RESEARCH REPORT

SWINE HEALTH

Title: Comparison of the Immune Response of Pigs to either a Modified Live Virus or an Inactivated PRRS Virus Vaccine – **NPB #97-1978**

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Abstract

Experiments were conducted to evaluate the characteristics of the PRRS virus immunity induced in pigs by vaccination with either a modified-live virus (MLV) vaccine (RespPRRS™; Nobl) or an inactivated vaccine (PRRomiSe™; Bayer). The effects of an oil-in-water adjuvant (Imugen II; Oxford Laboratories) on the kinetics and intensity of the immune response to the MLV vaccine were also examined. Pigs within treatment groups (n=5) received two injections (4 weeks apart) of either MLV, MLV mixed with adjuvant, or inactivated PRRS virus vaccine. Following vaccination, the cell-mediated immune (CMI) response was measured using an ELISPOT assay for the detection of PRRS virus-specific IFN-□ secreting cells. The humoral immune response was measured using the IDEXX PRRS ELISA. Two weeks after a single immunization with either the MLV vaccine or the MLV vaccine mixed with adjuvant high levels of humoral immunity were readily detectable. In contrast, the inactivated vaccine did not induce a detectable humoral response even after two immunizations. Booster immunization with either of the two MLV vaccine formulations failed to stimulate a secondary antibody response, instead the antibody titers declined. Following primary immunization with either vaccine the cellular immune response was rather weak and there were no significant differences between any of the groups as measured by the IFN-□ ELISPOT assay. In response to the secondary vaccination, pigs receiving either of the MLV formulations developed a similar and significantly higher frequency of PRRS virus-specific IFN-□ secreting cells than did the pigs receiving the inactivated vaccine. These frequencies however, were still lower than those usually seen in response to immunization with a pseudorabies virus MLV vaccine. These results indicate that a PRRS MLV vaccine is much more efficient than an inactivated vaccine at inducing

either humoral or cellular immunity. The addition of an adjuvant to a PRRS MLV vaccine does not seem to rescue it from its poor ability to induce virus-specific IFN-□-secreting cells.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is considered to be the most significant animal health problem currently facing pork producers in the United States. This emerging disease is caused by an Arterivirus (Terpstra et al., 1991; Wensvoort et al., 1991). The disease syndrome caused by PRRS virus is now recognized as a major cause of reproductive loss and respiratory disease in swine throughout North America, Europe and Asia. Clinically, infection in swine with PRRS virus results in acute reproductive failure in sows and gilts and is manifested primarily by anorexia, abortions, early farrowings (107-112 days of gestation), fetal mummification, partially autolyzed fetuses, stillborn pigs, weak live-born pigs, and delayed return to estrus. In neonates, the disease is characterized by respiratory distress, fever, interstitial pneumonia, and increased preweaning mortality (Christianson et al., 1993 and 1992; Gordon 1992; Hopper et al., 1992; Mardassi et al., 1994; Plana et al., 1992; Stevenson et al., 1993; and Zeman et al., 1993). PRRS virus exhibits a high degree of transmissibility and infectivity (Zimmermann et al., 1997), and may lead to chronic infection in swine herds as evidenced by isolation of virus from the oropharyx up to 157 days after experimental infection (Wills et al., 1997). Clearly PRRS virus has inflicted significant economic losses to the swine industry.

An evaluation of the potency of vaccines currently available in the United States against PRRS virus has unfortunately revealed significant deficiencies (Osorio et al., 1998. Proc. Allen D. Leman Swine Conference). When pregnant sows previously immunized with either of two modified-live virus (MLV) vaccines or an inactivated virus vaccine were challenged with a highly virulent strain of PRRS virus, only 50 and 10% of the offspring, respectively, were protected from the lethal effects of the virus (Osorio et al., 1998). Given that vaccination against this virus is one of the major strategies to control this disease, the poor performance of these vaccines under experimental conditions is troubling. In as much as the objective and subjective evaluation of the protection obtained by the currently available vaccine formulations has been characterized as "chaotic" (Lager et al., 1997), a better understanding of the mechanisms that regulate immunity to this virus will aid in the development of more effective vaccines. The use of MLV vaccines is also controversial, as illustrated by the adverse outcome with the use of the Ingelvac PRRS vaccine in Denmark. Clearly the rational development of an effective PRRS vaccine requires knowledge of the immunobiology of this virus. Our approach to address and understand the issue of the apparent inability of PRRS vaccines to provide protective immunity has been to examine the characteristics of the immunity induced by either infection with wild type virus or vaccination with MLV or inactivated PRRS virus vaccines. We believe this approach will yield information required for the development of a more effective vaccine formulation. Given the economic losses attributed to the PRRS virus this information is urgently needed.

