The effect of lectins and immune serum on the porcine immune response to a pseudorabies subunit vaccine

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The effect of lectins and immune serum on the porcine
immune response to a pseudorabies subunit vaccine

by

Frederick Philip Philips

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements of the Degree of
MASTER OF SCIENCE

Department: Veterinary Microbiology and Preventive Medicine
Major: Veterinary Microbiology

Signatures have been redacted for privacy

Iowa State University
Ames, Iowa
1986
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This thesis is divided into two separate sections. Section I describes the effect of the mitogenic lectins, Concanavalin A and Lens culinaris agglutinin, on the immune response of pigs to a pseudorabies subunit vaccine. Section II describes the effect of pseudorabies virus immune serum on the immune response of pigs to a pseudorabies subunit vaccine. Each section is presented separately and consists of the following subsections: abstract, introduction, materials and methods, results, discussion, and bibliography. A review of the literature concerning pseudorabies virus, vaccines, and potential adjuvants of vaccines precedes these sections. A supplemental literature citation pertaining to this review follows section II.
GENERAL INTRODUCTION

The principal method of control of pseudorabies is vaccination. Several effective attenuated and inactivated vaccines have been developed over the years. However, these vaccines do not prevent infection by pseudorabies virus (PRV), the establishment of a latent infection, or subsequent recrudescence of the virus in latently infected pigs. Furthermore, infected vaccinated pigs cannot be serologically differentiated from non-infected vaccinated pigs, since current vaccines contain all viral epitopes. Consequently, pigs that are vaccinated with currently available vaccines must be considered potential carriers of the virus. Subunit vaccines, a new generation of inactivated vaccines, contain a limited number of the total viral epitopes. Therefore, virus infected vaccinated pigs, that have antibody to all viral epitopes, can be serologically differentiated from non-infected vaccinated pigs. This ability would facilitate PRV control programs and could ultimately lead to the eradication of PRV from the swine population.

A disadvantage of subunit vaccines is their cost of production. In addition, some viral subunits may not be as immunogenic in their free form as they are when they exist as an integral part of the virus. It may be possible to overcome these disadvantages by developing ways to enhance the immune response of pigs to the subunit vaccines. In the following study, the lectins, Concanavalin A and Lens culinaris agglutinin, and PRV immune serum were evaluated as immunopotentiating agents of a PRV subunit vaccine in pigs.
Pseudorabies (Aujeszky's disease) is an economically significant disease of swine caused by a herpesvirus.\textsuperscript{36} The virus is found worldwide.\textsuperscript{37} Pseudorabies virus (PRV) causes fatal infections in swine, cattle, dogs, cats, and other mammals.\textsuperscript{3, 37} Man is apparently refractive to PRV infection, although a few suspect cases have been reported in Europe.\textsuperscript{113} The incidence of pseudorabies has been increasing in the United States\textsuperscript{38, 112} and throughout the world\textsuperscript{114} during the past decade and is rapidly reaching endemic proportions. A national survey of sera from hogs slaughtered in 1974 indicated that 0.56\% were positive for antibodies to PRV.\textsuperscript{27} Similar surveys revealed that the incidence of PRV infection in pigs rose to 3.73\% in 1978 and to 8.39\% in 1981.\textsuperscript{103} The economic losses due to pseudorabies in 1981 were conservatively estimated to cost the Iowa swine industry $33.9 million.\textsuperscript{8} A 1985 study on the cost of pseudorabies to the Iowa swine industry estimated the annual cost of the disease to be $107 to $117 million if losses due to reduced sales of breeding stock are included.\textsuperscript{40}

Vaccines have been extensively used to control the disease. A significant disadvantage of the traditional vaccine is that it is not possible to determine whether or not a vaccinated pig is a carrier of the virus. In 1984, Platt described a PRV subunit vaccine that was produced by extracting viral glycoproteins from the membranes of virus infected
cells. The subunit vaccine is unique because it allows the serological differentiation of infected vaccinated pigs from non-infected vaccinated pigs. The cost of producing the vaccine on a commercial basis is relatively high. The unit cost of the vaccine could be reduced if a smaller dose of antigen could be used. One means of accomplishing this goal would be to enhance the immune response of pigs to the vaccine antigen. Both lectins and immune complexes could be potential immunomodulating agents. The following review will focus on the virus, the disease that it causes, methods of control, and the use of lectins and immune serum as immunopotentiating agents.

Pseudorabies Virus

The virus

Pseudorabies virus, also known as Herpesvirus suis\(^2\), \(^{107}\) or Suid herpesvirus type \(^1\), belongs to the family Herpesviridae. The virus has been placed in the subfamily alpha-herpesvirinae based on its wide host range, short replication cycle, ability to cause rapid cell death, and ability to establish latent infections.\(^9\), \(^{104}\) The virulence of the virus is correlated to thymidine-kinase activity.\(^5\), \(^3\) The virus contains double stranded DNA which is surrounded by an icosahedral capsid that contains 162 capsomeres. The size of the capsid ranges from 110 to 230 nm in diameter.\(^3\) The capsid in turn is surrounded by a glycoprotein rich envelope that is derived from nuclear and possibly cytoplasmic membranes of infected cells.\(^7\), \(^2\)
A minimum of 20 viral envelope proteins have been described. Four of these proteins are glycosylated. Eight of these proteins are highly sulfated, major structural components with molecular weights of 125K, 98K, 74K, and 58K. The 125K, 74K, and 58K glycoproteins are covalently linked by disulfide bridges. These three proteins also share extensive amino acid sequence homology and probably originate from a single precursor protein. Three minor glycoproteins with molecular weights of 130K, 98K, and 62K are noncovalently linked to a 115K nonglycosylated protein. All seven glycoproteins are exposed on the surface of the intact virion. Monoclonal antibody studies by Hamp! et al. suggest that the major 98K glycoprotein is responsible for inducing serum-virus neutralizing antibody. However, Wathen et al. cited evidence that the 58K glycoprotein also plays a role in inducing serum-virus neutralizing antibody.

A non-structural 90K glycoprotein has also been described by Kaplan and Ben-Porat. This protein is sulfated to a greater extent than the other major proteins and is excreted from infected cells into the culture media. Erickson and Kaplan proposed that the 90K protein plays a role in forming the intracellular matrix that is apparently involved in the assembly of PRV virions. This protein may be involved in protecting the virus from the immune response of the host by promoting cell fusion or reacting with circulating antibody. A similar and possibly identical protein has been described by Platt et al. who used it as a diagnostic antigen to identify virus infected subunit vaccinated pigs.
The disease

Pseudorabies was originally called Aujeszky's disease in honor of Aladár Aujeszky, who first described the disease in cattle and demonstrated the filterable nature of the agent in 1902. As cited by Hurst, Marek named the disease infectious bulbar paralysis in 1904 as a result of experimental studies in rabbits in which the medulla oblongata was affected. Sabin noted that Nicolic proposed the name pseudorabies in 1932 in the mistaken belief that PRV was a variant of the rabies virus. Subsequently, Shope demonstrated through serological techniques that the cattle disease described by Aujeszky and Nicolic was identical to the cattle disease known as 'Mad Itch' in Iowa. Shope also demonstrated that the virus causing 'Mad Itch' produced clinical disease in swine, which are now recognized as the natural host of PRV.

Clinically, PRV is most severe in pigs under three months of age. The predominant manifestations of the disease in this age group are central nervous system dysfunction and respiratory system involvement. Neurological signs range from slight depression to severe disorientation which is frequently followed by convulsions and respiratory failure. Newborn piglets often die within 48 hours after developing respiratory signs. Older pigs are generally inapparently infected, although fatal infections can occur. Typical signs in swine, three months or older, include; inappetence, listlessness, lassitude, and prostration often accompanied with paddling of the extremities. The virus can also cause fetal death and resorption during the first trimester of pregnancy. Abortions,
stillbirths, and mummified fetuses are common during the last two trimesters.³⁷, ⁴⁷

Methods of control

Several factors must be considered in order to successfully control PRV. These factors include: shedding and subsequent transmission between animals, virus survival in the environment, and latency. Pseudorabies virus is spread between pigs primarily by nasal aerosols and fomites.²⁴, ²⁶ Rabbits, mice, and rats are not thought to play an important role in the natural transmission of the disease.⁶² Virion survival in the environment was found to be dependent on pH level and temperature. Davies and Beran demonstrated that an initial inoculum of 10⁷ PFU of PRV remained infectious for 10 days at 37 C to 120 days at 4 C at optimum pH levels of 6 to 8.²⁴ The virus is sensitive to repeated freezing and thawing, drying, and ultraviolet light.²⁴ Pigs that recover from the disease are frequently latently infected with the virus.⁹, ⁹⁰ These pigs play a major role in the epidemiology of the disease by periodically shedding virus following periods of stress, such as farrowing,²³ starvation, fighting, bacterial infection³⁶, ⁶³ or treatment with immunosuppressive agents such as dexamethasone.⁷⁴, ¹⁰⁴

There are two general approaches currently being used to control pseudorabies: elimination of PRV from infected herds through specific management programs and vaccination to prevent clinical disease, while accepting the presence of the virus.
Management programs

The management programs used to date have been thoroughly described by Thawley et al.\textsuperscript{103} These programs include: test and removal, offspring segregation, and depopulation-repopulation. Test and removal programs involve the serological testing of all pigs in a herd every 30 days for six months. All seropositive pigs are systematically culled from the herd. This program does not require separate confinement quarters and is easily administered. Disadvantages of this program are that it does not allow for the salvaging of valuable genetic lines and the frequent serological testing required is expensive.

Offspring segregation programs involve the removal and isolation of seronegative weanling pigs from the infected herd. The isolated pigs are monitored for PRV antibodies for at least 60 days. If the pigs remain seronegative, they are retained and used as breeding stock for a new herd. The main advantage of this method is that it permits the salvaging of valuable genetic lines. A disadvantage is that the weanling pigs may be latently infected with PRV in utero\textsuperscript{68, 109} or pre-weaning\textsuperscript{,5, 89} show no clinical signs, and remain seronegative until recrudescence of the virus.\textsuperscript{110} A modification of the offspring segregation method involves transferring embryos from selected infected sows to PRV free sows.\textsuperscript{12}

Recently, offspring segregation has been combined with controlled vaccination.\textsuperscript{56} In this program, seropositive pigs are removed from the herd. The remaining seronegative pigs are vaccinated with a killed vaccine. The first set of offspring that are produced are segregated and monitored for PRV antibody. If the segregated pigs remain seronegative for 60 days, they are used for the foundation of a new breeding herd.
The use of vaccine in the offspring segregation program has increased success rates.56

Depopulation-repopulation programs involve the complete removal of all swine from the contaminated premises, which are subsequently cleaned, disinfected, and left vacant for a minimum of 30 days. Pseudorabies free pigs are used to repopulate the premises. These pigs are monitored for at least 30 days and if they remain seronegative, the herd is considered to be PRV free. This method is expensive in terms of financial loss due to disrupted production and the loss of valuable genetic lines.

**Vaccination** Traditionally, two types of vaccines have been used for PRV: the modified live or attenuated vaccine and the inactivated or killed vaccine. The primary advantage of an attenuated vaccine is that the virus multiplies within the host and generates its own antigenic mass. Consequently, the immune response of the host is stimulated in such a manner that a more competent and longer lasting immunity is established than that induced by inactivated vaccines.44

The use of an attenuated virus vaccine has two distinct disadvantages. First, the use of such vaccines perpetuates the presence of the virus in the swine population. Secondly, the attenuated virus has the potential of reverting to a virulent form.5 In addition, vaccines that are attenuated for one species of animals may still be virulent for another species.105 For example, the BUK strain of PRV is attenuated with respect to pigs but is virulent for sheep.109 Currently, three PRV attenuated vaccines are available. Norden Laboratories, Lincoln, NE,
markets the attenuated BUK strain originally developed by Zuffa and Polak. Tech American corporation, Omaha, NE, markets the BUK strain that has been further attenuated by deleting the TK gene. Bioceutic corporation, Kansas City, MO, markets the Bartha K strain which was first described by Kojnok and Bartha in 1962.

