. Passive administration of serum containing a high titer of neutralizing antibody can prevent reproductive failure (Osorio et al. 2002) but, as previously noted, primary infection is characterized by PRRSV in the presence of neutralizing antibody and low levels of neutralizing antibodies may even enhance infection (Yoon et al. 1996). PRRSV-specific memory T cells also would be expected to provide immune surveillance and protection against reinfection, but there is no satisfactory model to explain their role given that there is no correlation between T cell responses and viral clearance (Xiao et al. 2004). Whether neutralizing antibodies or cytotoxic T cells are essential for protection or even play a key role under natural conditions of re-infection is not known.

Regardless of the mechanisms by which it is obtained, immune memory exists to provide protection against PRRSV re-infection. Lack of knowledge of the precise mechanisms impedes development of improved vaccines and other preventive measures. Nevertheless, it is reasonable to expect that strategies that stimulate the full range of immune responses induced by virulent infection, such as vaccination with live, attenuated viruses, will be more effective than strategies that seek to stimulate selected immune pathways, such as antibody responses to inactivated vaccines.

Since PRRSV is rapidly evolving, attenuated vaccine strains will always be different from current field isolates, and cross-protection against heterologous field isolates will always be the key issue in disease prevention strategies that include vaccination. Experimental studies consistently demonstrate a high level of protection for long periods of time, including the commercial lifetime of sows, against re-challenge with homologous strains of an immunizing virus (Lager et al., 1997a, 1997b, 1999; Mengeling et al., 1999). Attenuated, live vaccines also have been effective in reducing disease severity, duration of viremia, virus shedding and the frequency of heterologous PRRSV infection (Nielsen and Bøtner 1997; Dee and Molitor 1998; Christopher-Hennings et al. 1997; van Woensel et al. 1998; Mavromatis et al. 1999; Mengeling et al. 1999, 2003a, 2003b; Lager et al. 1999, 2003). Still, episodic field observations of chronic and endemic PRRS and of “vaccine failure” suggest that protective immunity may be a variable feature of the immune response to heterologous PRRSV isolates.

Three significant issues need to be considered in analyzing cross-protection against PRRSV. First, attenuated vaccine viruses are the same as virulent field viruses immunologically. They interact equally with the immune system, expressing the same antigens in the same way. Vaccinated animals mount innate and adaptive immune responses that are the same as immune responses to field viruses. Vaccinated animals harbor virus persistently in lymphoid tissues and shed the virus to other pigs, as evidenced by reisolation of vaccine strains from commercial herds.

Second is variability in the intensity of immune response to vaccine, including lack of seroconversion. It is feature of PRRSV that is not specific to vaccines. For example, substantial variation, including non-responsiveness, in the induction of neutralizing antibodies by virulent PRRSV has been reported repeatedly (Yoon et al. 1995; Loemba et al. 1996; Vezina et al. 1996; Nielsen and Bøtner 1997). A definition of protective immunity and the ability of vaccines to induce a state of protection must await a better understanding of the precise mechanisms of protective immunity to PRRSV.

Third, PRRS disease occurs in three different developmental stages, fetus, young and growing pig, and adult gilts and sows. The immune system of pigs is developing in the fetus and does not become fully competent until 4-8 weeks after birth. Pigs that are infected in utero and born alive are weak, frequently die before weaning, and are persistent carriers. Pigs infected early in life suffer respiratory disease, whereas gilts or sows are severely affected with reproductive disease if infected late-term, but do not appear to have significant disease if infected when nonpregnant or early in pregnancy. These observations would suggest that the immune response improves with age, but is less effective in more severely affected animals. However, the few prospective studies that have been performed do not provide a clear answer. Infection of fetuses elicits PRRSV-specific antibody production, contrary to the expectation that antigens presented in utero might be perceived as self (Butler et al. 2001). Furthermore, no differences were observed in respiratory disease or immune response in pigs infected at 1- , 4- or 10-weeks of age (Rossow et al. 1994). At this point we do not know if animal age or stage of development contributes significantly to the induction of anti-PRRSV immunity. Even if it does, the tremendous genetic and antigenic variation in PRRSV in the field may mask a smaller effect of animal age or developmental stage. However, the question remains relevant for vaccine protection against PRRS. Since there is no significant antigenic variation in the vaccine strain of virus and protection must be produced against heterologous viruses, differences in host animals that affect the potency of vaccinal immunity may be important.