Objectives

Given the importance the swine industry has placed on vaccination as a means to control the disease caused by the PRRS virus, we thought it was important to evaluate the early kinectics and the intensity of the immune response obtained by vaccination. Therefore, the objective of this investigation was to conduct a head to head comparison between the immune response generated between a commercially available MLV vaccine (RespPRRS®; Nobl) to a commercially available inactivated vaccine (PRRomiSe®; Bayer) utilizing quantitative methods.

Materials and Methods

Sixteen, 8-week-old crossbred pigs were separated into 4 groups within a positive pressure isolation facility. Pigs within treatment groups (n=5) received two injections (4 weeks apart) of either the MLV (RespPRRS®; Nobl) vaccine, the same vaccine mixed with 20% v/v of the oil-in-water adjuvant Imugen II (Oxford Laboratories), or the inactivated (killed virus) vaccine (PRRomiSe®; Bayer). One pig remained unvaccinated and in a separate pen. This control animal was given injections of PBS at the time of the primary and secondary vaccination. Groups receiving MLV vaccine were separated from the pigs receiving either inactivated virus vaccine or saline by 3 pens (i.e. roughly 40 feet). The kinetics and intensity of the cell-mediated and humoral immune response was followed for 2.5 months. All pigs in this study were PRRS virus seronegative and negative for PRRS virus-specific IFN-□ producing cells prior to immunization. The humoral immune response against PRRS virus was measured using the IDEXX PRRS ELISA. To measure accurately the intensity of the cellular immune response, we utilized both a standard lymphoproliferation assay (Zuckermann and Husmann, 1996) and an IFN-□ ELISPOT assay to enumerate the frequency of PRRS virus-specific memory T cells (Zuckermann et al., 1998). In both assays the *in vitro* recall response to PRRS virus was induced by stimulation with homologous vaccine virus strain VR2332. Since stimulating with the homologous virus induces the *in vitro* cellular immune response to recall-viral antigen, the dose of recall viral antigen was carefully titrated and the assays were performed utilizing sub-optimal doses of the virus. Significant differences between groups were estimated by Fisher's PLSD test.

Results and Discussion

To accurately compare the immune response generated to the three PRRS virus vaccine formulations tested here, we chose to utilize measures of cellular as well as humoral immunity. The humoral immune response was measured by the use of the IDEXX PRRS ELISA. This assay measures the PRRS virus-specific antibody levels present in serum samples, but does not differentiate between neutralizing and non-neutralizing antibodies. Nevertheless, the IDEXX ELISA is easy to perform and provides a general indication of the immune response to a vaccine. In addition, we wanted to measure a parameter that would accurately reflect the level of cell-mediated immunity (CMI). Our test of CMI rests in the application of an enzyme-linked immunospot (ELISPOT) assay to detect single interferon (IFN)-γ-secreting memory T cells responding to PRRS viral antigen. This assay is very sensitive and can detect even low frequencies (as low as 0.001%) of PRRS virus-specific lymphocytes isolated from peripheral blood. The unique application of this test to quantitate the degree of CMI gives a precise enumeration of the population of memory T cells capable of secreting IFN-□ in response to stimulation with recall antigen. The pivotal role of IFN-□

as a mediator of the cellular immune response is well characterized in the immunological literature. Since treatment of porcine macrophages with IFN-□ renders them resistant to infection by PRRS virus, it is likely that measurement of PRRS virus-specific IFN-□-producing T cells would provide a good indicator of protective immunity.

Figure 1 shows the ability of the different vaccine formulations to induce the production of PRRS virus-specific antibodies. While there was no significant difference in the antibody response generated by animals immunized with the MLV vaccine alone or the MLV mixed with adjuvant, there was a significant difference with the animals receiving the inactivated vaccine. At all time points, the inactivated vaccine failed to generate a positive antibody titer as measured in this assay. Although the intensity of the humoral response generated against the MLV vaccine formulations appears robust, other studies have shown that the type of antibody being measured early in the response is not neutralizing. On the contrary, the antibody produced may actually enhance PRRS infectivity. For example, it has been shown both *in vitro* and *in vivo* that the presence of low levels of PRRS virus-specific antibodies may exacerbate infection through a mechanism called antibody dependent enhancement, or ADE (Choi et al., 1992; Christianson et al., 1993; Yoon et al., 1994; Yoon et al., 1996; Yoon et al., 1997). Although the role of antibody in protective immunity remains to be examined, the concurrent detection of antibodies and viremia suggests inadequate protection provided by the humoral immune response.

