CHAPTER V

DICUSSION, COMMENTS AND CONCLUSION

Discussion and Comments

The antibody response in vaccinated pigs with different level of maternal antibody revealed the different pattern. Those with low maternal antibody titer yielded the higher antibody response than the other group which was in agreement with other previous studies (Metzger et al., 1978; Parchariyanon et al., 1994; Suvintarakorn et al., 1993). When challenged with high virulent strain CSFV, most of the vaccinated pigs significantly developed high level of seroconversion within two weeks. Even though, all pigs in group B and some in group A yielded Ab less than completely protective level (<32) on the day of challenge, all pigs still survive (Terpstra and Wensvoort, 1988). This implies that it might be the other mechanisms, apart from serum Ab, which can prevent the disease. In previous study, it has been reported that the number of specific interferon-γ secreting cells in vaccinated pigs was higher than non-vaccinated ones (Suradhat et al., 2001). Endsley et al. (2003) has previously reported that vaccination against BVD in young calves with high level of maternal Ab could activate production of memory B cells and many of T cell lines; CD4+, CD8+, and γδ T, despite undetectable Ab. However, both CMI and HMI response in CSFV vaccinated pig, tended to be affected by maternal Ab as well as the age of pigs (Suradhat and Damrongwatanapokin, 2003; Tiyasatkulkovit et al., 2002). How does the maternal immunity interfere Ab production and which cells are affected have not been so far understood. It may be due to pig has a distinct either structure or function of lymphoid tissues, especially a lot of subpopulation of lymphoid cells, differed from other mammals (Binns and Pabst 1996; Saalmuller et al., 2002). One of mechanisms accountable for low immune response after vaccination affected by maternal Ab is the neutralization effect to the vaccine virus. Hence, infection of vaccine virus to permissive cells was thwarted. Subsequently, the Ag-Ab complex would induce an active suppression through one of immunosuppressive cytokines; IL-10, processed by cross-linking of FcγR on antigen presenting cell (APC).

Furthermore, one of the crucial factors is age of the animal, that young animals would relatively show low function of APC. Thus, the response of low co-stimulatory signals will be presented. And also T-cell activation without such those signals, T-cell apoptosis would ensure to be occurred (Bot and Bona, 2002; Harris and Ronchese, 1999). Other possible mechanisms for low humoral immune responses in young animal may be related to the limitation of IFN production by helper T-cells, decreased IL-12 production affected to induction of $CD4^+$ T-cells as well as antigen-induced up-regulation of CD40-Ligand which could restricted the interactions of helper T-cells and B-cells (Gans et al., 2003).

Assumption of our study revealed that the CSF vaccination can actually prevent pigs in all vaccinated groups (A and B) from developing clinical signs and even death. It has not been yet to be concluded that vaccination can prevent white blood cell destruction in early stage and viremia. Some vaccinated pigs were developed leukopenia and viremia in 3dpi. It is noted that 2/6 pigs with viremia and leukopenia in group B were those which had relatively low SN titer in comparison to the others in the same group on the challenging day. The pigs with viremia and leukopenia either group A or B had a relatively slow seroconversion when compared to others in the same group as shown in Table A3, appendix A. Since, CSFV can induce leukopenia prior viremia; the depletion of white blood cell in infected pigs with no viremia possibly resulted from apoptosis (Summerfield et al., 1998). Many mechanisms might be involved for the induction of the apoptosis such as production of glycoprotein E^{ms}, activation of cytokines, etc. The glycoprotein E^{ms} created by virus itself in early stage at primary site of replication can induce both adjacent and distant cells to develop apoptosis (Bruschke et al., 1997; Le Bon et al., 1999; Weiland et al., 1999). Apoptosis can also be promoted by cytokines, IFN-γ and TNF-α, secreted from infected cells through the process of Fas Ag expression up regulation (Benedict et al., 2003; Oyaizu et al., 1994). In case of CSF, major cytokines that strongly related to the infection are TNF-α, IL-1α while IL-1β and IL-6 are the minor mediators (Sanchez-Cordon et al., 2002). Those cytokines-induced apoptosis has been previously reported in PRRSV and African swine

fever infection (Choi and Chae, 2002; Labarque et al., 2003; Salguero et al., 2002). However, apoptosis might be resulted from applying attenuated vaccine as well, such as CSFV Chinese strain causing glycoprotein E^{ms} which was detected in serum and lymphoid tissues (Lorena et al., 2001).

