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Recombinant fowlpox virus vaccines for poultry

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Summary The intensive poultry industries rely heavily upon the use of vaccines for disease control. Viral vector based vaccines offer new avenues for the development of vaccines for effective disease control in poultry. Techniques developed for the construction of recombinant vaccinia viruses have been readily adapted to the construction of recombinant viruses based on fowlpox virus (rFPV). The ability to insert several genes into the large genome of fowlpox may enable the development of multivalent vaccines and vaccines incorporating immune response modifiers such as lymphokines. Newcastle disease, avian influenza, infectious bursal disease and Marek's disease antigens expressed by rFPV have been shown to be effective vaccines in poultry. None appear, however, to provide a substantial improvement in vaccine efficacy. Recombinant FPV will be a valuable adjunct to conventional vaccines currently in widespread use. Whether rFPV or other vector based vaccines can circumvent the problems of vaccination in the presence of high maternally derived antibodies is yet to be resolved. The observation that avipoxvirus recombinants may be suitable for the vaccination of non-avian species provides an added dimension to vaccines based on FPV or other avipoxviruses. Recombinant FPV will find a useful role in poultry disease control when used in conjunction with conventional vaccines.

Key words: fowlpox virus, poultry, recombinant fowlpox virus, vaccines.

Introduction

The intensive poultry industries (for meat and egg production) rely heavily upon the use of vaccines for disease control. Because of the cost competitive nature of these industries vaccines must be very cheap to purchase and administer. In addition, to provide effective protection against disease the vaccines must be applicable at an early age — preferably at 1 day of age when the cost of vaccine administration can also be kept low. One day old birds are exposed to heavily contaminated environments immediately they are placed in production sheds. If vaccines cannot be delivered at 1 day of age, it is desirable that they be deliverable by mass administration methods at later times (e.g. via the drinking water or by aerosol spray). The majority of vaccines are used to control viral diseases and to be cost effective are based on live attenuated viruses. In layer and breeder birds, where it is desirable to re-vaccinate to maintain protection and to generate high levels of yolk sac antibodies to protect hatching chickens, the primary live virus vaccines are usually followed by inactivated adjuvanted vaccines. The laying bird may receive as many as 10 to 20 vaccinations during its production life.

The classical poultry pathogens of avian influenza (AI) and Newcastle disease viruses (NDV) remain serious disease threats where they occur. Fortunately Australia has remained free of virulent NDV since the 1930s. We have, however, seen three outbreaks of AI in the past in Victoria. The multiple serotype

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variants of infectious bronchitis virus (IBV) that have emerged in Europe and North America pose difficult vaccination problems. Marek's disease virus (MDV) continues to be effectively controlled by vaccination with the related herpes virus of turkeys (HVT) or type II attenuated MDV. Application of the type II attenuated MDV vaccines is particularly difficult since the virus is cell-associated, expensive to produce and must be transported and stored in liquid nitrogen. Infectious bursal disease virus (IBDV) now poses an increasing threat to our poultry industries. Past strains have been responsible for immunosuppression due to damage to the bursa of Fabricius with subsequent disease being cause by secondary infection with other pathogens. Virulent strains of IBDV have emerged in the past few years in Europe and Asia which appear to cause significant mortalities on their own. Fortunately, Australian poultry flocks have remained free of virulent IBDV strains. Avian coccidiosis remains the major parasitic disease of poultry whose control is totally reliant upon the prophylactic use of chemicals. Although there have been very substantial efforts to identify coccidial antigens suitable for vaccine use, so far none have been found effective.

The development of recombinant vaccinia viruses[1,2] for the expression and delivery of vaccine antigens to mammalian species soon led to the realization that other host-specific viruses would also be suitable vectors for vaccine delivery. A number of viruses of poultry are being developed as potential vaccine vectors. Poxviruses, herpesviruses and adenoviruses appear to be the most attractive candidate vector viruses.[3] Our work has concentrated on the development and evaluation of recombinant fowlpox viruses (rFPV) to deliver vaccine antigens to poultry. In this paper we review the efficacy of rFPV as poultry vaccines and attempt to compare their efficacy with currently available vaccines.