The IFN-γ response induced by the different vaccine formulations is shown in Fig. 2. The number of IFN-γ-secreting memory T cells generated after the primary vaccination with the inactivated PRRS virus vaccine was not significantly different from that obtained by immunization with the MLV vaccine or the same MLV vaccine mixed with adjuvant. In all cases, however, the frequency was low (<50 IFN-γ-secreting cells /10^6 PBMC) as compared to the frequencies of IFN-□-secreting cells typically obtained in response to vaccination with a pseudorabies virus (PRV) MLV vaccine. In the latter, the response usually averages 200 IFN-γ-secreting cells /10^6 PBMC (Zuckermann et al., 1998). Following the booster immunization, the frequency of IFN-γ-secreting memory T cells generated in response to the MLV increased and was significantly higher than that obtained by the inactivated vaccine, but was not different from that observed in response to immunization with the MLV mixed with adjuvant. In fact, the adjuvanted MLV vaccine was intermediate in its effectiveness to induce IFN-γ-secreting memory T cells. This was a surprising result given that we have previously observed a dramatic enhancement of IFN-γ-secreting memory T cells when the same adjuvant was given mixed with a PRV MLV vaccine (Zuckermann, unpublished observations). In addition, pigs receiving the booster immunization with the PRRS MLV or the MLV + Imugen vaccine exhibited an anamnestic response, whereas pigs receiving the inactivated vaccine failed to do so. Although the booster immunization with PRRS MLV vaccine did increase the frequency of IFN-γ-secreting cells (110±45 IFN-γ-secreting cells /10^6 PBMC), it was still low by PRV standards. When pigs are immunized with a MLV PRV vaccine the secondary response usually reaches >500 IFN-γ-secreting cells /10^6 PBMC (Zuckermann et al., 1998; Zuckermann, NPPC 1998 report)

It is reasonable to assume that both the intensity and quality of the immune response developed following vaccination is an important predictor of the level of

protection that any particular vaccine will provide. In the case of an infection by a lytic virus, neutralizing antibodies and IFN-□ production are thought to be important in mediating protective immunity (Zinkernagel, 1997). Our laboratory has previously shown that in the pig a robust IFN-□ response to pseudorabies (PRV) virus, but not neutralizing antibodies, is highly correlated to protection from subsequent challenge (Zuckermann, et al., 1998). We predict that a strong CMI response would also equate to protection against the PRRS virus-induced disease. This is based, in part, on the fact that PRRS virus preferentially infects macrophages, and that these cells can be made more resistant to infection by exposure to IFN-γ. It is thus reasonable to suggest that a strong vaccine-induced virus-specific IFN-γ response to PRRS virus will be necessary if vaccine formulations capable of establishing protective immunity to this virus are to be made. The data presented indicates that the currently available PRRS vaccine formulations are not as efficient at inducing cell-mediated immunity as the PRV MLV vaccine. Accordingly they are also not very efficient at conferring protection from challenge (Osorio et al., 1998). We have observed that there is a delay in the generation of the CMI response in young pigs infected with wild-type PRRS virus (Zuckermann and Osorio, unpublished observations). Although in the case of immunization with a PRRS MLV vaccine this delay is not as pronounced, the IFN-□ response is initially rather weak, increasing gradually in intensity over a period of months. Similarly, although a strong PRRS virus-specific antibody response following either infection or vaccination can be readily detected (as measured in the IDEXX ELISA), there is a significant delay in the generation of potentially “protective” neutralizing antibodies. These observations add to the puzzle surrounding the understanding of the PRRS virus immune response. We hypothesize that the apparent inability of vaccine and wild type PRRS virus to induce IFN-□-producing cells and neutralizing antibodies are the result of immunomodulatory properties of the PRRS virus.