The principal advantage of inactivated vaccines is their safety. Reversion of virus to a virulent form cannot occur and the vaccine can be used safely in a wider range of animals. In addition, killed vaccines can be prepared from virulent strains of PRV that may have antigenic epitopes not present in attenuated viruses. The primary disadvantage of inactivated vaccines is that they require a relatively large antigenic mass for immunization. Also, multiple doses are usually required to produce effective immunity. Currently, inactivated vaccines are produced by Norden Laboratories, Lincoln, NE, and Salsbury Laboratories, Charles City, IA.

Although both attenuated and inactivated vaccines prevent or reduce the severity of clinical disease, they do not prevent superinfection of pigs with virulent PRV and subsequent virus shedding. For example, Maes, et al. demonstrated that virulent virus was shed without clinical signs in vaccinated pigs for seven to ten days post infection. Consequently, all vaccinated pigs must be considered potential carriers of the virus. Furthermore, serological differentiation of virus infected vaccinated pigs from non-infected vaccinated pigs is not possible because current vaccines contain all the antigenic components of the virus. It is for this reason that subunit vaccines have attracted considerable attention.
Subunit vaccines represent a new generation of inactivated virus vaccines that contain a limited number of the antigenic components of the whole virus. They are inherently safe due to their lack of nucleic acids and may be less toxic than the whole virion preparation. Subunit vaccines of enveloped viruses consist of major structural glycoproteins, that are usually involved in cell attachment and/or penetration, and are capable of inducing an immune response in the host. Antibodies developed against these glycoproteins inhibit virus adsorption and penetration, which limits the course of infection and its severity in the host. The limited antigenic composition of subunit vaccines makes them ideal for use in control programs because virus infected vaccinates can be identified by serologically testing for antibody to non-vaccine viral antigen.

Subunit vaccines can be prepared by several methods. These methods include: extracting specific viral proteins from intact virions or from virus infected cells, synthesizing specific viral peptides, and recombinant techniques. To date all published accounts of PRV subunit vaccine development have dealt with extracted glycoproteins. The extraction of virus proteins from in vitro infected cells yields larger quantities than extraction from the virion. The extraction methods used for this purpose were originally developed for purifying cell-bound receptors and transplantation antigens. These methods involved the use of non-ionic detergents, Nonidet P-40 or Triton X-100.
One of the first evaluations of PRV glycoprotein extracts was done by Rock and Reed in 1980. These investigators were able to protect mice from lethal PRV challenge by immunizing them with a preparation prepared by solubilizing the membranes of virus infected cells with Triton X-100. Turner et al. were also able to induce protection by immunizing mice with membranes of infected cells solubilized with Nonidet P-40. Maes and Schutz reported that NP-40 extracted PRV glycoproteins induced significantly higher serum-virus neutralizing antibody titers than attenuated or inactivated virus vaccines. Subsequently, Platt demonstrated that lectin extracted PRV glycoproteins were highly immunogenic in the pig. Furthermore, pigs that were immunized with the lectin-extracted glycoproteins did not develop antibodies to a PRV-specific early excreted non-structural protein. As a result of this finding, Platt et al. used the early protein as a diagnostic antigen to differentiate virus infected vaccinated pigs from non-infected vaccinated pigs.

**Immunopotentiation of Subunit Vaccines**

Edelman noted that non-replicating, purified subunit vaccines are likely to be weak immunogens that will require immunopotentiation if they are to be effective. Two potential groups of immunopotentiators are lectins and antigen specific immune serum.
Lectins

Lectins are a class of proteins that exhibit specific carbohydrate binding specificities\textsuperscript{59} that have been compared to the reaction of antibody with antigen.\textsuperscript{98} The majority of lectins have been isolated from plants\textsuperscript{58} although lectins of mammalian origin are also known.\textsuperscript{13} Lectins were first discovered to induce mitogenic activity in lymphocytes by Nowell in 1960.\textsuperscript{73} Subsequently, lectins were used to quantitate and evaluate the cell-mediated immune system of mammalian species, including pigs, by lymphocyte blastogenesis.\textsuperscript{75, 95, 111} Most lectins have been found to have mitogenic activity and Sharon believes that all lectins may prove mitogenic if tested in suitable systems.\textsuperscript{95} Coulson and Chambers suggested that lectins "bypass" the requirement for antigenic recognition and induce cells to undergo immunological activation.\textsuperscript{17} Two lectins that may be particularly useful as immunopotentiators include Concanavalin A (ConA) and \textit{Lens culinaris} agglutinin (LCA).

Concanavalin A was purified by Sumner and Howell in 1936.\textsuperscript{101} It was the first lectin purified and is one of the better characterized T lymphocyte stimulating lectins.\textsuperscript{10, 16, 35} It is composed of dimeric, tetrameric, and higher-order forms of identical asymmetrical subunits with molecular weights of 25.5K.\textsuperscript{49} The carbohydrate binding sites of ConA are specific for alpha-D-mannoside-like residues of glycoproteins.\textsuperscript{83} Prolonged exposure of lymphocytes to ConA is necessary for the induction of mitosis and blast transformation.\textsuperscript{1, 6, 72} This induction may be reversed by exposing lymphocytes to competing saccharides\textsuperscript{72, 84} or antibody to ConA.\textsuperscript{71}
In vitro studies have demonstrated that lymphocytes transformed by ConA release various lymphokines, including: gamma-interferon, granulocyte-macrophage colony stimulating factor, interleukin-2, macrophage cytotoxicity factor, and macrophage migration inhibition factor. Other in vitro studies have demonstrated that ConA mediates the maturation of B cells by facilitating the focusing of helper T cells on the surface of responding B cells.

Lens culinarius agglutinin was first described by Landsteiner and Raubitschek in 1908, and purified by Howard and Sage in 1969. It consists of two subunits, that have similar structure and properties to ConA. The molecular weight of each subunit is 49K. The binding affinity of LCA is 50 times lower than the binding affinity of ConA for the same sugar residues.

The in vivo effects of lectins on the immune response of the host to specific immunogens is not well characterized. Egan et al. demonstrated that 500 µg of ConA suppressed the humoral immune response in mice to sheep red blood cells (RBC) when given at the same time and route. However, if the ConA was given two days prior to the first injection of sheep RBC, there was an apparent enhancement of the immune response to a second injection of sheep RBC given 7 to 21 days later. Barry and Hinrichs showed that ConA exerted a positive effect on the immune system of mice by enhancing the adoptive transfer of resistance to L. monocytogenes. Naspitz and Richter extensively reviewed the in vivo effect of phytohemagglutinin and found that while some authors detected an immunosuppressive effect, others did not.
**Immune serum**

Henry and Jerne demonstrated that 19s anti-sheep RBC antibodies given IV one to two hours prior to sheep RBC enhanced the primary immune response of mice to the sheep RBC 15 fold. In contrast, the same procedure using the 7s antibodies suppressed the immune response. The findings of Henry and Jerne were verified by Dennert et al., who were able to enhance the specific antibody response of mice to sheep RBC by giving 19s antibody prior to or in combination with the sheep RBC. Similiarly, Lehner et al. enhanced the antibody response of mice to sheep RBC by pretreating mice with sheep RBC specific monoclonal IgM.

In contrast to the immunopotentiation effect of 19s or IgM antibody reported by the above workers, Finkelstein and Uhr reported that antigen specific 19s and 7s antibody suppressed the antibody response to a bacteriophage. However, this immunosuppression was more pronounced with the 7s antibody than the 19s antibody.
SECTION I.

THE EFFECT OF THE MITOGENIC LECTINS, CONCANAVALIN A AND LENS CULINARIS AGGLUTININ, ON THE IMMUNE RESPONSE OF PIGS TO A PSEUDORABIES SUBUNIT VACCINE
The effect of the lectins, Concanavalin A (ConA) and Lens culinaris agglutinin (LCA), on the immune response of pigs to pseudorabies virus (PRV) subunit vaccine was evaluated utilizing 118 pigs. Neither lectin enhanced the immune response of the pigs to the subunit vaccine. In fact, both lectins appear to have had a slight immunosuppressive effect, ConA more so than the LCA.

The PRV subunit vaccine consisted of 15 ug of viral glycoproteins and 150 ug of the respective lectin prepared with or without Freund's incomplete adjuvant. Two inoculations were given subcutaneously, three weeks apart. Subsequently, the pigs were challenged with $10^{7.3}$ PFU of virulent PRV. The immune response of the pigs was determined by the development of serum-virus neutralizing (SN) antibody. In addition, the cell-mediated immune response was assessed by a lymphocyte blastogenesis assay in the adjuvanted subunit vaccine treatments. Furthermore, the clinical response of the pigs to PRV challenge was ascertained by survival rates, weight gain responses, and virus shedding patterns. The PRV infection was most evident during days 4 through 8 post-virus challenge (pc).

The slopes of the development of the SN antibody ranged from 0.082 to 0.122 log$_2$-day$^{-1}$, with no statistically significant differences being observed. Overall mean virus stimulation indexes for the post-challenge lymphocyte blastogenesis assay were 2.77, 2.65, and 1.91 for the lectin-free, LCA, and ConA treatments, respectively. No differences were
observed between the mean weight gain responses of the treatments. The mean weight gain responses for the adjuvanted lectin-free, LCA, and ConA treatments were 0.440, 0.298, and 0.297 Kg-day\(^{-1}\) for the days 4 to 8 time period. The slopes of the virus shedding patterns ranged from -0.249 to -0.357 log\(_{10}\)-day\(^{-1}\), with no significant differences being observed. However, the lectin-free and LCA treated pigs consistently shed lower quantities of virus and stopped shedding virus 48 hours before the ConA treated pigs. The survival data indicated that 100% of the pigs receiving adjuvanted subunit vaccine, with or without lectin, survived the PRV challenge, while 72%, 75%, and 70% of the pigs receiving non-adjuvanted lectin-free, LCA, or ConA subunit vaccine treatments, respectively, survived the PRV challenge.
Pseudorabies is an economically important disease of swine. Estimated losses from reduced weight gains, reduced conception rates, abortions, and deaths due to pseudorabies cost the Iowa swine industry $33.9 million in 1981. In 1985, the cost to the Iowa swine industry, including sales restrictions on breeding stock, was estimated to be $107 to $117 million. Major efforts to control the spread of pseudorabies depend on vaccination and management. Vaccination of pigs with attenuated or inactivated virus is effective. However, traditional vaccines prevent the free movement of swine because pseudorabies virus (PRV) infected vaccinated pigs cannot be differentiated from non-infected vaccinated pigs. This disadvantage can be overcome with the use of subunit vaccines for PRV which would permit the serological identification of virus infected pigs by using non-vaccine viral components as diagnostic antigen.

In 1984, Platt described an effective PRV subunit vaccine, that consisted of viral envelope glycoproteins. The viral glycoproteins were extracted by lectin affinity chromatography from detergent solubilized membranes of virus infected cells. As little as 16 ug of vaccine antigen was shown to induce a protective immune response. The vaccine was also found to be free of an early non-structural viral glycoprotein that was subsequently used as a diagnostic antigen to identify PRV infected subunit vaccinated pigs.
Although the subunit vaccine provides protection at relatively low dose levels, the extraction method may prove too expensive for commercial production. However, immunopotentiating agents may enhance the immune response of pigs to the viral glycoproteins, making it possible to reduce the amount of antigen necessary for the subunit vaccine, and consequently reduce its cost of production.