Construction of FPV recombinants

The strategies adopted for the construction of rFPV are based on the approaches developed for vaccinia virus recombinants.[4,5] Several sites have been used for the insertion of vaccine antigen genes including the thymidine kinase (TK) gene,[6] the terminal inverted repeat regions,[7] the site homologous to the vaccinia virus TK region from which the FPV TK appears to have been translocated[8] and several sites identified by shot-gun insertion strategies.[9] It is necessary to provide a poxvirus promoter to ensure expression of the inserted vaccine antigen. Promoters derived from FPV and other poxviruses all appear to operate to some level in rFPV.[10] To facilitate rFPV construction and identification, dominant selectable makers have been developed.[6] The β-galactosidase gene has been extensively used to allow rapid identification of recombinants using a chromogenic substrate.[8] Details of rFPV construction strategies have recently been reviewed and will not be dealt with in detail here.[11]

As many of the rFPV reported have been constructed using different FPV parent strains, insertion sites and promoters it is very difficult to determine the impact of these features upon their efficacy for vaccination of poultry. For example, promoter strength may be an important issue regarding the quantity of vaccine antigen delivered[10] and insertions in some regions could lead to attenuation of the rFPV in comparison with the parent FPV strain used for recombinant construction. The residual virulence of the rFPV could have a significant impact upon vaccine efficacy.

Recombinant FPV vaccines

Infectious bursal disease vaccine

The primary target of IBDV (a birnavirus) infection is the bursa of Fabricius. Infection of young birds causes depletion of the germinal follicles leading to immunosuppression and susceptibility to other infections. Current vaccines are based on low virulence field strains as live vaccines or on inactivated virus derived from infected bursae. Protection of the young chick during the first 3–5 weeks of life relies on generation of high antibody titres in the hen and transfer of these to the hatching chick via the yolk sac of the egg. Current vaccines do not generate protective immunity in day old birds, particularly in the presence of the

high levels of transferred maternal antibodies. Highly virulent strains of IBDV have emerged in Europe and Asia.[12] Unlike the previous strains, these highly virulent strains of IBDV directly cause significant levels of mortality in young birds.

The VP2 protein of IBDV, previously shown to induce protective immunity in poultry, has been expressed by a rFPV as a fusion protein with β-galactosidase.[13] When used to vaccinate 1 and 14 day old poultry this rFPV protected against mortality induced by the homologous IBDV strain or a highly virulent strain. It did not protect against bursal infection and damage. Our own studies with a rFPV expressing the VP2 protein from the Australian IBDV 002/73 strain have shown that this recombinant induces antibodies to IBDV, albeit at a level lower than an oil adjuvanted, killed IBDV vaccine.[14] This FPV-VP2 recombinant did provide protection against bursal infection and damage by the homologous 002/73 strain in poultry vaccinated at 1 or 21 days of age. The level of protection was less than that provided by the oil adjuvanted, killed IBDV vaccine. Neither of the reported recombinant FPV-IBDV vaccines appears to be as effective as currently available vaccines.

Infectious bronchitis vaccine

IBV (a coronavirus) is the cause of an acute, highly infectious respiratory disease in chickens. In Australia infection is also associated with a severe nephrosis. Production losses are associated with mortality, and in laying birds egg production drops occur with decrease in egg quality. Attenuated live viruses are used as vaccines for disease control. The complex serological and cross protection relationships of IBV isolates complicates the selection and use of strains for vaccination. Field experiences suggest vaccine failures are due to the emergence or selection of serotypes different from the vaccines in use. There is a need for improved IBV vaccines to provide broad protection and protection against emerging strains.

