Immunity Against Aujeszky's Disease

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I. Abstract.

The type of immune response induced by vaccination is critical for the development of a highly protective immunity against any infectious agent. For protection against Aujeszky's disease virus (ADV), a herpesvirus of swine, two types of commercial vaccines available: inactivated virus and modified live ADV. Previously it has been demonstrated that the latter vaccine is more effective in providing protective immunity. Since either type of vaccination results in the production of a comparable amount of virus neutralizing antibodies, it has been assumed that the difference in their protective abilities is reflected by the level of cell mediated immunity induced. Indeed, we have obtained evidence that the inactivated vaccine is not as efficient as the modified live virus at inducing cell mediated immunity. The type of immunity was measured by both the intensity of an ADV-specific lymphoproliferative response and the number of ADV-specific inteferon-γ producing cells present in the peripheral blood of pigs vaccinated twice with either type of vaccine (n=5 per group). The intensity of the bastogenic response in the peripheral blood mononuclear cells (PBMC) of animals vaccinated with the modified live virus vaccine was two fold higher than those vaccinated with the inactivated vaccine. Similarly, the average frequency of interferon-γ producing cells, as measured by ELISPOT, was 5 fold higher in the blood of animals inoculated with the modified live ADV vaccine. Interestingly, the titer of virus neutralizing antibodies in both groups was identical. Although both vaccines were able to provide protection against lethal challenge, the inactivated virus-vaccinated pigs gained less weight within the 7 days following challenge, indicating a lower level of resistance. These observations in combination with the know superior ability of modified live virus vaccines to reduce the amount of virus shedding, are strong arguments for the use of modified live virus vaccines in programs aimed at the control of Aujeszky's disease.

II. Studies on the immune response to Aujeszky's disease virus.

Aujeszky's disease virus is an alpha herpesvirus, for which the pig is its natural host. The infection of pigs with this virus causes Aujeszky's disease, which is characterized by a fatal encephalitis in newborn pigs and a milder disorder in older swine, which is mostly characterized by severe depression, anorexia, pyrexia, occasional mild ataxia, and respiratory distress (1). The clinical response to virus challenge depends on the immune status and age of the animal. Protective immunity can be induced by inoculation with both live and inactivated PrV vaccines, although the latter are not as effective (2,12,13) Although both vaccines prevent death from the virus challenge, the clinical response has measurable differences. For example, PrV challenge of vaccinated animals can result in a reduction in the rate of growth (weight gain), and even weight loss, depending on the severity of the challenge and level of protective immunity conferred by the vaccine. Many researchers have arrived to the conclusion that weight changes within seven days after PrV challenge, is the most sensitive and reproducible parameter which allows quantification of the level of protective immunity conferred by a vaccine (12,13,15,16). This method of potency measure for a PrV vaccine has been validated in a highly reproducible quantitative test which is based on the difference between the mean weight gain during the seven days after virus challenge (ΔG7) of vaccinated or naive control pigs (12). Using this method, inactivated vaccines have been shown to be less effective than modified live virus at inducing protective immunity (2,12,13). However, the mechanism(s) responsible for differences in the level of protective immunity conferred to a pig as a result of vaccination with either a modified live or inactivated virus (protection from death and reduced clinical symptoms) are unknown. For the past six years, our laboratory has been studying the immune reponse of swine to PrV and the development of T-cell memory (17-20) with the ultimate goal of determining the immunologic mechanism(s) responsible for protective immunity against this virus in pigs. We have demonstrated that immunization with live virus vaccines generates a robust cytotoxic T lymphocyte response (17). Our studies and those of others have also shown a strong lymphoproliferative response to vaccination (14-20). We have performed extensive characterization of the T-cells mediating these responses and have demonstrated that the virus-specific cytotoxic lymphocyte response is mediated by CD8 single positive (SP) lymphocytes, while the lymphoproliferative response is mediated by both CD4 SP and CD4/CD8 double positive lymphocytes (17,20). Others have examined the humoral immune response and found that it is strongly induced by both infection and vaccination with either a modified live or inactivated virus.