The ability of lectins to induce immunoglobulin\textsuperscript{11, 20, 26, 27} and interleukin-\textsuperscript{18} production in vitro make them excellent candidates for immunopotentiating agents. In vivo studies indicate that lectins may either suppress or stimulate the immune response. Egan et al. presented evidence that Concanavalin A (ConA) could enhance the antibody response of mice to sheep red blood cells.\textsuperscript{10} Martin demonstrated that it was possible to enhance the cytotoxic T lymphocyte response of mice to tumor cells by immunizing with homologous tumor cells linked to ConA.\textsuperscript{17} Similarly, ConA-stimulated murine spleen cells significantly enhanced the adoptive transfer of resistance to \textit{L. monocytogenes}.\textsuperscript{2} Concanavalin A has been shown to activate porcine lymphocytes in vitro.\textsuperscript{31} To date there have not been similar in vivo studies in food animals. The following study was done to determine if the immunity induced by the PRV subunit vaccine described above could be enhanced by incorporating either ConA or \textit{Lens culinaris} agglutinin (LCA) into the vaccine preparation.
MATERIALS AND METHODS

Virus, Cells, Media, and Buffer Solutions

Pseudorabies virus strain BE was used as the source of vaccine antigen and for pig challenge. The virus was originally isolated from a dog that died of pseudorabies\textsuperscript{25} and was subsequently plaque purified three times in Madin Darby bovine kidney (MDBK) cells. Prior to its use as a source of subunit vaccine antigen, the strain had been passaged a maximum of 14 times through MDBK cells and seven times through porcine kidney (PK) la cells. To insure that the strain maintained its virulence for challenge, it was back passaged once through a pig, reisolated in MDBK cells, and passed once in PK la cells. All virus was stored in aliquots at -70 C in tissue culture media.

Porcine kidney la cells were obtained from the Veterinary Medical Research Institute (Ames, IA). Madin Darby bovine kidney cells were obtained from the National Animal Disease Center (Ames, IA). Both cell lines were maintained in this laboratory in excess of 10 years and propagated at 37 C in a 5% CO\textsubscript{2} humidified incubator. Growth medium (GM) consisted of Eagle's minimum essential medium with Earle's salts and L-glutamine (MEM) (Gibco Laboratories, Grand Island, NY) supplemented with 10% heat inactivated fetal calf serum (FCS). Maintenance medium (MM) was GM with 2% FCS. Both GM and MM contained 100 units penicillin, 100 ug streptomycin and 3 ug amphotericin B per ml. Medium M199 (M199) containing 25 mM hepes buffer, Earle's salts and L-glutamine (Gibco
Laboratories, Grand Island, NY) was supplemented with 15% FCS, and contained 100 units penicillin, and 100 µg streptomycin per ml. Calcium and magnesium free Hank's balanced salt solution (HBSS) was supplied by Gibco Laboratories (Grand Island, NY).

Saline G consisted of 1.1 g dextrose, 8.0 g sodium chloride, 1.0 g lactalbumin hydrolysate, 1.2 mg phenol red, 400 mg potassium chloride, 153.3 mg sodium phosphate dibasic, 150 mg potassium phosphate monobasic, 150 mg magnesium sulfate crystal, and 16 mg calcium chloride crystal in deionized water q.s. to one liter. Tris-Tricine (TT) buffer, pH 8.6, 0.025 M, was supplied by Bio-Rad Laboratories (Richmond, CA). Extraction buffer (EB) consisted of TT buffer with 1% Triton-X 100. Acid-citrate dextrose (ACD) 2X solution consisted of 8 g citric acid monohydrate, 22 g sodium citrate and 25 g dextrose in 500 ml deionized water. Cleaning fluid consisted of MEM containing 1% FCS, 10 mM hepes, 100 units penicillin, 100 µg streptomycin, and 6 µg amphotericin B per ml. Phosphate buffered saline (PBS), pH 7.2, consisted of 8.5 g sodium chloride, 1.15 g sodium phosphate dibasic, and 270.8 mg potassium phosphate monobasic in deionized water q.s. to one liter.

Experimental Animals

Four to eight-week-old pigs were obtained from a secondary specific pathogen free herd maintained at the College of Veterinary Medicine (Iowa State University, Ames, IA). The pigs were maintained on a Ralston Purina (St. Louis, MO) standard grower ration and given free access to
feed at least seven days prior to virus challenge so that all pigs would be growing at a maximum rate at the time of virus challenge.

Subunit Vaccine Preparation

The PRV subunit vaccine, consisted of 15 μg of viral glycoproteins per dose, and was produced as previously described by Platt. Briefly, PK 1a cells were grown in 850 cm² roller bottles and infected with PRV at a multiplicity of infection greater than 5.0. This multiplicity of infection resulted in more than 95% of the cell monolayer showing cytopathic effects by 24 hours post inoculation. At this time, the virus infected cells were harvested using sterile glass beads, washed three times in MEM, and packed by centrifugation at 1900 x g. The cells were subsequently resuspended in EB at a ratio of 3.0 ml buffer to 1.0 ml of packed cell volume.

The cell suspension was sonically disrupted with a Branson Sonifier 350 (Danbury, CT) equipped with a microtip using 20 pulses at a 50% duty cycle at an output setting of two. The preparation was then gently agitated overnight at 4 C. Unsolubilized material was removed from the preparation by centrifugation at 500 x g for 15 minutes. A second centrifugation at 10^5 x g for 90 minutes, resulted in the formation of three distinct layers. These layers consisted of a cloudy upper layer, a clear middle layer and a sediment layer which composed roughly 20%, 75% and 5% of the total volume, respectively. Lipids were removed from the upper cloudy layer by freon extraction. The lipid free upper cloudy
layer was then pooled with the clear middle layer. Together these two layers represented crude viral antigen (CVA).

Pseudorabies viral glycoproteins were extracted from CVA by lectin affinity chromatography utilizing *Lens culinaris* agglutinin A and B covalently immobilized on agarose beads (E-Y Lab, San Mateo, CA). The column was prepared for use by washing with three column volumes of EB containing 2.5% mannose followed by 10 column volumes of TT buffer. The CVA was loaded onto the column and allowed to adsorb overnight at 4 C. Following adsorption, the column was rinsed with five column volumes of EB followed by five column volumes of TT buffer to remove free Triton X-100. The viral glycoproteins were then eluted from the column with four column volumes of TT buffer containing 2.5% mannose. The eluant containing the viral glycoproteins was simultaneously concentrated five to 10-fold and dialyzed against TT buffer at 4 C using a Pro-DiMem PA-10 membrane, with a 10 K mw cutoff, in a negative pressure micro dialysis concentrator (Bio-Molecular Dynamics, Beaverton, OR). Protein content was determined by the dye binding method described by Bradford.\(^7\)

**Virus Assay**

Virus was assayed by the plaque method.\(^9\) Duplicate 250 ul samples of 10-fold diluted virus suspensions were added to MDBK cell monolayers grown in 24-well tissue culture plates (Costar, Cambridge, MA) and incubated at 37 C for 90 minutes. The inoculum was then removed and the cell monolayers were rinsed twice with saline G before being overlaid
with MM containing 0.8% carboxymethylcellulose. The plates were reincubated at 37 C for 48 hours, fixed with PBS containing 6.0% formalin, and stained with crystal violet. Virus titer (PFU) was expressed as the log_{10}.

Serum-virus Neutralizing Antibody Test

Serum-virus neutralizing (SN) antibody titers were determined by a microtiter procedure utilizing 96-well flat bottom microtiter plates (Costar, Cambridge, MA) according to a modification of the microtiter method described by Hill et al.\textsuperscript{15} All sera were heat inactivated at 56 C for 30 minutes prior to titration. Duplicate two-fold dilutions of test sera were made leaving 100 ul of diluted serum in each well. Subsequently, 50 ul of saline G, containing 300 PFU of PRV, were added to individual wells with the exception that serum control wells received 50 ul of saline G only. The plates were gently shaken and incubated for one hour at 37 C in a 5% CO\textsubscript{2} humidified incubator. Following incubation, 150 ul of MEM, containing approximately 10\textsuperscript{5.6} MDBK cells per ml, were added to each well and the plates reincubated for 72 hours. Cell monolayers were then fixed with PBS containing 6% formalin and stained with crystal violet. One titer determination was made for each serum on two separate days. The 50% SN endpoints were determined and expressed as the geometric mean of the two determinations in log\textsubscript{2}.

Serum-virus neutralizing antibody titers for the non-adjuvanted lectin experiment were determined by the State Veterinary Diagnostic
Laboratory (College of Veterinary Medicine, Iowa State University, Ames, IA) using a microtiter technique.15  

Lymphocyte Blastogenesis

Lymphocytes were harvested aseptically from whole blood essentially as described by Boyum.6 Approximately 8.5 ml of blood were collected into a vacutainer tube containing 1.5 ml of ACD. Six ml of the citrated blood were gently diluted three-fold in PBS and layered over eight ml of Histopaque-1077 (Sigma Diagnostics, St. Louis, MO) contained in a siliconized 50 ml glass centrifuge tube. An opaque layer of lymphocytes was collected from the surface of the histopaque following centrifugation at 400 X g for 30 minutes at 25 C, and washed in 15 ml of HBSS. The lymphocytes were pelleted by centrifugation at 250 X g for 10 minutes and resuspended in 15 ml of 0.85% ammonium chloride solution for 10 minutes to lyse contaminating red blood cells. The lymphocytes were washed a second time in 15 ml of HBSS and resuspended in one ml of HBSS. The lymphocyte suspension was quantified using a Colter counter (Coulter Electronic Inc., Hialeah, FL) and standardized to $10^6.4$ lymphocytes per ml in M199.

The lymphocyte blastogenesis assay was performed essentially as described by Kaeberle and Roth.16 Parameter treatments were run in triplicate in 96 well flat bottom microtiter plates. Individual wells received 200 ul of the standardized lymphocyte suspension and 25 ul of the parameter treatment solution. Virus and mitogen treatments,
consisted of $10^7$ PFU of heat inactivated PRV and 0.625 ug ConA prepared in M199, respectively. The control treatment consisted of fresh M199 alone. The plates were incubated four days at 37 C in a 5% CO$_2$ humidified incubator and then labelled overnight with 0.375 uCi of methyl-H$^3$-thymidine (Amersham Corp., Arlington Heights, IL) contained in 25 ul of M199. The lymphocytes were harvested onto glass fiber pads. Beta emissions were counted for each well and were expressed as counts per minute (cpm). Mean cpm were determined for each treatment and compared.

Experimental Design

**Non-adjuvanted lectin experiment**

The effect of lectins on the porcine immune response to the PRV subunit vaccine was evaluated by comparing the SN antibody response and the post-viral challenge clinical response of pigs vaccinated with: a) the viral antigen alone, and b) the viral antigen mixed with 150 ug of either LCA or ConA. Pigs were randomly assigned to one of four treatments: unvaccinated control pigs that served as sentinels for adventitious exposure to PRV, lectin-free vaccinates, vaccinates receiving LCA, and vaccinates receiving ConA. Each group consisted of a minimum of three pigs and was replicated three to six times. All inoculated pigs received two, one ml doses of immunogen subcutaneously 42 and 21 days prior to intranasal virus challenge with $10^7.3$ PFU of PRV.
The SN antibody response was assessed by comparing the mean SN antibody titers of the treatment groups during immunization and post-virus challenge (pc). Serum-virus neutralizing antibody titers were measured at weekly intervals during immunization and following virus challenge. The mean rate of antibody formation was calculated from SN antibody titers determined on days 0 through 14 pc.