Protective immunity and endemic PRRS

Vaccines are used not only to reduce the risk of infection by PRRSV, but also to intervene in an infected herd to halt the spread of virus and reduce disease severity. Vaccine intervention to treat PRRS in an infected herd is based on the expectation that a second exposure to antigen will boost the level of immunity and that a stronger immune response will more rapidly and more thoroughly eliminate the virus. However, re-challenge of pigs previously exposed to PRRSV has little or no effect on the level of anti-PRRSV antibody responses (Foss et al. 2002). As described above, pigs that are exposed to PRRSV are largely resistant to re-infection and demonstrate little or not potentiation of immunity. Inability to boost immunity is consistent with the concept that

resistance is present at the level permissive target cells. Lack of infection would result in absence of antigen to restimulate memory antigen-specific B and T lymphocytes. If true, one might predict that neutralizing antibody levels could be increased by boosting with killed vaccine or recombinant proteins. While use of inactivated virus or proteins to boost immunity is logical, the approach has not been successfully applied. Perhaps the amounts of antigen are not sufficient, the compositions fail to properly stimulate immune recognition, or the preparations do not contain the appropriate targets for protection.

Maternal immunity to PRRSV

No specific study has evaluated the effect of maternal immunity on piglet susceptibility to PRRSV infection. We do not know the relationship between time of vaccination or infection of prebreeding or pregnant swine and the transfer of antibodies, or lymphocytes or PRRSV in colostrum and milk to nursing piglets. Nor do we know the effect of time and dose of previous PRRSV exposure, via vaccine or virulent field virus, on protection of nursing piglets against PRRSV infection or disease. Indirect inferences suggest that immune sows provide maternal protection to piglets. Anti-PRRSV antibodies are present in colostrum at the same concentration as in blood (Eichhorn and Frost 1997) and PRRS is reported to increase in pigs when maternal antibodies become undetectable (Albina et al. 1994; Houben et al. 1995; Chung et al. 1997). Therefore, piglets may have been protected by maternal immunity since healthy piglets are equally susceptible to PRRS at 1 and 10 weeks of age (Rossow et al. 1994). However, maternal immunity does not prevent transplacental infection (Lager et al. 2003), Also, PRRSV can be shed in milk (Wagstrom et al. 2001), and neutralizing antibodies, particularly at low concentrations, may exacerbate PRRSV infection by antibody-dependent enhancement (ADE) (Yoon et al. 1996). At present, the role of maternal immunity in protection of piglets against PRRS is not fully resolved.

Evidence of past exposure to PRRSV

Antibodies are produced to a wide variety of structural and nonstructural proteins of PRRSV following infection. The appearance and kinetics of antibody responses vary widely. Antibodies are produced to nucleocapsid and nsp2 within 7-10 days, whereas antibodies to GP5 and M are not usually detected before 20 days of infection. Antibody titers also show large differences over time. The nsp2 antibody titer is maintained at high levels in persistent infection, whereas anti-nucleocapsid titers decrease substantially at the end of acute infection but persist for extended periods thereafter. These varying patterns and differences in antibody production might be useful in developing diagnostic tools to better understand infection patterns in the field. Similarly, genetic differences among PRRSV strains is reflected in serological differences, which are known to occur between European and North American PRRSV strains, but also between vaccine and field viruses. Knowledge of viral gene sequences, combined with recombinant DNA technology and protein expression capabilities, will facilitate the development of differential sesrodiagnostics to not only detect previous exposure to PRRSV, but to provide more information about the nature of the exposure.

Conclusions

The interaction of PRRSV with its host is different that the interaction of pigs with other viral pathogens. Evasion of innate immune responses is followed by a prolonged acute, viremic infection of 4-6 weeks, followed by a persistent infection of lymphoid tissues for months. An adaptive, antigen-specific immune response is produced but in ineffective in elimination of PRRSV from the pig. The difficulty of achieving consistent and reliable control and prevention of PRRS with live, attenuated vaccines emphasizes our incomplete understanding of PRRS immunology. Serious deficits exist in our knowledge of the events initiating immunity at the time of infection, of key immunologic targets for both antibody and cytotoxic T cell-directed protection, of the molecular and cellular mechanisms regulating induction and maturation of the immune response, of the consequences of genetic diversity in PRRSV on immune protection, and of host genetic variation in pig populations on immune resistance to PRRSV. Thus, significant challenges remain in our efforts to develop better tools for diagnosis, prevention and control of PRRS.

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