Review

Factors critical for successful vaccination against classical swine fever in endemic areas

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Abstract

Classical swine fever (CSF) or hog cholera, caused by the classical swine fever virus (CSFV), is one of the most important viral diseases that cause serious economic loss to the swine industry worldwide. During the past 5 years, several techniques for measuring porcine cell-mediated immunity (CMI) were applied, in conjunction with other conventional techniques, to study factors that influence the induction of CSFV-specific immunity. Information, obtained from a series of experiments, demonstrated cell-mediated immune responses in providing protective immunity against CSF infection. Although it has been confirmed that commercially available modified live CSF vaccines are able to induce complete protection in vaccinated pigs, several factors including maternal immunity, the age of primary vaccination, vaccination protocol and complications caused by other pathogens, can greatly affect the effectiveness of CSF vaccines in the field.

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1. Introduction

Classical swine fever (CSF), formerly known as hog cholera, is one of the major diseases, causing serious economic loss to the swine industry worldwide (Moennig, 2000). The disease is caused by a Pestivirus named classical swine fever virus (CSFV), an enveloped, single-stranded RNA virus (Moennig, 2000). Classical swine fever virus can be classified genetically into three genogroups (Paton et al., 2000). All the genogroups have been isolated in Thailand, with the major prevalence being genogroups 1 and 3 (Parchariyanon et al., 2000). However, during the last decade, there has been an increased incidence of subacute and chronic CSF outbreaks caused by the moderately virulent CSFV, genogroup 2.2. The CSFV genogroup 2.2 is closely related to the European CSFV and was first isolated in Thailand in 1996. Since then, this genogroup has contributed to over 50% of the isolates during the 1990s (Parchariyanon et al., 1999). This newly emerged genogroup has become the major strain circulated in the area, causing milder clinical symptoms ranging from subacute to chronic forms of CSF (Parchariyanon et al., 1999; Damrongwatanapokin et al., 2002).

In the highly endemic areas routine vaccination against CSF is the most common means used for prevention and control. However, the increased incidence of subacute CSF during the 1990s in Thailand raised several concerns whether the vaccines and the vaccination programs were still effective. Since the year 2000, our group has carried out a research program, conducting several challenge studies to assess the efficacy of commercially available CSF vaccines and to evaluate the factors critical for successful vaccination against CSF. In this article, the immunological mechanisms involved in vaccine-induced disease protection and factors related to successful vaccination against CSF will be further discussed.

2. Immunological mechanisms underlying vaccine-induced protective immunity

Most of the information regarding the immunological mechanisms involved in protection against CSFV infection were derived from an experiment using the modified live, Chinese (C) strain vaccine. The C strain vaccine is generally accepted to be very safe in pigs of all age groups, providing complete protection, i.e. sterile immunity in vaccinated pigs. The C strain vaccine usually induces detectable neutralizing antibodies in vaccinated pigs 2–3 weeks following primary vaccination (Precausta et al., 1983; Terpstra et al., 1990). However, pigs vaccinated with the C-strain vaccine appear to be completely protected against virulent CSFV challenge as early as 1 week following vaccination (reviewed in Van Oirschot, 2003). It should be noted that, in some cases, vaccine-induced protection was achieved in the absence of neutralizing antibodies at the time of challenge (Launais et al., 1978; Rümenapf et al., 1991). These findings implicate the vital role of cell-mediated immunity (CMI) in viral protection during the early phases of immunity.