The clinical response was evaluated by comparing survival rates, mean weight gain responses, and the virus shedding patterns of the treatment groups. Weights were recorded and nasal swabs were collected in cleaning fluid for virus assay on days 0, 2, 4, 6, 8, 10, 12, 14, and 21 pc. Mean daily weight gain responses were determined over four time periods: days 0 through 4, days 4 through 8, days 8 through 12, and days 12 through 21 pc. The mean rate of viral clearance was calculated from the virus titers of nasal swabs collected on days 4, 6, and 8 pc.

Adjuvanted lectin experiment

The effect of combining lectins with adjuvanted subunit vaccine was also evaluated. Treatment groups consisted of a minimum of three randomly assigned pigs. Each treatment was replicated three to four times. Vaccine inoculums were identical to those described above with the exception that they contained 50% (v/v) Freund's incomplete adjuvant. The effect of lectins on the porcine immune response to the subunit vaccine was assessed as previously described with the exception that the cell-mediated immune (CMI) response was also evaluated. In addition, SN antibody titers were also measured on days -4, 4, and 10 pc.
The CMI response was determined by two methods. First, the CMI response of individual pigs to PRV was measured on days -4, 0, 4, 7, 10, 14, and 21 pc by the lymphocyte blastogenesis assay. The mean control, virus, and mitogen parameter cpm of the individual samples from a given treatment were pooled into pre-challenge (days -4 and 0) and post-challenge (days 4 through 21 pc) periods. The data were balanced by separating the replications that contained the LCA treatment from the replications that contained the ConA treatment. Overall treatment means were then calculated and compared.

Secondly, the CMI response was evaluated more conservatively by determining the number of pigs of each treatment that had a positive CMI response during three periods: days -4 and 0, days 7 and 10, and days 14 and 21 pc. Only pigs with a virus stimulation index ≥ 2.0, on both days of a period, were considered to have a positive PRV specific CMI response for that period. Mean SN antibody titers of the pigs with positive and negative CMI responses were calculated and compared to determine if the SN antibody response was correlated to the CMI response.

Data Analysis

The methods of data analysis used in this study take into account the multiple observations from a single individual. Only data collected from pigs surviving the virus challenge for each treatment were pooled for final analysis of each parameter, with the exception of the CMI data where all available data were included. By excluding data from pigs that
did not survive the virus challenge, comparisons between the treatments were not biased by impending death loss. Each replication was run under identical conditions and significant variation between the replications was not observed. Therefore, the data from all replications were pooled for analysis. Treatment means of each parameter were compared by an ANOVA procedure using a general linear model. Conservative degrees of freedom, i.e., one degree of freedom for each pig involved in the comparison minus one, were used in determining the statistical significance.

A Chi-square analysis was used to compare clinical survival data and the conservative method of determining PRV specific CMI responses of the treatment groups.
RESULTS

Non-adjuvanted Lectin Experiment

**Serum-virus neutralizing antibody**

The mean SN antibody titers are summarized in Table 1 and represented graphically in Figure 1. No statistically significant differences were observed between the treatments when their rates of antibody formation were compared (Table 2). It is interesting to note that the mean SN antibody titer for the ConA treatment was less than the mean SN antibody titers for the lectin-free or LCA treatments between days 0 and 14 pc. For example, on day 7 pc the mean SN antibody titers were 2.15, 1.25, and 0.86 log₂ for the lectin-free, LCA, and ConA treatments, respectively. The absence of a SN antibody response in the unvaccinated control pigs prior to virus challenge, and the absence of an anamnestic response pc indicates that all the pigs were free of PRV until challenged.

**Clinical response**

**Survival** The response of pigs to PRV challenge was most evident between days 4 and 8 pc, when most of the death loss occurred and clinical signs of PRV infection were most apparent. The number of pigs surviving the PRV challenge for the lectin-free, LCA, and ConA treatments were 13 of 18 (72.2%), 9 of 12 (75.0%), and 7 of 10 (70.0%), respectively. In contrast, only 9 of 21 (42.9%) unvaccinated control pigs survived the PRV challenge.
Table 1. The effect of lectins on the serum-virus neutralizing antibody response of pigs immunized with pseudorabies virus subunit vaccine without adjuvant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>-42</th>
<th>-21</th>
<th>-14</th>
<th>-7</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lectin-free</td>
<td>13</td>
<td>0.00</td>
<td>0.00</td>
<td>0.31</td>
<td>0.23</td>
<td>0.15</td>
<td>2.15</td>
<td>4.15</td>
<td>4.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.00</td>
<td>+0.00</td>
<td>+0.13</td>
<td>+0.17</td>
<td>+0.10</td>
<td>+0.39</td>
<td>+0.30</td>
<td>+0.39</td>
</tr>
<tr>
<td>Lens culinaris agglutinin</td>
<td>8</td>
<td>0.00</td>
<td>0.00</td>
<td>0.25</td>
<td>0.13</td>
<td>0.13</td>
<td>1.25</td>
<td>5.25</td>
<td>5.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.00</td>
<td>+0.00</td>
<td>+0.16</td>
<td>+0.13</td>
<td>+0.13</td>
<td>+0.49</td>
<td>+0.45</td>
<td>+0.42</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>7</td>
<td>0.00</td>
<td>0.00</td>
<td>0.14</td>
<td>0.14</td>
<td>0.29</td>
<td>0.86</td>
<td>3.86</td>
<td>4.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.00</td>
<td>+0.00</td>
<td>+0.14</td>
<td>+0.14</td>
<td>+0.29</td>
<td>+0.55</td>
<td>+0.55</td>
<td>+0.36</td>
</tr>
<tr>
<td>Unvaccinated control</td>
<td>9</td>
<td>0.00</td>
<td>0.00</td>
<td>0.11</td>
<td>0.00</td>
<td>0.00</td>
<td>0.77</td>
<td>3.44</td>
<td>4.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.00</td>
<td>+0.00</td>
<td>+0.11</td>
<td>+0.00</td>
<td>+0.00</td>
<td>+0.40</td>
<td>+0.34</td>
<td>+0.46</td>
</tr>
</tbody>
</table>

*Day 0 is the day of virus challenge.

*N equals total number of pigs surviving virus challenge from all replications.
Figure 1. The effect of lectins on the serum-virus neutralizing antibody response of pigs immunized with pseudorabies subunit vaccine without adjuvant. Day 0 is the day of virus challenge. Treatment groups include: lectin-free — , N=13; Lens culinaris agglutinin— , N=8; Concanavalin A—— , N=7; and unvaccinated control— — N=9. Points represent the mean of all pigs surviving virus challenge from all replications.
Table 2. The effect of lectins on the rate of antibody formation in pigs immunized with pseudorabies virus subunit vaccine without adjuvant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N^a</th>
<th>X Slope^b</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lectin-free</td>
<td>14</td>
<td>0.091</td>
<td>0.009</td>
</tr>
<tr>
<td>Lens culinaris agglutinin</td>
<td>8</td>
<td>0.122</td>
<td>0.009</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>7</td>
<td>0.085</td>
<td>0.015</td>
</tr>
<tr>
<td>Unvaccinated control</td>
<td>9</td>
<td>0.082</td>
<td>0.008</td>
</tr>
</tbody>
</table>

^a N equals total number of pigs surviving virus challenge from all replications.

^b The rate of antibody formation is represented by the slope of the curve described by the equation y=mx + b. Slope (m) was calculated by regressing antibody titer on time, i.e., days 0, 7, and 14. Day 0 is the day of virus challenge.

Weight gain response

The weight gain responses are summarized in Table 3. There were no statistically significant differences observed between the treatments over the time periods analyzed. The mean weight gain response of days 4 to 8 pc, for the lectin-free, LCA, and ConA treatments were -0.040, 0.069, and -0.051 Kg-day^-1, respectively. In contrast, unvaccinated controls lost an average of 0.526 Kg-day^-1. Following day 8 pc, the weight gain responses of all treatments were approximately equal indicating recovery from the virus challenge.
Table 3. The effect of lectins on the weight gain response of pigs immunized with pseudorabies virus subunit vaccine without adjuvant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N^a</th>
<th>Day b 0-4</th>
<th>Day 4-8</th>
<th>Day 8-12</th>
<th>Day 12-21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lectin-free</td>
<td>14</td>
<td>0.025</td>
<td>-0.040</td>
<td>0.604</td>
<td>0.634</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.067</td>
<td>+0.122</td>
<td>+0.061</td>
<td>+0.063</td>
</tr>
<tr>
<td>Lens culinaris</td>
<td>8</td>
<td>0.016</td>
<td>0.069</td>
<td>0.781</td>
<td>0.621</td>
</tr>
<tr>
<td>agglutinin</td>
<td></td>
<td>+0.178</td>
<td>+0.143</td>
<td>+0.221</td>
<td>+0.107</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>7</td>
<td>0.082</td>
<td>-0.051</td>
<td>0.666</td>
<td>0.629</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.219</td>
<td>+0.174</td>
<td>+0.150</td>
<td>+0.093</td>
</tr>
<tr>
<td>Unvaccinated control</td>
<td>9</td>
<td>-0.011</td>
<td>-0.526</td>
<td>0.543</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.172</td>
<td>+0.177</td>
<td>+0.188</td>
<td>+0.104</td>
</tr>
</tbody>
</table>

^aN equals the total number of pigs surviving virus challenge from all replications.

^bDay 0-4, 4-8, 8-12, and 12-21 pc are time periods over which the weight gain responses were calculated. Day 0 is the day of virus challenge.

Virus shedding patterns The virus shedding patterns are summarized in Table 4 and represented graphically in Figure 2. No statistically significant differences were observed between treatments when the rates of virus clearance, between days 4 and 8 pc, were compared (Table 5). The greatest amount of virus was shed from on day 4 pc, at which time the mean virus titers of the lectin-free, LCA, ConA, and unvaccinated control treatments were 4.62, 4.89, 4.75, and 4.11 log_{10}, respectively. Virus was recovered from all treatments through day 10 pc. All LCA treated vaccinates ceased virus shedding by day 12 pc, while 1 of
Table 4. The effect of lectins on the clearance of virus from the nasal cavities of pigs immunized with pseudorabies virus subunit vaccine without adjuvant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nb</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lectin-free</td>
<td>13</td>
<td>0.00</td>
<td>2.78</td>
<td>4.62</td>
<td>3.84</td>
<td>1.19</td>
<td>1.06</td>
<td>0.32</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.00</td>
<td>±0.31</td>
<td>±0.16</td>
<td>±0.27</td>
<td>±0.26</td>
<td>±0.47</td>
<td>±0.32</td>
<td>±0.02</td>
<td>±0.00</td>
</tr>
<tr>
<td>Lens culinaris agglutinin</td>
<td>9</td>
<td>0.00</td>
<td>2.46</td>
<td>4.89</td>
<td>3.48</td>
<td>1.40</td>
<td>0.54</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.00</td>
<td>±0.41</td>
<td>±0.29</td>
<td>±0.26</td>
<td>±0.52</td>
<td>±0.24</td>
<td>±0.00</td>
<td>±0.00</td>
<td>±0.00</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>7</td>
<td>0.00</td>
<td>3.81</td>
<td>4.75</td>
<td>4.42</td>
<td>1.76</td>
<td>1.06</td>
<td>0.65</td>
<td>0.22</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.00</td>
<td>±0.39</td>
<td>±0.34</td>
<td>±0.31</td>
<td>±0.77</td>
<td>±0.58</td>
<td>±0.42</td>
<td>±0.19</td>
<td>±0.00</td>
</tr>
<tr>
<td>Unvaccinated control</td>
<td>9</td>
<td>0.00</td>
<td>3.32</td>
<td>4.11</td>
<td>3.60</td>
<td>2.88</td>
<td>2.60</td>
<td>0.77</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.00</td>
<td>±0.31</td>
<td>±0.35</td>
<td>±0.29</td>
<td>±0.61</td>
<td>±0.63</td>
<td>±0.43</td>
<td>±0.03</td>
<td>±0.00</td>
</tr>
</tbody>
</table>

\(^a^\) Day 0 is the day of virus challenge.

