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Viral Vectors for Veterinary Vaccines

MICHAEL SHEPPARD

Animal Health Biological Discovery, Pfizer Central Research, Groton, Connecticut 06340

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I. Introduction and Background

Numerous reviews have described the use of viral vectors for possible vaccine delivery (e.g., Cavanagh, 1985; Sheppard and Fahey, 1989; Wray and Woodward, 1990; Graham and Prevec, 1992; Boyle and Heine, 1993; Hilleman, 1994; Martin, 1994; Dorner, 1995; Babiuk et al., 1996; Perkus and Paoletti, 1996). However, in this review I will focus solely on the use of viral vectors for delivery of veterinary vaccines. It is without question that vaccination plays an essential role in veterinary medicine, providing the major and often the only prophylactic approach for the control of infectious diseases. In spite of the vast array of currently available vaccines veterinarians and the livestock producers continue to express the need for vaccines that not only maintain the best features of killed or subunit vaccines (such as safety) as well as the best features of conventional modified live vaccines (such as...
efficacy) but improve on them. As well as the need for continual improvement of vaccines there exists a need for new vaccines either to new diseases (e.g., chicken anemia virus or porcine reproductive and respiratory syndrome virus) or to old diseases for which vaccines are not available or no longer meet the requirements of the end user (e.g., bovine virus diarrhea virus vaccines). As well as new vaccines there is also need for vaccines with special features that allow potential customers to design disease control programs that suit their specific needs on top of offering greater safety and improved protection. The design and construction of these new veterinary vaccines is a major challenge facing the field of vaccinology. With the continued demand of improving vaccines and producing new ones it is easier for potential vaccine candidates to fail to meet the increased level of requirements that are expected. The failure of some vaccines can result from problems associated with delivery, such as insufficient or no induction of the appropriate protective immune response. The development of delivery systems to produce vaccines that are more effective, offer greater safety, are convenient to administer, and are compatible with customer practices is part of the challenge for vaccinologists. The development of safe and convenient live viral vectors for the delivery of veterinary vaccines is one possible way of meeting some of these challenges. Recombinant DNA technology has allowed more detailed characterization of the genetic organization of many viruses to such an extent that regions suitable for insertion of foreign genetic material have been identified. This has resulted in the development of numerous types of viral vectors from a wide variety of viral families. Some of these viral vectors have been developed with the potential for delivering and expressing gene(s) from a foreign pathogen and so act as a vaccine vector (Table I). The viral vector is often genetically attenuated or cannot complete its replication cycle in the animal to be immunized, and thus produces no clinical disease. Although initially the majority of viral vector development centered around poxviruses, especially vaccinia (Panicali and Paoletti, 1982; Macket et al., 1982), it was not long before viral vector development witnessed a virtual explosion in the types of viruses developed into vectors. These included herpesviruses (Post et al., 1982), adenoviruses (Berkner and Sharp, 1982), retroviruses (Wei et al., 1981), papovviruses (Southern and Berg, 1982), polyoma virus (Fried and Ruley, 1982), picornaviruses (Kitson et al., 1991), Semliki Forest virus (SFV; Zhou et al., 1994), Sindbis virus (Pugachev et al., 1995), and even some plant viruses (Jagadish et al., 1996; Dalsgaard et al., 1997).
TABLE I

CHARACTERISTICS OF THE MORE COMMON VIRUS GROUPS USED AS VECTORS

| Characteristics | Pox viruses | Adenoviruses | Herpes viruses | Retroviruses |
|-----------------|-------------|--------------|---------------|--------------|
| Genome          | 180–300 kb  | 30–45 kb     | 150–200 kb    | 9.2 kb       |
| Max. Insert     | >30 kb      | >5 kb        | 30 kb         | 8 kb         |
| Max. Titer      | $10^7$–$10^9$ | $10^8$–$10^{11}$ | $10^9$–$10^8$ | $10^9$–$10^9$ |
| Administration  | Scarification/injection | Injection/aerosol/oral | Injection/water | Injection |
| Safety          | Problems with immuno-suppressed | Inflammation | Latency | Genomic insertion |
| Background expression by vector | Yes | Yes | Yes | No |