Cell-mediated immunity (CMI) is known to have both an effecter and a regulatory role on the immune system and is believed to be essential for immunity against intracellular pathogens, including viruses (Janeway et al., 1999). Previous information regarding the role of CMI in vaccine induced, viral protection in pigs has been very limited, due to the lack of reagents and the impracticality of the conventional techniques for the detection of porcine CMI. Nevertheless, antigen-specific lymphoproliferative activity was demonstrated in peripheral blood lymphocytes from vaccinated pigs that were protected against the CSFV challenge (Remond et al., 1981). The existence of CSF-cytotoxic T lymphocytes (CTL) in the peripheral blood mononuclear cells (PBMC), from immunized pigs, has been demonstrated (Pauly et al., 1995; Armengol et al., 2002; Piriou et al., 2003). An alternative technique for assessing CMI is to measure the production of cytokines that are known to influence or directly relate to cellular immunity. Among these cytokines, the role of interferon-gamma (IFN-\(\gamma\)) for the induction of CMI has been well characterized. IFN-\(\gamma\) has several immunoregulatory effects that are involved in the induction of anti-viral immunity, including the activation of CTL, natural killer (NK) cells and phagocytes (Janeway et al., 1999). The measurement of viral-specific IFN-\(\gamma\) production is believed to be a direct reflection of viral-specific lymphocyte activities (Mateu de Antonio et al., 1998; Zuckermann et al., 1998). Furthermore, viral-specific IFN-\(\gamma\) production remains detectable for
a long period of time, whereas lymphocyte proliferation tends to diminish due to the decreased ability of T cells to produce IL-2 (Mateu de Antonio et al., 1998). Previously, an ELISPOT assay for measuring porcine IFN-γ producing cells was established and used for determining the number of pseudorabies virus (PRV)-specific, IFN-γ producing cells. Several reports have shown that the number of viral-specific IFN-γ producing cells is a good indicator of anti-viral immunity (Mateu de Antonio et al., 1998; Zuckermann et al., 1998, 1999). Subsequently, an ELISPOT assay for determining CSFV-specific IFN-γ production was established and has been used for the assessment of the CSFV-specific cellular immunity by several research groups (Suradhat et al., 2001; Armengol et al., 2002; Rau et al., 2006). In our experience, CSFV-specific IFN-γ secreting cells could be detected in the PBMC from pigs vaccinated with the lapinized C strain vaccine, as early as 6 days, and lasted for up to 140 days, following vaccination (Suradhat et al., 2001). Furthermore, disease protection could be demonstrated in pigs carrying high numbers of CSFV-specific IFN-γ cells at the time of challenge, while neutralizing antibodies were still undetectable (Suradhat et al., 2001; Suradhat and Damrongwatanapokin, 2003). These findings highlight the role of vaccine-induced cellular immunity in viral protection during the early phases of immunity. In addition, the role of CMI in protection against CSFV infection was indicated even in the presence of CSFV-specific neutralizing antibodies. When the pigs were challenged 140 days after a single vaccination, good correlation occurred between the level of protection and the level of IFN-γ production, more than the antibody titer (Suradhat et al., 2001). Despite the protective role of the cellular immune response at the time of challenge, the role of CSFV-specific antibody following such challenge should not be excluded, since there was a significant increase in the SN titers of the vaccinated pigs, but not the control unvaccinated pigs, following the challenge (Suradhat and Damrongwatanapokin, 2003; Suradhat et al., 2001). Generally, CSFV infection causes severe B-cell depletion (Suša et al., 1992), possibly by the induction of massive lymphocyte apoptosis (Summerfield et al., 1998). Protected pigs were probably able to control the establishment of viral infection, and therefore, B cell function was not affected. The rapid anamnestic antibody response following the CSFV challenge was certainly advantageous in reducing viral spreading in the protected animals.

Although an ELISPOT assay can be used to monitor overall IFN-γ production in response to CSFV, the assay cannot differentiate the lymphocyte subpopulation responsible for cytokine production. Thus, it was not clear which cellular subpopulation (helper T cell; Th, CTL, etc.) was responsible for the cytokine production observed in the previous ELISPOT study. This limitation has been overcome by the application of flow cytometry to analyze the cellular markers and the intracellular cytokine production, simultaneously. Recent studies revealed that PBMC from pigs vaccinated with the C-strain vaccine produced IFN-γ in response to CSFV and that the double-positive (DP), CD4⁺CD8⁺ population was the major IFN-γ producer (Suradhat et al., 2005). This finding is in agreement with previous reports demonstrating that DP cells, which are the memory Th population, can produce high levels of IFN-γ in response to a recall antigen or polyclonal T cell activator (Rodriguez-Carreno et al., 2002; Saalmuller et al., 2002). The finding also implies that the IFN-γ producing cells detected by ELISPOT assay, following immunization with the C strain vaccine in the previous study, were indeed reflecting Th activity.