\(^b^\) N equals total number of pigs surviving virus challenge from all replications.
Figure 2. The effect of lectins on the clearance of virus from the nasal cavities of pigs immunized with pseudorabies subunit vaccine without adjuvant. Day 0 is the day of virus challenge. Treatment groups include: lectin-free — , N=13; Lens culinaris agglutinin — — , N=8; Concanavalin A — — , N=7; and unvaccinated control — — , N=9. Points represent the mean of all pigs surviving the virus challenge from all replications.
Table 5. The effect of lectins on the rate of virus clearance from the nasal cavities of pigs immunized with pseudorabies virus subunit vaccine without adjuvant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N&lt;sup&gt;a&lt;/sup&gt;</th>
<th>X Slope&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lectin-free</td>
<td>13</td>
<td>-0.286</td>
<td>0.028</td>
</tr>
<tr>
<td><em>Lens culinaris</em> agglutinin</td>
<td>9</td>
<td>-0.291</td>
<td>0.052</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>7</td>
<td>-0.249</td>
<td>0.076</td>
</tr>
<tr>
<td>Unvaccinated control</td>
<td>9</td>
<td>-0.046</td>
<td>0.091</td>
</tr>
</tbody>
</table>

<sup>a</sup>N equals total number of pigs surviving virus challenge from all replications.

<sup>b</sup>The rate of virus clearance is represented by the slope of the curve described by the equation y=mx+b. Slope (m) was calculated by regressing virus titer on time, i.e., days 4, 6, and 8. Day 0 is the day of virus challenge.

13 (7.7%) lectin-free vaccinates, 2 of 7 (28.6%) ConA treated vaccinates, and 5 of 9 (55.6%) unvaccinated controls were still shedding virus on day 14 pc. No virus shedding was detected in any surviving pig on day 21 pc.

Adjuvanted Lectin Experiment

**Serum-virus neutralizing antibody**

The SN antibody titers are summarized in Table 6 and represented graphically in Figure 3. No statistically significant differences were observed between treatments when the rates of antibody formation were compared (Table 7). However, the SN antibody response of ConA treated...
Table 6. The effect of lectins on the serum-virus neutralizing antibody response of pigs immunized with pseudorabies virus subunit vaccine with adjuvant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>-42</th>
<th>-21</th>
<th>-14</th>
<th>-7</th>
<th>-4</th>
<th>0</th>
<th>4</th>
<th>7</th>
<th>10</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lectin-free</td>
<td>15</td>
<td>0.00</td>
<td>0.00</td>
<td>0.82</td>
<td>0.97</td>
<td>0.87</td>
<td>0.87</td>
<td>0.82</td>
<td>4.62</td>
<td>5.65</td>
<td>5.75</td>
<td>5.82</td>
</tr>
<tr>
<td>Lens culinaris</td>
<td>12</td>
<td>0.00</td>
<td>0.00</td>
<td>0.27</td>
<td>0.54</td>
<td>0.43</td>
<td>0.27</td>
<td>0.33</td>
<td>4.00</td>
<td>5.33</td>
<td>5.71</td>
<td>5.83</td>
</tr>
<tr>
<td>Lens culinaris agglutinin</td>
<td>12</td>
<td>0.00</td>
<td>0.00</td>
<td>0.16</td>
<td>0.24</td>
<td>0.21</td>
<td>0.21</td>
<td>0.22</td>
<td>0.35</td>
<td>0.23</td>
<td>0.28</td>
<td>0.19</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>14</td>
<td>0.00</td>
<td>0.00</td>
<td>0.27</td>
<td>0.29</td>
<td>0.30</td>
<td>0.16</td>
<td>0.05</td>
<td>3.11</td>
<td>4.79</td>
<td>5.43</td>
<td>5.32</td>
</tr>
<tr>
<td>Unvaccinated control</td>
<td>7</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.14</td>
<td>2.68</td>
<td>5.21</td>
<td>5.46</td>
<td>5.30</td>
</tr>
</tbody>
</table>

Day 0 is the day of virus challenge.

N equals total number of pigs surviving virus challenge from all replications.
Figure 3. The effect of lectins on the serum-virus neutralizing antibody response of pigs immunized with pseudorabies subunit vaccine with adjuvant. Day 0 is the day of virus challenge. Treatment groups include: lectin-free ——— N=15; Lens culinaris agglutinin— — — N=12; Concanavalin A— — — N=14; and unvaccinated control— — — N=7. Points represent the mean of all pigs surviving virus challenge from all replications.
The effect of lectins on the rate of antibody formation in pigs immunized with pseudorabies virus subunit vaccine containing adjuvant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Na</th>
<th>X Slopeb</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lectin-free</td>
<td>15</td>
<td>0.099</td>
<td>0.006</td>
</tr>
<tr>
<td>Lens culinaris agglutinin</td>
<td>12</td>
<td>0.108</td>
<td>0.006</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>14</td>
<td>0.104</td>
<td>0.006</td>
</tr>
<tr>
<td>Unvaccinated control</td>
<td>7</td>
<td>0.091</td>
<td>0.006</td>
</tr>
</tbody>
</table>

A Na equals total number of pigs surviving virus challenge from all replications.

b The rate of antibody formation is represented by the slope of the curve described by the equation y = mx + b. Slope (m) was calculated by regressing antibody titer on time, i.e., days 0, 4, 7, 10, and 14. Day 0 is the day of virus challenge.

The SN antibody response of the unvaccinated control pigs prior to virus challenge, and the absence of an anamnestic response in these pigs pc indicates that all pigs were free of PRV until challenged.
Cell-mediated immune response

The CMI responses of vaccinated pigs treated with LCA and ConA are summarized in Tables 8 and 9, respectively. No statistically significant differences were observed between the lectin-free and LCA treatments during the pre- and post-virus challenge periods (Table 8). The pre-challenge mean virus counts per minute (cpm) of the lectin-free and LCA treatments were 1540.5 and 1105.3, respectively. In contrast a statistically significant difference of 524 virus cpm (P<0.01) was observed between the lectin-free and ConA treatments during the pre-challenge period, suggesting that ConA had a suppressive effect on the development of the CMI response to PRV (Table 9). No differences were observed between the mean virus cpm of lectin-free and lectin treatments during the post-challenge period. However, the mean virus cpm of the lectin-free and lectin treatments were at least two times greater than unvaccinated controls (Tables 8 and 9), indicating that the PRV subunit vaccine sensitized lymphocytes to PRV.

Results of the conservative method of evaluating the effect of the lectin treatments on the development of a PRV specific CMI response are summarized in Figure 4. Prior to challenge, 3 of 15 (20%) lectin-free pigs and 2 of 12 (17%) LCA treated pigs had demonstrated positive CMI responses for PRV. None of the 14 ConA treated pigs were CMI positive, again suggesting that ConA may be immunosuppressive. By the days 7 and 10 period, the number of positive CMI responders increased to 10 of 15 (67%) lectin-free pigs, 6 of 12 (50%) LCA treated pigs, and 6 of 14 (46%) ConA treated pigs. The number of positive CMI responders during the
Table 8. The effect of *Lens culinaris* agglutinin on the cell-mediated immune response of pigs immunized with pseudorabies virus subunit vaccine with adjuvant as determined by lymphocyte blastogenesis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LF</th>
<th>LCA</th>
<th>UV</th>
<th>LF</th>
<th>LCA</th>
<th>UV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>922.9</td>
<td>1138.7</td>
<td>1081.8</td>
<td>1432.4</td>
<td>1687.3</td>
<td>1761.7</td>
</tr>
<tr>
<td></td>
<td>+169.8</td>
<td>+311.8</td>
<td>+261.1</td>
<td>+146.9</td>
<td>+265.5</td>
<td>+414.2</td>
</tr>
<tr>
<td>Virus</td>
<td>1540.5</td>
<td>1105.3</td>
<td>770.2</td>
<td>4316.2</td>
<td>4466.7</td>
<td>1697.8</td>
</tr>
<tr>
<td></td>
<td>+207.6</td>
<td>+234.7</td>
<td>+200.3</td>
<td>+608.9</td>
<td>+568.1</td>
<td>+234.9</td>
</tr>
<tr>
<td>Mitogen</td>
<td>25610.3</td>
<td>21829.5</td>
<td>23356.7</td>
<td>42709.7</td>
<td>39357.8</td>
<td>32478.4</td>
</tr>
<tr>
<td></td>
<td>+4295.7</td>
<td>+5089.9</td>
<td>+5044.6</td>
<td>+5024.4</td>
<td>+6077.5</td>
<td>+6797.2</td>
</tr>
</tbody>
</table>

Pre-challenge values are pooled means calculated from data collected on days -4 and 0. Day 0 is the day of virus challenge.

Counts per minute.

Post-challenge values are pooled means calculated from data collected on days 4, 7, 10, 14, and 21 post-virus challenge.

LF = lectin-free, N=12; LCA = *Lens culinaris* agglutinin, N=12; UV = unvaccinated control, N=12 and 7 pigs at the beginning and end of the virus challenge period.

Control = 25 ul M199; Virus = 25 ul M199 containing $10^7$ PFU heat inactivated PRV; Mitogen = 25 ul M199 containing 0.625 ug ConA.
Table 9. The effect of Concanavalin A on the cell-mediated immune response of pigs immunized with pseudorabies virus subunit vaccine with adjuvant as determined by lymphocyte blastogenesis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-challenge(^a) mean cpm(^b) ± SE</th>
<th>Post-challenge(^c) mean cpm ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LF</td>
<td>ConA</td>
</tr>
<tr>
<td>Control</td>
<td>896.0</td>
<td>1133.6</td>
</tr>
<tr>
<td></td>
<td>±136.9</td>
<td>±233.4</td>
</tr>
<tr>
<td>Virus</td>
<td>1443.5</td>
<td>920.6</td>
</tr>
<tr>
<td></td>
<td>±174.4</td>
<td>±139.8</td>
</tr>
<tr>
<td>Mitogen</td>
<td>32461.7</td>
<td>29252.3</td>
</tr>
<tr>
<td></td>
<td>±4360.3</td>
<td>±4115.7</td>
</tr>
</tbody>
</table>

\(^a\) Pre-challenge values are pooled means calculated from data collected on days -4 and 0. Day 0 is the day of virus challenge.

\(^b\) Counts per minute.

\(^c\) Post-challenge values are pooled means calculated from data collected from days 4, 7, 10, 14, and 21 post-virus challenge.

\(^d\) LF = lectin-free, N=12; ConA = Concanavalin A, N=15; UV = unvaccinated control, N=12 and 7 pigs at the beginning and end of the virus challenge period.