**II. Viral Vector Construction**

Greater understanding of the structure and function of a wide range of viruses at the genetic level has opened up ways of designing novel viral vaccine vectors which should improve the quality and effectiveness of some future vaccines as major prophylactic tools. Viral vaccine vectors have really developed from a greater technological understanding of viruses at the genetic level, where today they have become a viable alternative strategy as one method for the delivery of vaccines.

The concept of viral vectors was first highlighted by Bernard Moss and others in the early 1980s (Mackett et al., 1982; Panicali and Paoletti, 1982), where they showed that vaccinia virus could be engineered to carry and express foreign genes (Panicali and Paoletti, 1982; Mackett et al., 1982). From the time when Moss and others first demonstrated that vaccinia virus could be developed as a vector for the expression of foreign genes, the technology has been exploited to apply to a variety of virus families as well as a variety of foreign genes including those that encode antigens from pathogens. As a result both DNA and RNA viruses have been developed as viral vaccine vectors (Table I).

To produce viral vaccine vectors it is first necessary to study the genome of the vector to a stage of understanding where at least one region suitable for insertion of foreign genetic material has been iden-
tified. Second, genes from pathogens that encode proteins that will induce an appropriate protective immune response and can be stably integrated into the vector’s genome and expressed need to be identified. Finally, it is necessary to insert the foreign gene(s) in such a way as to ensure the correct and sufficient expression of the foreign gene(s).

The ideal viral vaccine vector would have all or at the very least some of the following features:

- Safe and nonpathogenic for the vaccinate
- Evoke the appropriate protective immune response
- Single host or limited host range
- Stable genome
- No integration into the host genome
- Readily accessible region(s) for insertion of foreign genetic material
- Able to tolerate well insertion of foreign genetic material and expression of foreign gene(s)
- Convenient to deliver and fits with management practices
- Relatively simple and cost effective to produce
- Limited background gene expression by the vector

III. Advantages and Disadvantages of Viral Vectors for Vaccine Delivery

Live viral vectors offer several advantages for vaccine delivery compared to killed, subunit, or conventional modified live vaccines. First, because of the possibility of delivering divalent or even perhaps multivalent vaccines, using a single type of vector can result in a single manufacturing process rather than several and possibly even a single vaccination rather than several. Therefore, vectored vaccines have the potential to be less expensive to the manufacturer and the end user. Because the foreign gene is being expressed in the cells of its natural host, it is expected that any post-translational modifications required will be correct and produce an authentic antigen, as opposed to *Escherichia coli* or baculovirus systems (among others) that do not always produce authentic foreign proteins. Depending on the vector selected it may be possible to deliver the vectored vaccine more conveniently to the mammal or bird by spray or water or some other means rather than by needle injection. Such a mass administration approach may be particularly relevant to the poultry industry. The vector could also be constructed to deliver simultaneously an immunomodulator (e.g., gamma interferon), which could modify the type or
magnitude of the immune response to allow the vaccine to be success-
ful or more successful than it would be otherwise. The vector only
expresses the antigens from the pathogen that are required to elicit a
protective immune response and therefore reduces or eliminates the
chance of disease by being exposed to the whole pathogen as with a
killed or modified live vaccine. Finally, the appropriate viral vectors
will induce both cell-mediated and humoral immune responses and in
some cases are particularly suitable for inducing a local immune re-
response in the mucosal surfaces.