It should also be noted that the number of IFN-γ producing cells, following vaccination, was always lower than that observed following CSFV challenge (Suradhat et al., 2005, 2006). This finding is consistent with an other report that demonstrated that immunization with a modified live vaccine induces a lower level of cell-mediated response than the actual viral infection (Piriou et al., 2003). Interestingly, the CD4⁻CD8⁺ population was found to be the major IFN-γ producer, while there were a significantly less number of IFN-γ producing DP cells in the PBMC of the vaccinated pigs during the first week following CSFV challenge (Suradhat et al., 2005). As it has previously been suggested, porcine memory Th population preferentially homes to the secondary lymphoid organs (Zuckermann, 1999), thus, the low number of antigen-specific DP cells may simply reflect the difference in tissue homing preference among the lymphocyte subpopulations, during the effector phase of the immune response. The increased CD8⁺ activity in vaccinated pigs following challenge was possibly vaccine-induced anamnestic response of the CD8⁺
population. Alternatively, it is also possible that rapid IFN-γ production by CSFV-specific memory Th, following the challenge, might contribute to the generation of CTL (Piriou et al., 2003). Nevertheless, the data suggests that activation of memory CSFV-specific Th cells and priming of a viral specific CD4+CD8+ population, possibly CTL, occurred in the pigs vaccinated with the C strain vaccine. These cellular activities are likely be one of the protective immune mechanisms induced by the C strain vaccine, particularly during an early phase of immunity.

When vaccinated pigs were challenged at a later stage after vaccination, there seemed to be a good correlation between the presence of neutralizing antibodies at the time of challenge and viral protection (Terpstra and Wensvoort, 1988; Suradhat et al., 2001). It is generally accepted that vaccinated pigs with active neutralizing antibody titers of \( \geq 32 \) were considered to be protective (Terpstra and Wensvoort, 1988). It should be noted that this assumption was obtained from experiments using a modified live vaccine. As the vaccine antigens are able to enter both the endogenous and exogenous antigen presentation pathway, this type of vaccine should efficiently activate both humoral (neutralizing antibody) and cell-mediated immunity (CTL). In general, active CSFV-specific antibody production following vaccination, with the C strain vaccine, correlated well with the levels of IFN-γ production (Suradhat et al., 2001). Therefore, routine monitoring of SN titers, i.e. seroprofile, can still be used for assessing the effectiveness of a CSF vaccine program in the field. In fact, we strongly recommend routine monitoring of herd immunity to CSFV, in farms situated in a highly endemic areas.

As suggested above, neutralizing antibodies also contribute to viral protection, through the reduction of spreading and shedding of the infectious virions. The evidence that neutralizing antibodies, alone, could provide protection has also been reported. Piglets with exceptionally high levels of maternal derived neutralizing antibodies (\( >512 \)) are passively protected against the CSFV challenge (Parchariyanon et al., 1994). Furthermore, the E2 subunit vaccine, which mainly activated the CSFV-specific Th population and the production of neutralizing antibodies, confer clinical protection against the CSFV challenge (de Smit et al., 2001; Uttenthal et al., 2001; Dewulf et al., 2002). However, it should be noted that the information regarding the protective values of the E2 subunit vaccines were rather inconsistent (reviewed in Van Oirschot, 2003). As viral protection induced by the E2 subunit vaccine is likely to rely on the ability to induce CSFV-specific neutralizing antibodies (Van Oirschot, 2003), it may take a longer time (at least 2–3 weeks) following vaccination or more than one vaccination to obtain complete viral protection. A recent study in Thailand has shown that at least two vaccinations were required to induce clinical protection in pigs carrying a low level of maternal derived antibodies (MDA), at the time of vaccination. Nevertheless, most of the protected pigs still developed fever, leucopenia, and viraemia following the viral challenge (Damrongwatanapokin et al., 2006). The efficacy of the E2 subunit vaccine for prevention of horizontal and vertical viral transmission has also been previously placed in doubt (Dewulf et al., 2002, 2005).