\(^e\) Control = 25 ul M199; Virus = 25 ul M199 containing 10\(^7\) PFU heat inactivated PRV; Mitogen = 25 ul M199 containing 0.625 ug ConA.
Figure 4. The effect of lectins on the cellular immune response of pigs immunized with pseudorabies subunit vaccine with adjuvant. A positive response was indicated by a virus stimulation index > 2.00 on two consecutive determinations. Day 0 is the day of virus challenge. Treatments are; lectin-free ( ), N=15; Lens culinaris agglutinin ( ), N=12; Concanavalin A ( ), N=14; and unvaccinated control ( ), N=16, 9, and 7 on days -4 and 0, days 7 and 10, and days 14 and 21, respectively.
final period, days 14 and 21 pc, were 5 of 15 (33%), 6 of 12 (50%), and 5 of 14 (36%) for the three treatments, respectively. No positive CMI responders were detected among the unvaccinated control treatment, pre- or post-virus challenge. These differences were not significant by chi-square analysis.

The SN antibody titers of pigs with positive CMI responses, as determined by the conservative method, were compared to the SN antibody titers of pigs with negative CMI responses for each of the three periods analyzed. The results of these comparisons are summarized in Table 10. No significant differences were observed.

Clinical response

Survival The survival rates of 15 lectin-free, 12 LCA, and 14 ConA treated pigs were all 100%. In contrast, only 7 of 16 (43.8%) unvaccinated controls survived the virus challenge.

Weight gain response The weight gain responses are summarized in Table 11. No statistically significant differences were observed between the treatments. The mean weight gain responses of all treatment groups ranged from 0.016 to 0.024 Kg-day$^{-1}$ during first 4 days pc. Weight gains increased dramatically between day 4 and 8 pc to 0.440, 0.298, and 0.292 Kg-day$^{-1}$ in the lectin-free, LCA, and ConA treatment groups, respectively. In contrast, unvaccinated controls lost an average of 0.057 Kg-day$^{-1}$ during this period. Although the differences between the lectin-free treatment and the lectin treatments were not significant
Table 10. Correlation of the serum-virus neutralizing antibody and cell-mediated immune responses of pigs immunized with adjuvanted pseudorabies virus subunit vaccine with and without lectins

<table>
<thead>
<tr>
<th>Time period</th>
<th>LF&lt;sup&gt;a&lt;/sup&gt;</th>
<th>LCA</th>
<th>ConA</th>
<th>UV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CMI+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>CMI-&lt;sup&gt;c&lt;/sup&gt;</td>
<td>CMI+</td>
<td>CMI-</td>
</tr>
<tr>
<td>Pre-challenge</td>
<td>1.51 ± 0.54</td>
<td>0.70 ± 0.98</td>
<td>1.19 ± 1.68</td>
<td>0.20 NA ± 0.28</td>
</tr>
<tr>
<td>Early post-challenge</td>
<td>5.73 ± 0.08</td>
<td>3.95 ± 1.60</td>
<td>4.65 ± 1.02</td>
<td>4.60 ± 0.80</td>
</tr>
<tr>
<td>Late post-challenge</td>
<td>6.42 ± 0.40</td>
<td>5.46 ± 1.00</td>
<td>6.15 ± 0.62</td>
<td>5.38 ± 0.82</td>
</tr>
</tbody>
</table>

<sup>a</sup>LF = lectin-free, N=15; LCA = Lens culinaris agglutinin, N=12; ConA = Concanavalin A, N=14; UV = unvaccinated controls, N=16, 9, and 7 pre-challenge, early post-challenge, and late post-challenge, respectively.

<sup>b</sup>Pre-challenge = days -4 and 0; Early post-challenge = days 7 and 10; Late post-challenge = days 14 and 21. Day 0 is the day of virus challenge.

<sup>c</sup>CMI+ = stimulation index > 2.00 on two days; CMI- = stimulation index < 2.00 on at least one day.

<sup>d</sup>None available.

Between day 4 and 8 pc, they do suggest that the lectins may be immunosuppressive. Following day 8 pc, the weight gain responses of all treatments were approximately equal indicating recovery from the virus challenge.
Table 11. The effect of lectins on the weight gain response of pigs immunized with pseudorabies virus subunit vaccine with adjuvant

Mean weight gain + SE (Kg day⁻¹)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Day⁰⁻⁴</th>
<th>Day 4-8</th>
<th>Day 8-12</th>
<th>Day 12-21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lectin-free</td>
<td>15</td>
<td>0.025</td>
<td>0.440</td>
<td>0.497</td>
<td>0.673</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.109</td>
<td>+0.097</td>
<td>+0.097</td>
<td>+0.020</td>
</tr>
<tr>
<td>Lens culinaris</td>
<td>12</td>
<td>0.016</td>
<td>0.298</td>
<td>0.601</td>
<td>0.726</td>
</tr>
<tr>
<td>agglutinin</td>
<td></td>
<td>+0.101</td>
<td>+0.162</td>
<td>+0.041</td>
<td>+0.022</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>14</td>
<td>0.024</td>
<td>0.297</td>
<td>0.560</td>
<td>0.722</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.112</td>
<td>+0.035</td>
<td>+0.066</td>
<td>+0.026</td>
</tr>
<tr>
<td>Unvaccinated control</td>
<td>7</td>
<td>0.256</td>
<td>-0.057</td>
<td>0.421</td>
<td>0.572</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.071</td>
<td>+0.114</td>
<td>+0.088</td>
<td>+0.061</td>
</tr>
</tbody>
</table>

aN equals the total number of pigs surviving virus challenge from all replications.

bDay 0-4, 4-8, 8-12, and 12-21 pc are time periods over which the weight gain responses were calculated. Day 0 is the day of virus challenge.

Virus shedding patterns The virus shedding patterns are summarized in Table 12 and represented graphically in Figure 5. No statistically significant differences were observed between the treatments when the rates of virus clearance, between days 4 and 8 pc, were compared (Table 13). The greatest amount of virus was shed on day 4 pc, at which time the mean virus titers for the lectin-free, LCA, ConA treatment groups, and unvaccinated controls were 4.33, 4.78, 4.98, and 5.57 log₁₀, respectively. Virus was recovered from all treatments through day 8 pc. Lectin-free and LCA treated vaccinates ceased virus
Table 12. The effect of lectins on the clearance of virus from the nasal cavities of pigs immunized with pseudorabies virus subunit vaccine with adjuvant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N^b</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lectin-free</td>
<td>15</td>
<td>0.00</td>
<td>3.12</td>
<td>4.33</td>
<td>2.55</td>
<td>0.14</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.00</td>
<td>+0.43</td>
<td>+0.41</td>
<td>+0.40</td>
<td>+0.08</td>
<td>+0.00</td>
<td>+0.00</td>
<td>+0.00</td>
<td>+0.00</td>
</tr>
<tr>
<td>Lens culinaris agglutinin</td>
<td>12</td>
<td>0.00</td>
<td>3.92</td>
<td>4.78</td>
<td>3.11</td>
<td>0.63</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.00</td>
<td>+0.30</td>
<td>+0.34</td>
<td>+0.54</td>
<td>+0.25</td>
<td>+0.00</td>
<td>+0.00</td>
<td>+0.00</td>
<td>+0.00</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>14</td>
<td>0.00</td>
<td>4.32</td>
<td>4.98</td>
<td>3.70</td>
<td>0.70</td>
<td>0.19</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.00</td>
<td>+0.29</td>
<td>+0.18</td>
<td>+0.37</td>
<td>+0.29</td>
<td>+0.19</td>
<td>+0.00</td>
<td>+0.00</td>
<td>+0.00</td>
</tr>
<tr>
<td>Unvaccinated control</td>
<td>7</td>
<td>0.00</td>
<td>4.13</td>
<td>5.57</td>
<td>4.64</td>
<td>3.31</td>
<td>1.12</td>
<td>0.65</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.00</td>
<td>+0.28</td>
<td>+0.24</td>
<td>+0.45</td>
<td>+0.57</td>
<td>+0.62</td>
<td>+0.44</td>
<td>+0.00</td>
<td>+0.00</td>
</tr>
</tbody>
</table>

^a Day 0 is the day of virus challenge.

^b N equals the total number of pigs surviving virus challenge from all replications.
Figure 5. The effect of lectins on the clearance of virus from the nasal cavities of pigs immunized with pseudorabies subunit vaccine with adjuvant. Day 0 is the day of virus challenge. Treatment groups include: lectin-free — — , N=15; Lens culinaris agglutinin—— , N=12; Concanavalin A—— , N=14; and unvaccinated control—— — , N=7. Points represent the mean of all pigs surviving the virus challenge from all replications.
Table 13. The effect of lectins on the rate of virus clearance of pigs immunized with pseudorabies virus subunit vaccine with adjuvant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>X Slope</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lectin-free</td>
<td>15</td>
<td>-0.350</td>
<td>0.033</td>
</tr>
<tr>
<td>Lens culinaris agglutinin</td>
<td>12</td>
<td>-0.346</td>
<td>0.031</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>14</td>
<td>-0.357</td>
<td>0.030</td>
</tr>
<tr>
<td>Unvaccinated control</td>
<td>7</td>
<td>-0.188</td>
<td>0.054</td>
</tr>
</tbody>
</table>

aN equals total number of pigs surviving virus challenge from all replications.

bThe rate of virus clearance is represented by the slope of the curve described by the equation $y = mx + b$. Slope (m) was calculated by regressing virus titer on time, i.e., days 4, 6, and 8. Day 0 is the day of virus challenge.

shedding by day 10 pc. In contrast, 2 of 14 (14.3%) ConA treated vaccinates shed virus on day 10 pc, and 3 of 9 (33.3%) surviving unvaccinated control pigs were still shedding virus on day 12 pc.
DISCUSSION

The primary objective of this experiment was to determine if the incorporation of lectin in a PRV subunit vaccine would enhance the porcine immune response to the vaccine. Results of the above experiments indicated that neither LCA nor ConA enhanced the immune response of pigs to the subunit vaccine. In fact, the data suggest that both lectins may have actually been slightly immunosuppressive. For example, although no statistically significant differences were observed in the development of the SN antibody response between the treatments, the mean SN antibody titers of lectin-treated pigs were consistently lower by day than the corresponding titers of the lectin-free vaccinates. This trend was apparent between days -14 and 7 pc among pigs inoculated with vaccine without adjuvant (Table 1) and between days -14 and 10 in pigs inoculated with adjuvanted vaccine (Table 3).

Lectin-treated pigs also appeared to be more severely affected clinically than lectin-free pigs following virus challenge. This effect was most apparent between days 4 and 8 pc among treatments receiving adjuvanted vaccine. The mean daily weight gain of the lectin-free vaccinates was 0.440 Kg-day\(^{-1}\) in contrast to 0.298 and 0.297 Kg-day\(^{-1}\) for the vaccinates receiving LCA and ConA, respectively (Table 11). A similar trend was also evident in the virus shedding patterns of the adjuvanted treatments between days 4 and 8 pc. During this period the overall mean virus titer shed daily by the lectin-free vaccinates was 2.34 log\(_2\) in contrast to 2.84 and 3.13 log\(_2\) of LCA and ConA treated vaccinates, respectively (Table 12).
Further evidence suggesting that lectins may be immunosuppressive in the pig was provided by studies on the CMI response of pigs receiving adjuvanted vaccine. Prior to virus challenge, the mean virus stimulated cpm of the lymphocyte blastogenesis assay were greater in the lectin-free vaccinates than in vaccinates receiving lectins (Tables 8 and 9). However, this difference was only significant when the ConA treatment was compared to the lectin-free treatment (p<0.01). This comparison suggests that ConA may be more immunosuppressive than LCA. When the CMI data were analyzed by the conservative method, a similar effect was observed. Prior to virus challenge, none of 14 ConA treated pigs were CMI positive. In contrast, 3 of 15 (20%) lectin-free pigs and 2 of 12 (17%) LCA treated pigs were CMI positive.