One of the main disadvantages of using viral vectors for vaccine
delivery is that like subunit vaccines each vector can only deliver one
or a relatively small number of foreign antigens to the host animal and
therefore rely on those being able to elicit a completely protective im-
mune response. Also the only antigens that can be delivered are those
that are encoded by nucleic acid. Thus such things as lipopolysac-
charides are not deliverable. With any vector, regardless of type, only a
limited amount of foreign genetic material can be inserted into the
vector’s genome stably and expressed appropriately. One must always
be wary of altered tissue tropisms due to the expression of the foreign
gene(s). Of course the effectiveness of a viral vector is limited by preex-
isting immune response in the animal from prior exposure to the virus
used to construct the vector. Finally, as with all live vaccines there is
the problem of shelf life and compatibility with other vaccine prepara-
tions.

IV. Construction of Safer Viral Vectors for Vaccine Delivery

To produce viral vaccine vectors successfully it is necessary to ensure
that the vector itself does not pose any disease threat to the animal
that receives the vaccination or to the person delivering the vaccine to
the animal. Most often this is achieved by attenuating the viral vector
in some way. Until recent times the means of generating a live attenu-
ated virus had been entirely empirical. This process usually involved
the passaging of the virus in cell culture or animals that were not the
natural host, followed by testing of the resulting viruses for decreased
virulence in the natural host. The basis for attenuation is most often
unknown, and may be a result from as minor as a single base change,
and thus the chance of reversion back to virulence is always a possi-
bility. This type of traditional method for generating a live attenuated
virus is not necessarily the most attractive method for generating a
viral vaccine vector. With the advent of molecular biology and our
improved knowledge of viruses at the genetic level it is now possible to generate live attenuated viruses with precise genetic changes, improving their safety and thus make them more suitable as vectors for vaccine delivery.

A. DELETION OF NONESSENTIAL GENES

A good example is the deletion of the thymidine kinase (TK) gene. While the deletion of the TK gene has little or no effect on virus growth in cell culture, TK deleted viruses can be significantly attenuated in vivo ((Buller et al., 1985; Kit et al., 1985, 1986; Becker et al., 1986). This feature has been exploited successfully for the development of live attenuated herpesvirus vaccines (McGregor et al., 1985; Kit et al., 1985; Marchioli et al., 1987; Moorman et al., 1990) as well as safer herpesvirus and poxvirus vectors (e.g., Buller et al., 1985; Bayliss et al., 1991; Mulder et al., 1994; Hu et al., 1997).

B. DELETION OF ESSENTIAL GENES

If an essential gene is deleted from a virus, the virus can only grow if the gene or gene product is provided in trans. This virus is phenotypically normal but genotypically defective and cannot replicate in the host because the deleted gene product is not available. This type of virus can replicate in vitro with the help of a genetically engineered supporting cell line that expresses the deleted gene product. The stage of the virus life cycle of which the gene product is required will govern how far through the replication cycle a virus will proceed. In some cases (e.g., if the essential deleted gene is required for virus penetration of the cell) the virus will complete a single round of replication in the host but the progeny viruses will not be able to invade any other cell. (Farrell et al., 1994; McLean et al., 1994; Peeters et al., 1994). However, if the deleted essential gene is an early gene that is required to activate other viral genes, then the number of viral proteins synthesized may be limited and the viral genome may not be able to complete even a single cycle of replication (Chen and Knipe, 1996; Brehm et al., 1997; Da Costa et al., 1997).

C. REPLICATION LIMITED VIRUS

A third alternative, which has been exploited successfully, is to use a virus that can only completely replicate in one species as a vector in another species, where it cannot complete an entire cycle of replication but can commence a replication cycle sufficiently to allow expression of
the foreign gene (Tartaglia et al., 1992). The canarypox virus (CPV) vector, termed ALVAC, has successfully been exploited to the degree of commercial success. The CPV is restricted to avian cells only for productive replication but can be used to vaccinate mammals where it can elicit an immune response to the foreign gene product without completing an entire cycle of replication (Tartaglia et al., 1992, 1993; Taylor et al., 1995). The human adenovirus type 5 (HAV-5) has also been exploited in a similar fashion to the CPV (Table III) but has the disadvantage that this virus is a human pathogen and so has yet to be exploited commercially.