Immunohistochemistry of CSFV in lymphoid tissue detected the CSFV-viral antigen was demonstrated in many cell types of all tissues, especially in lymphoid follicular germinal center of tonsil where markedly severe lymphoid depletion was occurred, which was similar to previous study (De Las Mulas et al., 1997; Narita et al., 2000). The difference of susceptibility to infection in each organ might be depended on amount of viral receptors of glycoprotein E^{ms} or E2 (Hulst and Mooreman,1997; Hulst et al., 2001). By the limitation of MAb affinity, Ag masking, and tissue condition, some positive sections could not be detected clearly. The quantitation of such positive cells is limited. In case of autolytic tissue, only well demarcated cells could be counted. There was no difference for the period of death among all affected pigs. Upon the study, no viral Ag or any histopathological change could be detected in all vaccinated pigs' lymphoid tissues at the end of the study (21 dpi). Apoptosis detection in lymphoid tissues showed in all pigs, either vaccinated or non-vaccinated group developed quantitatively appearances. Pigs from group B gave the highest mean of apoptotic cells in various lymphoid tissues when those from group C gave the lowest number. In addition, the thymic lymphoid tissue of pigs in group C was the most affected among the others because they were rich of T-cell population for apoptosis at the day of challenge (Bianchi et al., 1992; Summerfield et al., 1998). Interestingly, the correlation between apoptotic cells and CSFV-infected cells was low negative correlation ($r^2 = 0.21$). This is implied that the areas with higher number of CSFV-infected cells would have lesser chance of apoptosis to occur, which was different from the previous study (Sato et al., 2000). The reason of low number of apoptosis detected in challenged pigs may be the observation time. Generally, the apoptosis occurs very fast and the apoptotic bodies are rapidly removed by phagocytic cells with in a few hours (Scott et al., 2001). In the case of CSF, apoptosis is detected with in a few days after infection (Summerfield et al., 1998)

and their apoptotic bodies might have been removed before the pig die. That may be the reason why we could not detect the apoptotic cells induced by CSFV at the end of disease. The other reason was probably due to the technical problems such as the infected tissue conditions, poor counterstaining. Apoptotic cell count per whole-field cell count (0.1 mm^2) might yield more accuracy than that per one field. It is possible to conclude that the pigs in group C developed low apoptosis resulting from the decrease of lymphoid cells in those areas investigated and the late of time phase to detect apoptosis at the end of observation (21 dpi). This is agreed with earlier study, that the number of apoptotic cells per area of investigation in CSFV-infected pigs' thymus would reach the highest sum during 4-7 dpi and then declined, as a result of decrease in whole cell amount (Sanchez-Cordon et al., 2002). For more exact result, the lymphoid tissues would be collected in various time of infection such as every 2 days until the end of experiment (21 dpi).

Lymphoid tissue apoptosis which was more prevalent to be seen in group B than group A might be the result of higher lymphocyte activation in the pigs which showed a higher immune response as supported by the evidence that development of lymphocyte apoptosis had high positive correlation with SN titer (r^2 = 0.81). When calculating the relation between antibody response and apoptosis, it was showed the relationship of high level of apoptosis in vaccinated pigs with high SN antibody level at the end of experiment (21 dpi) (Figure 12). Thus, the immune system regulates the number of activated lymphocyte to normal homeostasis by activation-induced cell death (AICD) (Green et al., 2003; Zhang et al., 1999). Moreover, when compared the number of apoptotic cells in both group A and B to the other experiments, the apoptosis of lymphoid tissues in vaccinated pigs were higher than unvaccinated ones (Resendes et al., 2004; Sanchez-Cordon et al., 2002). Though, apoptosis can be noticeable found in the lymphoid tissues of vaccinated pigs, the protection was not affected. It is revealed that all pigs could survive from the challenging. However, upon this study, it could not either specify the cell type developing apoptosis or explain different function of immune cells between both vaccinated groups.