III. Protective immunity against Aujeszky's disease.

Immunization of pigs with an inactivated or a modified live ADV vaccine clearly results in the induction of a virus-specific immune response which provides different levels of protection from lethal virus challenge (12). However, despite the numerous experimental vaccination/challenge studies which have conducted with ADV, it is difficult to determine the extent to which individual mechanisms contribute to the protection of the animal from disease. That humoral immunity is capable of mediating protection was indicated by the demonstration that passive immunization of swine with mAbs specific for PrV was able to provide protection from lethal virus challenge (5). However, other evidence suggests that humoral immunity might not be the main mechanism mediating protection. For example, there is a poor correlation between the titer of vaccine-induced virus-neutralizing antibody in pigs and the level of protection against disease (4,6). Furthermore, humans suffering from agammaglobulinemia are not predisposed to severe life-threatening viral infection with either measles or herpes simplex virus (HSV) infection. This contrasts with the outcome in patients suffering from T-cell immunodeficiency such as Di George syndrome (congenital absence of the thymus) or in nude athymic mice, where infections with HSV are more severe and life-threatening. Although passive neutralizing antibodies may delay the process, it is the adoptive transfer of HSV-specific T cells that protects the nude mice by resolving the infection (11). Direct evidence that cell-mediated immunity is essential in mediating protective immunity against herpesviruses has been obtained in murine experimental models of HSV and PrV infection. For HSV, adoptive transfer experiments performed by several groups of investigators have shown that both CD4 and CD8 cells are able to provide protective immunity (7,8,14). For PrV, the administration of interferon-γ-neutralizing antibody at the time of vaccination, significantly decreased vaccine-induced protective immunity (10), suggesting that the development of T helper cells producing interferon-γ response was necessary for the generation of protective immunity.

Based on these observations, a strong case for cell-mediated immunity as a major contributor to protective immunity against herpesviruses can be made, at least in those experimental murine models. The role of interferon-γ producing cells, at least as an indication of the generation of protective immunity against ADV in pigs, is a major focus of this manuscript. Interferon-γ can have a direct antiviral effect by inhibiting virus growth and by inducing the expression of major histocompatibility complex (MHC) class II antigens. Both HSV and PrV are susceptible to the growth inhibitory effects of interferon-γ *in vitro* (9,11). The administration into mice of interferon-γ in combination with an inactivated PrV vaccine was able to modulate the immune response by increasing the production of virus-specific IgG2a and by enhancing the resistance to PrV challenge (10). Again, these observations indicate that cell mediated immunity, and in particular interferon-γ producing cells, are important in conferring protective immunity against herpesviruses, although the effector mechanism(s) by which these cells mediate their protective effect *in vivo* is unknown. Investigators in the field agree that interferon-γ producing cells (either CD4 or CD8 lymphocytes) have a central role in controlling herpesvirus infections and in the development of protective immunity (7,8). The effector functions by which these cells are likely to provide anti-viral protection include activation and attraction of phagocytic cells, B-cell helper activity for generating complement fixing antibodies, and direct cytolytic action on virus-infected cells.

IV. Cellular immune response to vaccination of pigs with inactivated or modified live virus. To address the issue of the importance of cell-mediated immunity in protection against Aujeszky's disease we conducted a comparative analysis of the T lymphocyte response to inactivated and modified live virus vaccines. To asses this response we measured the virus-specific lymphoproliferative response and the generation of virus-specific interferon-γ-producing cells in the peripheral blood of swine at weekly intervals following vaccination with these two types of vaccines. Our studies clearly show that there are differences in the quality and quantity of the immunity induced by an inactivated versus a modified Aujeszky's disease virus vaccine. While the inactivated vaccine was very efficient at inducing humoral immunity (Fig 1A), it only elicited a weak virus-specific interferon-γ response (Fig 1B). In contrast, the live vaccine not only induced a similarly intense humoral response (Fig. 1A) but also robust lymphoproliferative (not shown) and virus-specific interferon-γ responses (Fig. 1B). To test the degree of protection conferred by these two kinds of vaccines, the animals were challenged with a lethal dose of wild type Aujeszky's disease virus. The modified live virus vaccined provided superior protection as demonstrated by the difference in the mean weight gain during the seven days after challenge (Fig. 2) which as calculated according to method by Stellman et al. (12) gave a statistically significant ΔG7 value between the two vaccinated groups (p=0.02). It seems reasonable to speculate that differences in the level of virus-specific cellular immune response were responsible for the distinct levels of protective immunity conferred by these two types of vaccines. However, definitive ascription for the better protection to a specific cellular immune mechanism(s) requires further experimentation.