The peplomer or spike protein of IBV expressed by vaccinia virus induced low level antibody responses in vaccinated mice.[15] There have been no reports of successful use of rFPV expressing the peplomer antigen to protect chickens against challenge with IBV. We have successfully expressed the peplomer gene of IBV M41 strain in FPV. Expression of the antigen by both the vaccinia P.L11 promoter and the P.E/L FPV promoter was demonstrated by immunofluorescence. We have failed, however, to demonstrate induction of antibodies or protective immune responses in poultry vaccinated with these rFPV (D. B. Boyle & H. G. Heine unpubl. obs. 1993). Because of the multiple and complex serotypes of IBV and the negative results with FPV recombinants to date, the prospects for new IBV vaccines based upon viral vectors appear remote at this time.

Newcastle disease vaccine

Newcastle disease is considered to be the most significant exotic disease threat to the Australian poultry industries. Virus strains range from highly virulent to avirulent, however there is no strain specific immunity. All NDV vaccines protect against the different pathotypes. The strains of NDV present in Australian poultry flocks cause very minor or no disease problems. A wide variety of attenuated live and killed NDV vaccines are used outside Australia with the live attenuated vaccines being the most cost effective to deliver because they are very cheap to produce.

Several research groups have reported the construction and evaluation of rFPV[7,16–20] and pigeonpox virus[21] expressing NDV genes and protection has been demonstrated with rFPV expressing either the haemagglutinin-neuraminidase (HN) or fusion (F) gene. Protection provided by FPV-F recombinants appeared better than that provided by FPV-HN recombinants.[20] The protection provided by FPV-NDV recombinants was not as good as that induced by conventional NDV vaccines (D. B. Boyle unpubl. obs. 1993). The combined use of conventional NDV and rFPV-NDV vaccines generated better immune responses than either alone.[22]

Avian influenza vaccine

Classical fowl plague is caused by AI viruses predominantly of the H5 or H7 haemaggluti-

nin (HA) subtypes. Outbreaks of AI have occurred in southern Australia on three occasions in the past: 1976, 1986 and 1992. Disease control has relied upon quarantine and movement controls, and eradication has been achieved by slaughtering of all birds on infected farms. There are currently no commercially applicable vaccines available for the control of AI. Recombinant FPV expressing the HA subtype H5 and the nucleoprotein (NP) of AI have been evaluated in poultry for vaccine efficacy.[23–27] The HA H5 subtype expressed by FPV provided very good protection against AI disease in chickens and turkeys. In some but not all challenge situations AI virus replication and shedding was also substantially reduced. Protection was subtype specific with FPV-H5 recombinants protecting against H5 subtype viruses but not against H7 subtype virus. Immunization with a rFPV expressing the cross-reactive NP antigen did not provide protective immunity against AI. The results show that vaccination protection against AI is mediated by antibodies directed against the HA. Protection is provided against disease, but substantial virus replication and shedding can occur in the vaccinated and challenged bird. The failure of the NP to induce protection in poultry, in spite of its cross-reactivity across subtypes, reinforces the observations made in the mouse influenza model, where vaccinia virus NP recombinants failed to provide protection against disease and infection even though they were able to induce cross-reactive cytotoxic T lymphocytes.[28–30]

Marek's disease vaccine

All of the currently available vaccines against MDV, with the exception of those based on the related HVT, are delivered as cell-associated viruses requiring storage and transport on liquid nitrogen. A rFPV expressing the glycoprotein B of MDV has been shown to induce neutralizing antibodies to MDV, to reduce levels of cell-associated viraemia and to protect against lymphoma and death caused by challenge with homologous and heterologous MDV strains, including highly virulent strains.[31] Provided the FPV-MDV recombinant vaccines can provide protection against MDV in field applications, they may represent significant improvements over currently available vaccines.

Influence of maternally derived antibodies on vaccination with FPV recombinants

To provide protection as early as possible and to keep the costs of vaccine delivery as low as possible, the preferred time for primary application of many poultry vaccines is at 1 day of age, that is, when the birds leave the hatching incubators and immediately prior to placing in brooding and growing sheds. Vaccines applied at this age face two significant problems. The immunological immaturity of the day old birds reduces vaccine efficacy and maternally derived antibodies adsorbed via the yolk sac have the potential to interfere with active immunization at this early age. Some disease control strategies deliberately endeavour to maximize maternally derived antibodies by hyperimmunization of the laying hen to protect the newly hatched bird during the first 3–5 weeks of age. Vaccination is then carried out when maternally derived antibodies have waned sufficiently to no longer interfere with active immunization. In practice the proper timing of this vaccination is difficult since waning maternally derived protection can lead to a period in which many birds in a flock are susceptible to infection prior to protection being conferred by active immunization.