In practice, the CSFV infected farms in endemic area, there are various patterns of Ab response and also inconstant antibody level. Even in the same age group, the maternal Ab would undoubtedly interfered the vaccine. The use of DNA vaccines or virus-vectored vaccines might be able to solve this problem, which was successfully assured in many infectious diseases (Monteil et al., 1997). The satisfaction has not been sufficiently met in CSF since their capacity in preventing fever, and leukopenia which some population of T-cell was decreased until the end of observation (Markowska-Daniel et al., 2001). The suitable recommendation would be focused on the study of types and function in cellular or molecular level of immune system, especially in vaccinated pig with maternal interference, and the study of apoptosis effect that induced by immunization. Those informations would be applied for develop a better vaccine that can induce a good immune response even in neonatal animals for further benefit in effective disease prevention and control of CSF.

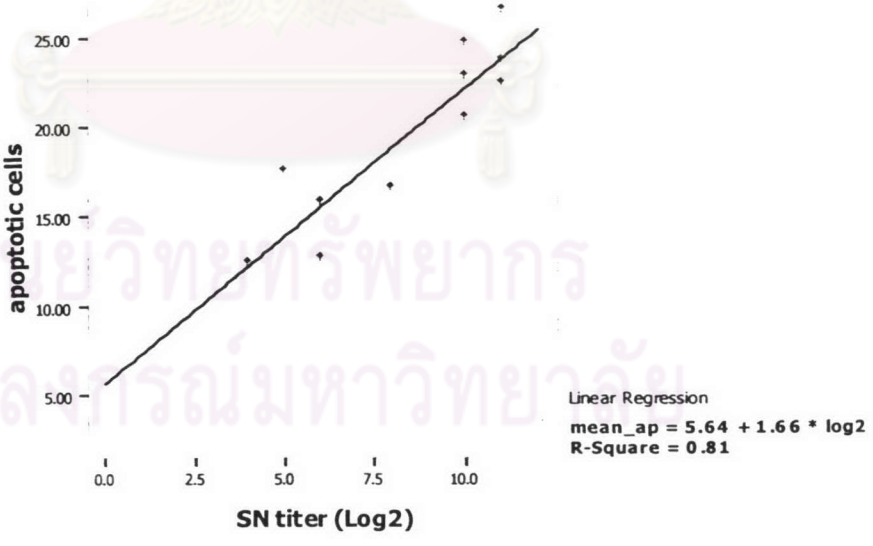

Figure 12 The associated scatter diagram indicating a linear relationship between SN titer and apoptotic cells of vaccinated pigs

Conclusion

Upon results of the study revealed that vaccinated pigs showed different of immunologic response after challenged, depending on their maternal antibody level at the day of vaccination. Vaccinated pigs with higher maternal antibody (log_2 of SN = 6.50±0.84) yielded low antibody response (5.33±1.75) while the other with lower antibody (2.50±0.55) would markedly develop high response (10.5±0.55) at 21 dpi ($P<0.05$). The titer of non-vaccinated group showed continuously decrease to undetectable level from 14 dpi. Though, variable immune responses were demonstrated in vaccinated groups, the SN titers in both groups A and B were sufficient to protect the pigs. However, leucopenia and viremia could be found at 3 dpi in some vaccinated pigs from both groups and eventually become undetectable at 7 dpi and throughout the observation.

Post mortem examination at the end of observation revealed the typical lesions of CSF in unvaccinated piglets such as petechiation and ecchymosis of lymph nodes, gastrointestinal mucosa, kidneys, strawberry lymph nodes, splenic infarction, and button ulcer. The histopathological study in lymphoid tissue showed severe lymphoid depletion in non-vaccinated pigs and no depletion in all vaccinated pigs. The immunohistochemistry showed no CSFV-Ag in lymphoid tissues of all vaccinated pig while remarkably presented of the CSFV-Ag positive (22.9±9.1 cells/0.1mm^2) was detected in non-vaccinated pigs in various cells including lymphoid cells, macrophages, reticuloendothelial cells and connective tissues. Apoptosis was found in lymphoid tissue especially in thymus with significantly difference among groups ($P<0.05$). The apoptotic cells were the most prevalent to be observed in group B (23.4±7.8 cells/0.1mm^2) and the least in group C (3.0±1.7 cells/0.1mm^2). The statistical correlation between apoptosis and CSFV-Ag infected cells showed low negative correlation at $\beta = -0.458$ and $r^2 = 0.21$. The high apoptosis in the high antibody response pigs suggested to be due to activation induced cell death for regulates homeostasis of activated cells. However all vaccinated pigs could survived and finally recovered from the CSFV challenge.