Findings on the efficacy of the E2 subunit vaccine suggest that nature of an antigen presented to the immune system (i.e. endogenously or exogenously produced form) can significantly affect the ability of the vaccines to induce protective immunity against CSFV. Generally, exogenous protein antigen does not efficiently activate CTL activity (Janeway et al., 1999). It should be noted that CSFV is cell-associated and a non-cytopathic virus. Replication is restricted in the cytoplasm of the cell and the virus can spread directly from the infected cell to adjacent cells (Van Oirschot, 1999). Thus, inefficient priming of viral-specific CTL could be one of the explanations for incomplete viral protection induced by the subunit vaccine. Interestingly, plasmid encoding E2 protein of CSFV conferred complete protection in the vaccinated-challenged pigs, even in the absence of neutralizing antibodies at the time of challenge (Ganges et al., 2005). Together, these findings emphasize the vital role of cell-mediated immunity (i.e. viral specific CTL activity) for controlling viral spreading in the challenged animals.

3. Factors critical for successful vaccination against classical swine fever in endemic areas

During the past years, we have conducted several CSF vaccine trials and challenge experiments to
investigate the factors critical for the induction of protective immunity against CSFV by modified live CSF vaccines. Some of the experiments were performed in order to verify common beliefs among swine practitioners, or to evaluate the off-labeled vaccine usage that was once widely practiced in the country. Several factors critical for a successful CSF vaccination program will be discussed further as follows.

3.1. The vaccines

The C strain, modified live vaccine (MLV) has been regarded as one of the most effective CSF vaccines that provides complete clinical and virological protection, i.e. sterile immunity, within a week of vaccination (Suradhat et al., 2001; Van Oirschot, 2003). It has been suggested that the C strain CSF-MLV is the vaccine of choice for an emergency vaccination protocol (Van Oirschot, 2003). Several strains of commercial CSF-MLV, mostly derived from genogroup 1, are available in the market. In our experience, a single vaccination of the CSF-MLV, including C strain (both lapinized and tissue culture derived) and GPE- strains, induced complete protection against CSFV infection as early as 6 days after vaccination. It should be noted that this protective effect was observed on the condition that the pigs had low levels of MDA (≤32) at the time of vaccination (Suradhat et al., 2001; Suradhat and Damrongwatanapokin, 2003).

Several genogroups of CSFV have recently been isolated in Thailand (Parchariyanon et al., 2001). Although it is well accepted that CSF-MLV can effectively induce complete protection against all of the CSFV strains, increased prevalence of the newly emerged genogroups during the last decade has raised concerns whether the available vaccines can induce complete protection to every CSFV genogroup found in Thailand. Our data, together with other reports, confirm that CSF-MLV can induce complete protection against all tested CSFV genogroups, including the newly emerged genogroups 2 and 3 (Parchariyanon et al., 2001; Suradhat and Damrongwatanapokin, 2003). Thus, the increased prevalence of new genogroups is unlikely to be due to any inability of inducing heterotypic protection by CSF-MLV.

Although, CSF-MLV could effectively induce protective immunity in pigs, certain conditions are required to achieve complete viral protection. Off-labeled vaccine usage, as for example, vaccination of the CSF vaccine combined in the same injection with other vaccines should be systemically tested prior to implementation. In one of our studies, combining the C-strain vaccine and a live eG-deleted, PRV vaccine resulted in a significant reduction in cellular response against CSFV, while there were no differences in the levels of SN antibody. Although the pigs vaccinated with the combined vaccine were clinically protected against CSFV challenge, there were clearly more fever days and pathological changes in this group, when compared to those from the pigs vaccinated with the CSF vaccine alone (Suradhat et al., 2001).