V. Conclusion

The evidence presented indicates that the use of a modified live vaccine is the most appropriate type of vaccine currently available to help control Aujeszky's disease. Inactivated vaccines, although safer, do not provide the type of immunity needed to effectively counter the illness effect of infection by wild type virus. Until more effective inactivated vaccines become available the use of a modified live vaccine should be the first choice.

VI. References.

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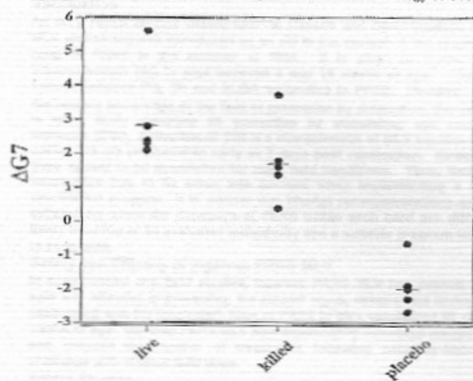


Figure 2. Potency testing of the modified live and inactivated PrV vaccine. The same pigs described in Fig. 1. were challenged ten days after the second immunization with 10 LD50 of wild type PrV. The animal were weighed at the time of challenge and seven days after challenge. The criterion ΔG7 was utilized as a mesure of the potency of the vaccine (i.e., the difference between the mean weight gain during the seven days after pathogenic challenge of vaccinated or control pigs) as described by Stellmann et al. Two of the pigs in the placebo group died at day seven. The difference in ΔG7 between the pigs vaccinated with the modified live and the inactivated is statistically significant ($p=0.02$). The vaccinated gropus are also significantly different from the placebo group ($p<0.001$).

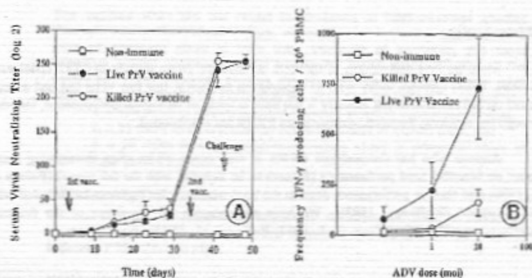
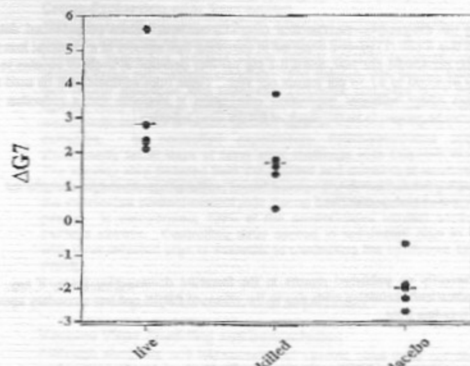


Figure 1. Serum virus neutralizing antibody response and frequency of interferon-gamma producing cells in the peripheral blood of pigs following vaccination with either an inactivated or live ADV vaccine. Three groups of eight week-old cross-bred pigs (five pigs per group) were immunized twice four weeks apart with either saline (open squares), a modified live (Tolvid, Upjohn; closed circles), or an inactivated (Triad, Oxford Labs.; open circles) ADV vaccine. Two weeks after the second immunization the animals were challenge with 10 LD50 of wild type ADV. A. Serum samples were collected before immunization (day 0), and several times after primary (days 9, 15, 23, 29) and secondary (day 41) immunizations and once after challenge (day 48). The titer of virus neutralizing antibodies was determined by standard procedures. V alues represent the mean and standard deviation of five pigs per group.
B. Ten days after the second vaccination peripheral blood mononuclear cells from these animals were isolated and the frequency of ADV-specific IFN-γ producing cells determined by ELISPOT. Cells were stimulated for 20 h with the indicated dose of ADV. Values represent mean ± standard deviation of five pigs.



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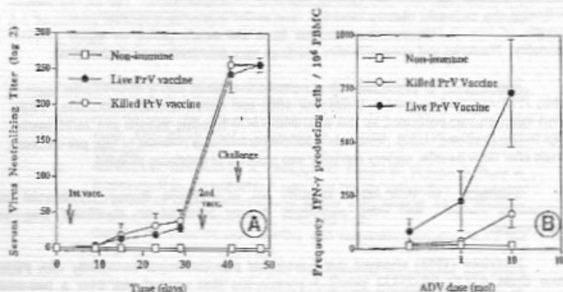

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