Although the immune mechanisms responsible for mediating protective immunity against PRRS virus remain unknown, it seems reasonable to suggest that a more immunogenic vaccine could provide better protective immunity. Importantly, the level of immunity in convalescent pigs as a result of a primary PRRS virus infection is apparently sufficient to prevent disease by secondary exposure, at least in sows being reinfected 6 months after the primary infection (Gorcyca et al., 1993; Lager et al., 1994). This observation agrees with the results of our own studies which indicate that several months after exposure to either wild-type or attenuated PRRS virus, virus-inhibitory immunity is generated. The apparent low immunogenicity of PRRS MLV and inactivated vaccines may be at least partially responsible for their poor performance under experimental conditions (Osorio et al., 1998), and field conditions (Mengeling et al., 1997). A strong cellular immune response to PRV, as demonstrated in the IFN-γ ELISPOT assay, is quickly established within two weeks after immunization with a MLV vaccine. This response is associated with a significant level of protective immunity (Zuckermann et al., 1998). In contrast, we have shown that the response after either vaccination with PRRS MLV or infection with wild-type PRRS virus is less pronounced and much slower to develop (Figure 2, and unpublished observations). All in all, it appears that the wild type PRRS virus or attenuated forms of this virus have a lower efficiency in stimulating T-cell responsiveness to the immunizing virus, when compared with a PRV MLV taken as a gold standard. The reason(s) behind the poor

immunogenicity of PRRS virus is currently being investigated in our laboratory. Among the possibilities being explored are either modulation or suppression of the immune response. We believe that clarification of this issue is essential for the development and formulation of a highly protective PRRS vaccine.

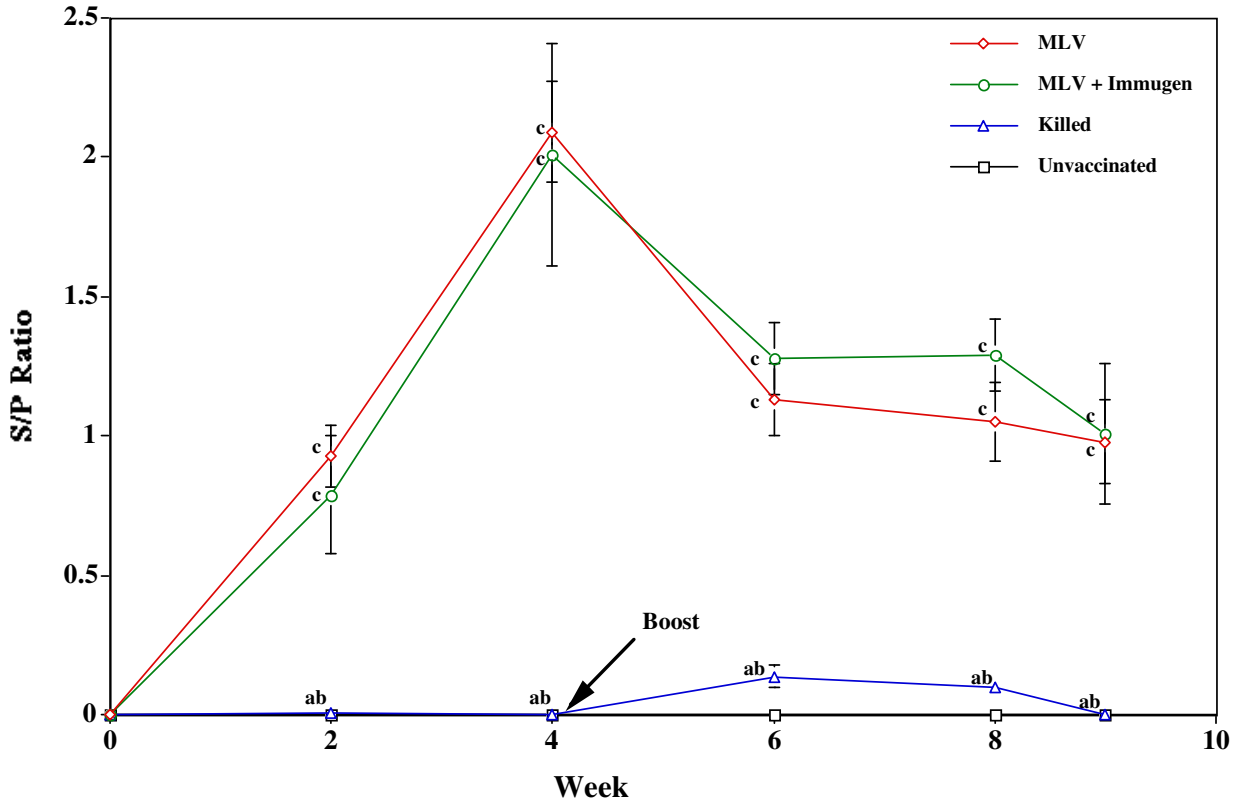

Figure 1. Comparison of the humoral immune response of pigs (n=5) vaccinated with PRRS MLV, MLV + Imugen, and killed vaccine as measured in the IDEXX PRRS ELISA assay. Vaccinated animals were immunized twice four weeks apart. Time-points in the MLV and the MLV + Imugen vaccine treatment groups marked with a (*) were significantly different than the unvaccinated control group by Fisher's PLSD test ($p<.05$). The sample to positive ratio (S/P ratio) in the killed vaccine treatment group never reached the minimum positive cut-off level (0.4).