Several other strategies are also available and in some cases have been exploited successfully in order to generate safe viral vectors for vaccine delivery. Table V (next section) provides a summary of some of these possible approaches.

V. Examples of Reported Viral Veterinary Vaccine Vectors

Even though there are a great many examples of viral vectors reported in the literature since they were first described in 1982, the number of publications reporting the use of viral vectors for veterinary vaccine delivery is not that large. After searching for published papers that describe viral vectors with veterinary vaccine applications, especially those that could be described as purposely developed for veterinary use, the obvious conclusion was that even though this research was first described in 1982 the veterinary side is still in its infancy. Publications describing viral vectors for veterinary vaccine delivery can be divided into several groups, which are represented in the following tables: Table II, poxvirus vectors; Table III, adenovirus vectors; Table IV, herpesvirus vectors; and Table V, other virus vectors. Although these four tables probably do not include every single publication describing viral vectors for veterinary vaccine delivery they do describe the majority of published papers and present the reader with an idea of the limited amount of research that has occurred in this field during the last 15 years.

VI. Commercially Available Viral Vaccine Vectors for Veterinary Use

At the time of writing this review only three viral vectored vaccines for use in the veterinary field have been licensed for release. All three are based on poxvirus vectors and the three vectors represent the
### TABLE II

**POXVIRUS VECTORS FOR THE DELIVERY OF VETERINARY VACCINES**

| Vector | Pathogen | Antigen | Test animal | Reference                  |
|--------|----------|---------|-------------|----------------------------|
| CPV    | RHDV     | Capsid  | Rabbit      | Fischer et al., (1997)     |
| RPV    | FPV/rabies | VP2/G   | Cat         | Hu et al., (1997)          |
| CPV/VV | CDV      | F/HA    | Ferret      | Stephensen et al., (1997)  |
| FPV    | NDV      | F/HN    | Chicken     | Taylor et al., (1996)      |
| Myxoma | Influenza | HA      | Rabbit      | Kerr and Jackson (1995)    |
| SPV    | PrV      | gp50/gp63 | Swine      | van der Leek et al., (1994) |
| CPV    | FeLV     | env/gag | Cat         | Tartaglia et al., (1993)   |
| VV     | Rabies   | G       | Fox         | Brochier et al., (1991)    |
| PPV    | NDV      | F       | Chicken     | Latellier et al., (1991)   |
| FPV    | NDV      | HA/NA   | Chicken     | Boursnell et al., (1990a)  |
| FPV    | NDV      | HN/F    | Chicken     | Boursnell et al., (1990b)  |
| VV     | BLV      | env     | Rabbit      | Ohishi et al., (1990)      |
| VV     | EHV-1    | gp13    | Mouse       | Guo et al., (1989)         |
| VV     | PrV      | gp50/63 | Mouse       | Kost et al., (1989)        |
| FPV    | Rabies   | G       | Dog/cat     | Taylor et al., (1988)      |
| VV     | FeLV     | env     | Cat         | Gilbert et al., (1987)     |
| VV     | Rabies   | G       | Fox         | Blancou et al., (1986)     |
| VV     | Rabies   | G       | Mouse       | Kieny et al., (1984)       |

**Key:** VV, vaccinia virus; FPV, fowl poxvirus; PPV, pigeon poxvirus; SPV, swine poxvirus; CPV, canary poxvirus; RHDV, rabbit hemorrhagic disease virus; CDV, canine distemper virus; FPV, feline parvovirus; PrV, pseudorabies virus; FeLV, feline leukemia virus; NDV, Newcastle disease virus; BLV, bovine leukemia virus; EHV, equine herpes virus.