Although the lectins appeared to be immunosuppressive at the dose levels tested, their overall effect on vaccinated pigs was only minor. The survival rates of the lectin-free and lectin treated pigs were essentially the same and markedly greater than the unvaccinated control pigs. Similarly, the weight gains of vaccinates were significantly greater (p<0.05) than the weight gains of unvaccinated control pigs between days 4 and 8 pc, when the clinical signs of the disease were most pronounced. In addition, an anamnestic antibody response was observed in the vaccinated pigs after virus challenge that was not observed in the unvaccinated controls. Furthermore, PRV specific CMI responses were detected in vaccinates, but not in unvaccinated control pigs following virus challenge. The overall mean virus stimulation indexes, calculated from the post-challenge data summarized in Tables 8 and 9, were 2.77,
2.65, and 1.91 for the lectin-free, LCA, and ConA treatments, respectively. In contrast, the overall mean virus stimulation index for the unvaccinated control treatment was only 1.01. When the CMI data were analyzed by the conservative method, 5 of 15 (33%) lectin-free pigs, 6 of 12 (50.0%) LCA treated pigs, and 5 of 14 (36%) ConA treated pigs were CMI positive two weeks after the virus challenge. No CMI response was detected by the conservative method in unvaccinated controls at this time. These differences in the immune and clinical responses of vaccinated and unvaccinated pigs, indicate that the adjuvanted PRV subunit vaccine sensitized the porcine immune system to PRV in the presence or absence of LCA or ConA.

The slight immunosuppressive effect of lectins observed in this study, may have been due to the concentration and time of lectin administration. Egan et al. demonstrated that ConA could enhance the immune response to an antigen if given three days prior to administration of the antigen. In contrast, ConA significantly depressed the antibody response of mice to sheep red blood cells if it was given at the same time and by the same route.\textsuperscript{10} The mechanism by which such immunosuppression occurs \textit{in vivo} has not been clearly defined. The normal development of a specific immune response depends on the interaction of antigen presenting macrophages and helper T (T\textsubscript{h}) lymphocytes.\textsuperscript{3} This interaction is facilitated through the binding of major histocompatibility complex and antigen specific receptors. As a result, interleukin-2 is produced which facilitates the expansion of B and T lymphocyte clones. The B lymphocytes further differentiate into
antibody producing plasma cells. Some investigators believe that B lymphocyte differentiation must be preceded by capping of membrane associated immunoglobulin molecules around an immunoglobulin-antigen complex. The duration and intensity of the immune response is also thought to be limited by suppressor T (T_s) lymphocytes. Consequently, any agent that induces T_s lymphocyte formation, or interferes with the expression or mobility of specific lymphocyte membrane receptors, can be expected to suppress the immune response. In vitro studies have demonstrated that ConA can stimulate the formation of T_s lymphocytes, \(^1\) as well as interfere with the expression, \(^8\), \(^14\) and the mobility of membrane bound immunoglobulin receptors. \(^13\), \(^33\)

The reduced immune response observed in the lectin treated pigs may not have been due to a direct effect of lectins on lymphocytes as described above. The antigen dose used in the present study was selected because it approached the minimal amount necessary to induce an immune response, \(^22\) and therefore any enhancement of the immune response of the pigs would have been more easily observed. Consequently, the immune response of the pigs may have been reduced if the amount of antigen available was reduced even slightly by complexing with either of the lectins. It is interesting to note that pigs which received ConA appeared to have a weaker immune response than pigs that received LCA. This difference may have been due to the fact that ConA binds glycoprotein 50 times stronger than LCA.\(^{29}\)


SECTION II.

THE EFFECT OF PSEUDORABIES VIRUS IMMUNE SERUM ON THE IMMUNE RESPONSE OF PIGS TO A PSEUDORABIES SUBUNIT VACCINE
The effects of immune serums on the immune response of pigs to a pseudorabies virus (PRV) subunit vaccine were evaluated utilizing 18 pigs. Fifteen pigs were randomly assigned to three groups, i.e., early immune serum, late immune serum, and normal serum. Three pigs served as sentinel controls. Neither the early or late immune sera markedly enhanced the immune response of the pigs. However, the early immune serum appeared to have had a positive effect as compared to the normal or late immune sera.

Early and late immune serums were collected on days 7 and 21 post-infection, respectively, from untreated pigs that had been experimentally infected with PRV. Normal serum was collected from the same pigs prior to the challenge. The normal serum and early immune serum were negative for serum-virus neutralizing (SN) antibody, while the late serum had a SN antibody titer of 3.0 log₂. The experimental regime consisted of two doses of vaccine. For the first dose, five ml volumes of the serums were administered with 100 ug of viral glycoproteins sc. A second dose containing the viral glycoproteins alone was given two weeks later. Three weeks after the second dose of vaccine, i.e., day 0, the pigs were challenged with 10^7.3 PFU of virulent PRV. The effects of the treatments were assessed by comparing the SN antibody response and the survival rates of the pigs post-virus challenge (pc).

Prior to day 7 pc, the mean SN antibody titers of the treatment groups were essentially zero. The mean SN antibody titer of the late
immune serum treatment was significantly (p<0.01) depressed on day 7 pc as compared to the normal serum and early immune serum treatment groups. Mean SN antibody titers on day 7 pc were 3.00±0.82, 3.00±0.7, and 1.40±0.24 log₂ for the normal, early, and late immune serum treatments, respectively. However, only 2 of 5 (40%) pigs treated with the normal immune serum survived through day 7 pc. In contrast, all five pigs of the early and late immune serum treatments survived through day 7 pc.
Pseudorabies is an economically important disease of swine.\textsuperscript{22} Estimated losses from reduced weight gains, reduced conception rates, abortions, and deaths due to pseudorabies cost the Iowa swine industry $33.9 million in 1981.\textsuperscript{2} In 1985, the cost to the Iowa swine industry was estimated to be $107 to $117 million if sales restrictions on pseudorabies virus (PRV) infected breeding stock were included.\textsuperscript{6} Major efforts to control the spread of PRV depend on vaccination and management.\textsuperscript{9, 19} Vaccination of pigs with attenuated or inactivated virus vaccines is effective.\textsuperscript{12, 23} However traditional vaccines prevent the free movement of swine because PRV infected vaccinated pigs cannot be differentiated from non-infected vaccinated pigs.\textsuperscript{13} This disadvantage can be overcome with the use of subunit vaccines for PRV which would permit the serological identification of virus infected pigs by using non-vaccine viral components as diagnostic antigen.\textsuperscript{15}

In 1984, Platt described an effective PRV subunit vaccine that consisted of viral envelope glycoproteins.\textsuperscript{14} The viral glycoproteins were extracted by lectin affinity chromatography from detergent solubilized membranes of virus infected cells.\textsuperscript{13} As little as 16 \textmu g of the viral glycoproteins were shown to induce a protective immune response.\textsuperscript{14} The subunit vaccine was also found to be free of an early non-structural viral glycoprotein that was subsequently used as a diagnostic antigen to identify virus infected subunit vaccinated pigs.\textsuperscript{16}
Although the viral glycoproteins provide protection at relatively low dose levels, the extraction method may prove too expensive for commercial production. The vaccine could be made more economical if the antigen dose required to induce protection could be reduced. This goal might be achievable through the use of immunomodulating agents that would enhance the immune response of pigs to the PRV subunit vaccine.

Several groups of investigators have demonstrated that early immune serum given separately or in combination with specific antigen can influence the immune response to that antigen. Henry and Jerne clearly demonstrated that the primary immune response of mice to sheep red blood cells (RBC) was increased 15 fold by pretreating mice with 19s (IgM) anti-sheep RBC antibodies. Dennert et al. demonstrated the same effect by either pretreating mice with IgM or combining IgM with the sheep RBC. Lehner et al. were also able to enhance the antibody response of mice to sheep RBC by pretreating with sheep RBC specific monoclonal IgM. The following study was done to determine if the immune response of pigs could also be enhanced by incorporating immune serum with the PRV subunit vaccine.
Pseudorabies virus strain BE was used both as the source of vaccine antigen and for pig challenge. The virus was originally isolated from a dog that died of pseudorabies\textsuperscript{17} and was subsequently plaque purified three times in Madin Darby bovine kidney (MDBK) cells. Prior to its use as a source of subunit vaccine antigen, the strain had been passaged a maximum of 14 times through MDBK cells and seven times through porcine kidney (PK) la cells. To insure that the strain maintained its virulence for challenge, it was back passaged once through a pig, reisolated in MDBK cells, and passed once in PK la cells. The virus titer was determined by a plaque assay method\textsuperscript{4} and stored in aliquotes of tissue culture growth media at \(-70\) C.

Porcine kidney la cells were obtained from the Veterinary Medical Research Institute (Iowa State University, Ames, IA). Madin Darby bovine kidney cells were obtained from the National Animal Disease Center (Ames, IA). Both cell lines were maintained in this laboratory in excess of 10 years and propagated at 37 C in a 5% CO\textsubscript{2} humidified incubator. Growth medium consisted of Eagle's minimum essential medium (MEM) with Earle's salts and L-glutamine (Gibco Laboratories, Grand Island, NY) supplemented with 10% heat inactivated fetal calf serum and contained 100 units penicillin, 100 ug streptomycin and 3 ug amphotericin B per ml.
Experimental Animals

Four to eight-week-old pigs were obtained from a secondary specific pathogen free herd maintained at the College of Veterinary Medicine (Iowa State University, Ames, IA). The pigs were maintained on a Ralston Purina (St. Louis, MO) standard grower ration.

Serum-virus Neutralizing Antibody

Duplicate, serum-virus neutralizing (SN) antibody titer determinations were performed by the State Veterinary Diagnostic Laboratory (College of Veterinary Medicine, Iowa State University, Ames, IA), according to a modification of the microtiter method described by Hill et al. 8 Briefly, two-fold dilutions of heat inactivated test sera were made so that 50 ul of diluted serum remained in each well of a 96-well microtiter plate. Subsequently, 50 ul of MEM containing approximately 300 TCID$_{50}$ of PRV were added to individual wells with the exception that serum control wells received 50 ul of MEM only. The plates were gently shaken and incubated for one hour at 37 C in a 5% CO$_2$ humidified incubator. Following incubation, 50 ul of MEM containing approximately $10^{5.6}$ MDBK cells were added to each well and the plates reincubated for 48 hours. Serum-virus neutralizing titers were expressed as the reciprocal of the highest dilution that produced complete neutralization of virus ($\log_2$).
Production of Immune Serum

Immune serum pools were made from unvaccinated control pigs from previous studies that survived intranasal virus challenge of $10^{7.3}$ PFU of PRV. Normal serum was collected from pigs prior to the virus challenge. Early and late immune serums were collected 7 and 21 days post-virus challenge (pc), respectively. Normal serum and early immune serum pools were negative for SN antibody. The SN antibody titer of the late immune serum pool was $3.0 \log_2$.

Subunit Vaccine Preparation

Pseudorabies virus glycoproteins were prepared as described by Platt, quantitated by the dye binding method of Bradford and used as subunit vaccine. The experimental regime consisted of two doses of vaccine. The first dose consisted of 100 ug of non-adjuvanted viral glycoproteins combined with five ml of normal serum, or early or late immune serum. The antigen-immune serum mixtures were incubated at 37 C for one hour to facilitate the formation of immune complexes. The second dose consisted of the 100 ug of non-adjuvanted viral glycoproteins alone.