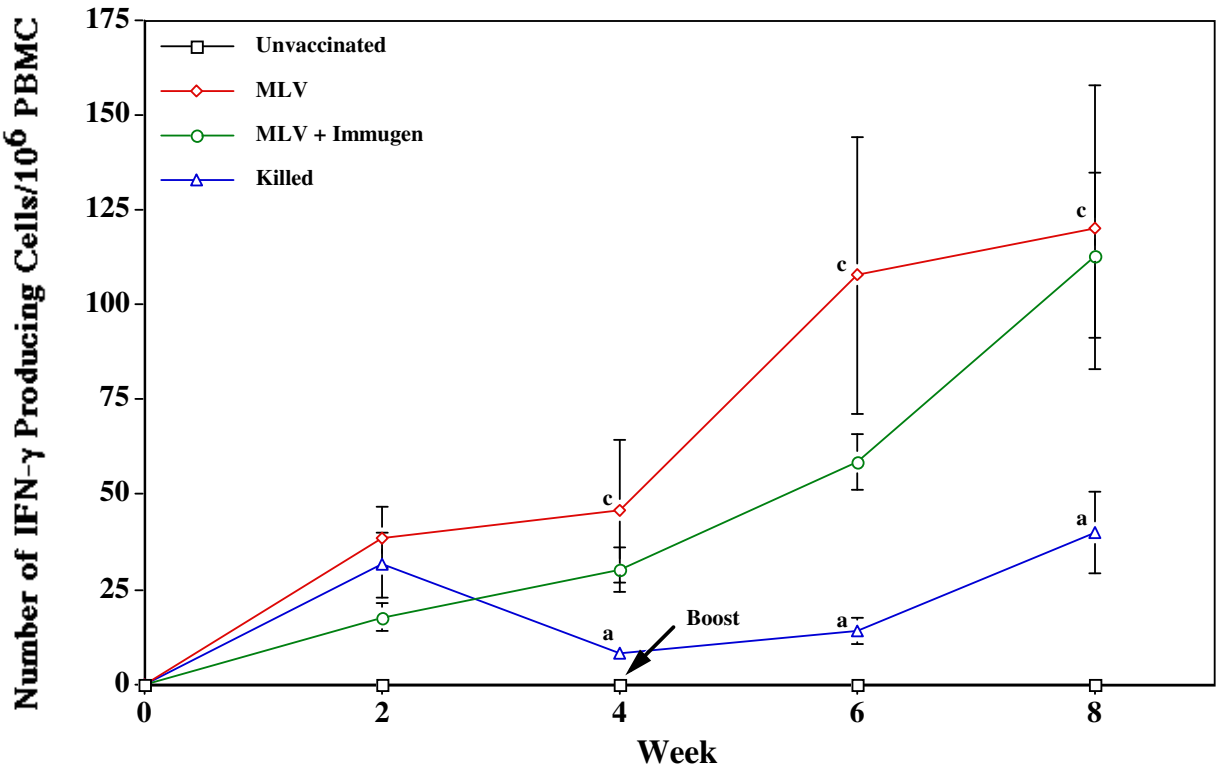

Figure 2. Comparison of the cell-mediated immune response of pigs vaccinated with PRRS MLV, MLV + Imugen, and killed vaccine as measured in the ELISPOT assay. This assay measures the frequency of PRRS virus-specific IFN-□-secreting memory T cells isolated from peripheral blood mononuclear cells (PBMC). PBMC from PRRS vaccinated and non-vaccinated pigs were cultured in 96 well Immulon II plates coated with the porcine IFN-□-specific mAb P2G10, in the presence of PRRS virus isolate VR2332 as recall antigen. After 18 hour incubations at 37 ^{0}C in a CO_2 incubator, the wells were first reacted with biotin-labeled mAb P2C11 which recognizes an epitope on porcine IFN-□ different from that of the capture mAb P2G10. The wells were then reacted with streptavidin-conjugated horse radish peroxidase (SA-HRP), followed by the substrate TMB membrane (KPL) to visualize the spots. IFN-□ secreting PRRS virus-specific memory T cells were enumerated at the indicated times after immunization. Time-points between treatment groups with different letters are significantly different based on Fisher's PLSD test ($p<.05$). Time-points without letters are not significantly different from the other two vaccinated groups. All time points after the first vaccination were significantly different from the unvaccinated control group.

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