Oral CSF-MLV vaccination was introduced by the European countries for the purpose of controlling CSF in wild boars (Van Oirschot, 2003). To obtain complete protection, the oral CSF vaccine formulation requires the addition of a vaccine stabilizer and a higher dose of the MLV virus (Kaden et al., 2000). In addition, more challenge studies will be needed to evaluate the effectiveness of this vaccine regimen in domestic pigs. Vaccine protocols using a commercially available CSF vaccine for oral immunization of domestic pigs are not, at the moment, recommended.

3.2. The pigs

In our experience, interference by maternal derived antibodies (MDA) is the most common factor affecting the induction of protective immunity against CSFV in the field. Piglets are born agammaglobulinaemia and acquire passive immunity by colostral intake. The levels of CSFV-specific MDA of the suckling pigs correlate well with the levels of serum neutralizing (SN) titer of the sows. Without any exposure to CSFV, MDA gradually declines with a half-life of approximately 2 weeks (Coggins, 1964). Generally, it is well accepted that optimal protection can be achieved by vaccination of piglets carrying MDA titers of ≤32 (Suvintrakorn et al., 1993; Parchariyanon et al., 1994). This can be problematic in a highly endemic area, where all the pigs are routinely vaccinated and viral circulation in the farm can be high. Natural CSFV exposure of the vaccinated sows leads to anamnestic antibody responses that are passed on to the piglets without any observed clinical signs. Although it has been reported that high levels of
MDA can confer some degree of protection, the MDA titers of more than 256 were required to obtain complete clinical protection in the piglets (Parchariyanon et al., 1994). Furthermore, it would take a longer time for the MDA, at this level, to decline to the level that does not interfere with CSF vaccine efficacy, i.e. longer window of susceptibility. Accumulating data indicates that MDA of more than 32 significantly affects the induction of protective immunity by CSF-MLV, and this leads to vaccine failure (Suvintrakorn et al., 1993; Parchariyanon et al., 1994; Suradhat and Damrongwatanapokin, 2003). In some experiments, piglets vaccinated in the presence of high MDA titers never showed seroconversion to the subsequent CSFV exposure (Parchariyanon et al., 1994; Suradhat and Damrongwatanapokin, 2003). Interestingly, in the challenge study using a moderately virulent CSFV (genogroup 2.2), all challenged pigs survived the challenge through to the end of the experiment. However, CSFV could be isolated from the sera of unvaccinated pigs and 50% of the pigs, vaccinated while having high levels of MDA. With an increase in the prevalence of subacute and chronic CSF during the past years, infected/unprotected pigs may survive for a long period of time without any obvious clinical signs of CSF. This clinical situation will certainly complicate any disease control program, as the infected pigs become an undetected source of infection on the farm. Again, these findings highlight the significance of routine serosurveillance and the use of other diagnostic tools for monitoring the herd immune status and plans for immunoprophylaxis.

Apart from MDA, the influence of age at the time of primary vaccination was also investigated. Piglets at the age range of 3–5 weeks, with comparable levels of MDA titers (<32), were vaccinated twice at a 2 weeks interval with the lapinized C strain vaccine. Levels of CSFV-specific, cellular and antibody responses, were determined every 2 weeks up to 10 weeks post-vaccination. The result indicated that piglets immunized at 5 weeks old developed a greater number of CSFV-specific, IFN-γ producing cells in the PBMC and higher CSFV-specific, SN titers than pigs immunized at 3 weeks old (Suradhat and Damrongwatanapokin, 2002). Although CSFV challenge was not performed in this study, the results clearly demonstrated that younger pigs are not as immunocompetent as the older ones. The finding is in accordance with previous knowledge that the porcine immune system is fully matured at the age of 4 weeks (Povey and Carman, 1997). Thus, when emergency vaccination protocol is implemented in very young piglets, during an outbreak, a booster vaccination may be required to achieve complete protection. Furthermore, it is highly recommended that emergency vaccination protocol should be removed as soon as the outbreak is over.