Experimental Design

Eighteen weanling pigs were randomly divided into three treatment groups of five pigs each and an uninoculated control group of three pigs.
All treated pigs were inoculated subcutaneously with an initial dose of antigen combined with normal, early immune, or late immune serum. A second dose of antigen without serum was given 14 days later. All pigs were nasally challenged with $10^{7.3}$ PFU of virus 21 days after the second antigen dose.

The effect of serum treatment was evaluated by comparing the mean SN antibody response of the treated groups during the immunization period, and following virus challenge. The survival rates of the three treatments were compared after virus challenge. Data was analyzed by an analysis of variance procedure (ANOVA) using a randomized complete block design. The data was blocked by day in the ANOVA procedure to control the day to day variability in the test results. Survival rates were compared by chi-square analysis.
RESULTS

The mean SN antibody titers of the treatments are summarized in Table 1. No SN antibody was detected in any pig prior to day 7 pc. On day 7 pc, the mean SN antibody titers were 3.00 ±0.82, 3.00 ±0.71, and 1.40 ±0.24 log₂ for the normal, early immune, and late immune serum treatments, respectively. The SN antibody titer of the late immune serum treatment was significantly less (p<0.01) than the normal and early immune serum treatments. All SN antibody titers of the treatment groups were essentially equal beyond day 7 pc. Unvaccinated control pigs remained free of SN antibody until day 7 pc when all surviving pigs had titers of 1.00 ±0.00 log₂. By day 10 pc, the mean SN antibody titer rose to 2.5 ± 0.50 log₂ which was markedly less than the titers of the vaccinated treatments. The absence of a SN antibody response in the unvaccinated control pigs prior to virus challenge, and the absence of an anamnestic response pc indicates that all the pigs were free of PRV until challenged.

The survival rates of the three treatment groups, that received the PRV subunit vaccine with normal, early immune, and late immune serum, were 2 of 5 (40%), 5 of 5 (100%), and 4 of 5 (80%) pigs, respectively. The survival rate of the unvaccinated control treatment was 2 of 3 (67%) pigs. Clinical disease appeared to be more severe in unvaccinated control and normal immune serum treated pigs than in early and late immune sera treated pigs. All death losses in the first two groups occurred between days 4 and 8 pc. In contrast, the one death among pigs treated with late immune serum occurred on day 10 pc.
Table 1. The effect of pseudorabies immune serum on the serum-virus neutralizing antibody response of pigs immunized with a non-adjuvanted pseudorabies virus subunit vaccine

Mean serum-virus neutralizing antibody titer ± SE (log2) by day^a

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N^c</th>
<th>-35</th>
<th>-21</th>
<th>-14</th>
<th>-11</th>
<th>-7</th>
<th>0</th>
<th>4</th>
<th>7</th>
<th>10</th>
<th>14</th>
<th>21</th>
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<tr>
<td>Normal serum</td>
<td>5</td>
<td>0.00</td>
<td>0.00</td>
<td>0.80</td>
<td>0.20</td>
<td>0.00</td>
<td>0.00</td>
<td>3.00</td>
<td>4.00</td>
<td>4.50</td>
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<tr>
<td></td>
<td></td>
<td>±0.00</td>
<td>±0.00</td>
<td>±0.05</td>
<td>±0.20</td>
<td>±0.00</td>
<td>±0.00</td>
<td>±0.82</td>
<td>±0.00</td>
<td>±1.00</td>
<td>±1.00</td>
<td></td>
</tr>
<tr>
<td>Early serum</td>
<td>5</td>
<td>0.00</td>
<td>0.00</td>
<td>0.40</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>3.00</td>
<td>4.10</td>
<td>4.80</td>
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<tr>
<td></td>
<td></td>
<td>±0.00</td>
<td>±0.00</td>
<td>±0.24</td>
<td>±0.00</td>
<td>±0.00</td>
<td>±0.00</td>
<td>±0.71</td>
<td>±0.37</td>
<td>±0.41</td>
<td>±0.19</td>
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</tr>
<tr>
<td>Late serum</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1.40**</td>
<td>4.30</td>
<td>4.88</td>
<td>5.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.00</td>
<td>±0.00</td>
<td>±0.00</td>
<td>±0.00</td>
<td>±0.00</td>
<td>±0.00</td>
<td>±0.24</td>
<td>±0.20</td>
<td>±0.13</td>
<td>±0.13</td>
<td></td>
</tr>
<tr>
<td>Unvaccinated control</td>
<td>3</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1.00</td>
<td>2.50</td>
<td>4.25</td>
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<tr>
<td></td>
<td></td>
<td>±0.00</td>
<td>±0.00</td>
<td>±0.00</td>
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<td>±0.00</td>
<td>±0.00</td>
<td>±0.50</td>
<td>±0.25</td>
<td>±0.00</td>
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</tr>
</tbody>
</table>

^a Day 0 is the day of virus challenge.

^b Normal serum = serum collected prior to virus challenge + vaccine; Early serum = serum collected on day 7 pc + vaccine; Late serum = serum collected on day 21 pc + vaccine; Unvaccinated control = no serum or vaccine.

^c N equals total number of pigs at start of experiment. N = 2 on days 7 through 21 pc for the unvaccinated control and normal serum treatments. N = 4 for the late serum treatment on days 14 and 21 pc.

** p<0.01.
The primary objective of this experiment was to determine if the incorporation of PRV immune serum with a PRV subunit vaccine would enhance the immune response of pigs to the vaccine. The results of this limited study indicate that PRV immune serum did not markedly enhance the immune response. However, the SN antibody response and survival rate of pigs receiving early immune serum suggest that some enhancement of the immune response may have occurred. All five (100%) pigs that received early immune serum with vaccine survived the virus challenge, while only 2 of 5 (40%) pigs receiving normal immune serum with the vaccine survived. The data also indicate that the immune response of pigs which received the late immune serum may have been slightly suppressed as indicated by the SN antibody response. On day 7 pc, the mean SN antibody titer of pigs treated with late immune serum was 1.60 log₂ less than the mean SN antibody titers of pigs treated with either normal or early immune serum (p<0.01).

These results were not unexpected assuming that IgM and IgG were the immunomodulating components of the early and late immune sera, respectively. Several investigators have documented that IgM can enhance the SN antibody response to a specific antigen if the immunoglobulin is presented to the host before or concurrently with the antigen.5, 7, 10 The immunosuppressive effect of IgG, when given prior to or concurrently with antigen has also been documented.7
It is recognized that these conclusions are statistically weak due to the small number of pigs involved in this study. Another deficiency of the study was the lack of knowledge of whether or not the antigen or the antibody was in excess in the inoculum. The ratio of antigen to antibody has been demonstrated to affect the outcome on the immune response. Insufficient antibody may not affect the immune response, while excessive antibody may actually depress the immune response. Also, high levels of IgM may actually suppress the SN antibody response, although the levels of IgM in the preparations were insignificant compared to the dilution achieved in the pigs. Other components of the immune serum may actually have been responsible for the observed effects of the treatments. Additional experimentation will be required in order to determine whether or not immune serum can be used as an economical and efficient immunopotentiating agent.
BIBLIOGRAPHY


SUMMARY

Two lectins, Concanavalin A (ConA) and Lens culinaris agglutinin (LCA), and pseudorabies early and late immune sera were evaluated for their effect on the porcine immune response to a pseudorabies subunit vaccine. The viral glycoproteins of the subunit vaccine are expensive to produce relative to attenuated and inactivated vaccines. The cost of the subunit vaccine could be reduced if a way of enhancing the porcine immune response to the viral glycoproteins was developed. A reduction in the dose of antigen required for the subunit vaccine to induce a protective immune response to PRV would subsequently reduce the cost of the vaccine. The lectins and immune sera did not enhance the porcine immune response to the pseudorabies subunit vaccine. In fact, the ConA and late immune serum treatments appeared to suppress the porcine immune response. However, the early immune serum appeared to confer a degree of protection, although that experiment was only a preliminary study.

The 150 ug of lectin, ConA or LCA, were incorporated into the pseudorabies subunit vaccine, containing 15 ug of viral glycoproteins, in the presence and absence of Freund's incomplete adjuvant. Mean serum-virus neutralizing (SN) antibody, cell-mediated immunity (CMI) and the clinical response of the pigs post-virus challenge (pc) were used to evaluate the different treatments. The LCA had no observable effect on the SN antibody or CMI, while the ConA appeared to reduce the SN antibody prior to day 7 pc and the post-challenge CMI. The mean SN antibody titers, on day 7 pc, were $4.62 \pm 0.42$, $4.00 \pm 0.35$, and $3.11 \pm 0.28 \log_2$.
for the adjuvanted lectin-free, LCA, and ConA treatments, respectively. A similar trend was observed in the non-adjuvanted study. The overall mean virus stimulation indexes for the post-challenge CMI were 2.77, 2.65, and 1.91 for the lectin-free, LCA, and ConA treatments, respectively. A stimulation index greater than 2.0 is considered to be a positive CMI. The CMI was not measured in the non-adjuvanted study.

The clinical response of the pigs yielded further evidence that the lectins may have reduced the porcine immune response. This effect was most apparent between days 4 and 8 pc, when the symptoms of the disease were most severe. The mean daily weight gains for this period were 0.440 ± 0.097, 0.298 ± 0.162, 0.297 ± 0.035 Kg-day⁻¹ for the adjuvanted lectin-free, LCA, and ConA treatments, respectively. In addition, the ConA treatment consistently shed virus 48 hours longer and in larger quantities than the lectin-free or LCA treatments.

The five ml of pseudorabies immune sera, normal, early, or late, were incorporated into the pseudorabies subunit vaccine, containing 100 ug of viral glycoproteins, in the absence of adjuvant. The normal and early immune sera were seronegative for SN antibody to PRV, while the late immune serum was seropositive with a SN antibody titer of 3.0 log₂. Mean SN antibody and survival of the virus challenge were used to evaluate the treatments. Normal and early immune sera had no apparent enhancing effect on the SN antibody response of the pigs to the pseudorabies subunit vaccine, while late immune serum appeared to suppress the SN antibody response pc. The SN antibody titers on day 7 pc were 3.00 ±0.82, 3.00 ±0.71, and 1.40 ±0.24 log₂ for the normal, early,
and late serum treatments, respectively. However, of the five pigs that started the experiment in each treatment, only two of the normal, and four of the late immune serum treated pigs survived the virus challenge as compared to 100% survival of the early immune serum treated pigs. The small number of pigs involved in the immune serum study requires that additional experimentation be performed to determine whether or not immune serum can be used as an economical and efficient immunopotentiating agent of the PRV subunit vaccine.
LITERATURE CITED


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ACKNOWLEDGEMENTS

I would like to thank my major professor, Dr. Kenneth Platt, for his enthusiastic support, time, and encouragement throughout these studies. The help and advice of my committee members, Dr. Donald Graves and Dr. Merlin Kaeberle, are also greatly appreciated.

Several individuals provided invaluable technical assistance and help in handling the animals. These included Randy Atkinson, Cindy Brough, Nancy Gorley, Martha Jeffrey, Ervin Johnson, Melinda Tolle, Carol Toussaint, and Anne Yeung. The cooperation and help of fellow graduate students is also appreciated. I thank Dr. Kramer, Verla Schoelkoph, and Heidi Anderson for the unabridged use of one of the word processors. Steve Pendry, Craig McClanahan, and the staff of Biomedical Communications department provided invaluable assistance in the Telegraf computerized production of the figures within the text. Dr. Jerome Sacks was very accommodating and helpful with his suggestions on handling the statistical calculations. Without my wife, Wanda, the chores of typing, proof-reading, and correcting the manuscript would have been overwhelming. I appreciate her more now than ever.

Thank you everyone.