Those few studies that have been reported with vaccinia virus recombinants have shown that passively acquired antibodies to the co-expressed antigen inhibit the generation of active immunity to that antigen. Serum from influenza immune mice transferred to mice subsequently immunized with a vaccinia-influenza HA recombinant suppressed the antibody response to the HA.[32] Vaccination of young infants who possess maternally derived antibodies to respiratory syncytial virus (RSV) with conventional RSV vaccines produces poor responses to the protective RSV glycoprotein antigens in spite of extensive replication of the virus. Cotton rats receiving hyperimmune serum to RSV glycoproteins prior to vaccination with vaccinia virus recombinants expressing RSV glycoproteins had suppressed antibody responses and were more susceptible to infection than control animals. Suppression was both qualitative and quantita-

tive with the total antibody responses to the glycoproteins being suppressed and the suppression was selective for epitopes involved in induction of neutralizing antibodies.[33–34] Passively acquired antibodies to the vector may also lead to suppression of immune responses to the vector and co-expressed antigen.[32] This immunosuppression appears to operate on both the B and T cell responses and may extend beyond the time after which residual maternally derived antibodies are no longer detectable.[35] Since application of poultry vaccines to 1 day old chickens is crucial to the successful control of disease in many cases, the impact of maternally derived antibodies upon vaccine efficacy warrants further detailed investigation.

Vaccination of non-avian species

The proposal that recombinant avipoxviruses may be suitable for the vaccination of non-avian species provided an added dimension to vaccines based on FPV or other avipoxviruses.[36] Recombinants expressing rabies and measles glycoproteins have been shown to be protective in non-avian species.[37–40] Recombinants based on canarypox were shown to be more effective than rFPV.[38] One of these recombinants has been evaluated in a Phase I human vaccination trial.[39] Avipoxvirus infection of mammalian cell lines and animals fails to produce infectious progeny virus, yet expression of early gene products occurs, and so provides a substantial safety advantage over recombinants based upon viruses generating productive infections.

Conclusions

Those studies reported to date on rFPV for vaccination of poultry suggest that they will be a valuable adjunct to conventional vaccines currently in widespread use. Some, such as AI, may provide a vaccine where none has existed before and others, such as MDV, may be significantly cheaper to produce and deliver. Where routes of inoculation have been compared, the most efficacious has been by wing web inoculation.[27] This has the disadvantages of being labour intensive, costly and best applied at 1 day of age. Mass vaccination strategies (e.g. via drinking water or aerosol) do not appear to be applicable to rFPV unless suitable FPV parent strains can be found. Vaccination and challenge experiments have shown that rFPV can induce protection against overt clinical disease. Their efficacy has not been directly compared with conventional vaccines and the longevity of responses has not been determined. With many poultry diseases, protection against disease alone is not sufficient to prevent production losses as infection without overt clinical disease may still lead to very significant production losses. Recombinant FPV may find a useful role in poultry disease control when used in conjunction with conventional vaccines. In the case of FPV-NDV recombinants the combined use of conventional and rFPV vaccines generated better immune responses than either alone.[22] The issue of whether rFPV or other vector based vaccines can circumvent the problems of vaccination in the face of high maternally derived antibodies is yet to be resolved but it remains a central issue in the application of vector based vaccines for poultry disease control. Other vector viruses (e.g. adenoviruses and herpesviruses) may be more suitable for mass administration in the face of maternally derived antibodies. However these vectors have yet to be fully established and evaluated. Recombinant FPV based vaccines do offer the potential to develop multivalent vaccines.

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