3.3. Complications by other pathogens

As shown through our series of experiments, the induction of protective immunity against CSFV infection depends on several factors. Most of the challenge studies were conducted in the isolation units where disease complications by other pathogens were minimized. The results and interpretations obtained from these experiments may underestimate clinical outcomes in the field, where pigs can be exposed to several pathogens at the same time. Several pathogens, mycotoxins and chemicals are known to negatively modulate the immune system, and therefore, significantly interfere with the effectiveness of any CSF vaccination protocol. In our experience, co-infection with PRV at the time of CSFV challenge resulted in fatal CSFV infection of the vaccinated pigs, despite successful immunization against CSFV, as determined by the presence of CSFV-specific, IFN-γ producing cells and SN titers, prior to the CSFV challenge (S. Suradhat, unpublished).

Since its emergence in the late 1980s, porcine reproductive and respiratory syndrome virus (PRRSV) has become one of the most economically important pathogens of the swine industry (Meng, 2000). In Thailand, the prevalence of PRRSV infection is believed to be more than 80% (Thanawongnuwech et al., 2004). Several lines of evidence suggest that PRRSV can negatively modulate the immune system. Our recent findings demonstrate that PRRSV induces systemic production of a very potent immunosuppressive cytokine, interleukin-10 (IL-10), during an early stage of infection (Suradhat and Thanawongnuwech, 2003). Furthermore, the presence of PRRSV in the culture suppressed IFN-γ production by the CSF-MLV primed PBMC when stimulated with the recall antigen; CSFV (Suradhat et al., 2003). In order to explore the effect of PRRSV infection on the efficacy
of CSF-MLV, we conducted the dual PRRSV-CSFV challenge experiment. Seventeen days old pigs were infected with a Thai PRRSV isolate a week before vaccination with the lapinized C strain vaccine. Three weeks after CSF vaccination, the pigs were challenged with the virulent CSFV. The results demonstrated that PRRSV infection significantly interfered with induction of CSFV-specific immunity which resulted in vaccine failure (Suradhat et al., 2006). The finding is in agreement with a previous report that PRRSV infection suppresses active antibody responses to CSF vaccination (Li and Yang, 2003). Interestingly, while measuring the levels of CSFV-specific, IFN-γ production, we observed that the numbers of IL-10 producing cells by the PBMC from PRRSV infected pigs were significantly increased during the first 14 days post-infection. The systemic IL-10 production, occurred at the time of CSF vaccination, was likely to interfere with CSF vaccine efficacy, and subsequently led to vaccine failure. We postulated that the PRRSV-induced, IL-10 production could result in the inhibition of immune responses to other antigens, to which they may be exposed during the same period of PRRSV infection. In fact, the negative effects of PRRSV infection on the efficacy of other porcine vaccines have been previously reported by many investigators (De Bruin et al., 2000; Thacker et al., 2000). This finding implied that CSF vaccination during the active stage of PRRSV infection should be avoided. Taken together, the above findings emphasize that the influence of other pathogens on the immune system and/or interactions among the pathogens should also be taken into consideration. Strict biosecurity and routine monitoring of the herd immune status will be crucial for preventing such complications.

4. Final remark

During the past years, we have explored several factors critical for successful CSF vaccination in the field. Our results, together with previous reports, confirm that CSF-MLV can effectively induce protective immunity against CSFV infection, when used properly. In our opinion, CSF vaccine failure that is observed in the field is primarily due to a lack of understanding of the herd immune status, mechanisms of immunological protection, viral pathogenesis, and epidemiology. Sharing such information among veterinary researchers, swine practitioners and farmers, together with a strengthening of the disease surveillance program is necessary for a successful CSF preventive and control program, which in our hope, will eventually lead to the eradication of CSF in the region.

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References