### TABLE III

**ADENOVIRUS VECTORS FOR THE DELIVERY OF VETERINARY VACCINES**

| Vector | Pathogen | Antigen | Test animal | Reference                  |
|--------|----------|---------|-------------|----------------------------|
| OAV    | Tinea ovis | 45W     | Sheep       | Rothel et al., (1997)      |
| HAV-5  | PRCV     | Spike   | Swine       | Callebaert et al., (1996)  |
| HAV-5  | TGE      | Spike   | Swine       | Torres Iet al., (1996)     |
| HAV-5  | Rabies   | G       | Skunk       | Yarosh et al., (1996)      |
| HAV-5  | BCV      | HEG     | Cotton rat  | Bacca-Estrada et al., (1995)|
| HAV-5  | FIV      | env     | Cat         | Gonin et al., (1995)       |
| HAV-5  | PRCV     | Spike   | Swine       | Callebaert et al., (1994)  |
| HAV-5  | PrV      | gD      | Swine       | Adam et al., (1994)        |
| HAV-5  | Rabies   | G       | Dog         | Prevec et al., (1990)      |
| HAV-5  | PrV      | gp50    | Rabbit/mouse| Eloit et al., (1990)       |

**Key:** OAV, ovine adenovirus; HAV-5, human adenovirus type 5; PRCV, porcine respiratory corona virus; TGE, transmissible gastroenteritis virus; BCV, bovine corona virus; PrV, pseudorabies virus.
### TABLE IV

**HERPES VIRUS VECTORS FOR THE DELIVERY OF VETERINARY VACCINES**

| Vector | Pathogen | Antigen | Test animal | Reference                |
|--------|----------|---------|-------------|--------------------------|
| HVT    | NDV      | HN/F    | Chicken     | Reddy et al., (1996)     |
| HVT    | MDV      | gpAB    | Chicken     | Reddy et al., (1996)     |
| FHV-1  | FeLV     | env     | Cat         | Willems et al., (1996)   |
| PrV    | HCV      | gpE1    | Swine       | Mulder et al., (1994)    |
| HVT    | MDV      | gpB     | Chicken     | Ross et al., (1993)      |
| BHV-1  | PrV      | gpC     | Swine       | Kit et al., (1992)       |
| FHV-1  | FeLV     | gag/env | Cat         | Wardley et al., (1992)   |
| BHV-1  | FMDV     | cp-epitopes | Cattle   | M. Kit et al., (1991)    |
| BHV-1  | FMDV     | cp-epitopes | Cattle   | S. Kit et al., (1991)    |
| PrV    | HCV      | gpE1    | Swine       | van Zijl et al., (1991)  |

*Key: HTV, herpes virus of turkeys; FHV, feline herpes virus; BHV, bovine herpes virus; PrV, pseudorabies virus; NDV, Newcastle disease virus; MDV, Marek's disease virus; FMDV, foot-and-mouth disease virus; HCV, hog cholera virus.*

The evolution in poxvirus vector development. The first vector approved was the vaccinia virus vector carrying the rabies G glycoprotein gene (e.g., Kieny et al., 1984; Blancou et al., 1986; Brochier et al., 1991). In terms of complying with the characteristics of a desirable vector for vaccine delivery in the veterinary setting, this vector has the greatest number of undesirable characteristics. However, it satisfied an unmet need and as a result was released in various parts of the world. The second vector to be licensed for release was the fowlpox virus vector. This vector delivers the Newcastle disease virus HN and F genes and is designed to vaccinate poultry (e.g., Boursnell et al., 1990a,b; Taylor et al., 1996). While this vector has the desirable characteristic of only replicating in poultry it also has some limitations that affect its use in the field. The third vector licensed is the canarypox virus vector and represents the state-of-the-art poxvirus vector. This vector was developed to deliver the HA and F genes of canine distemper virus and is the most recently available of the three vector vaccines (e.g., Stephensen et al., 1997).

### VII. Summary

Whatever strategy is adopted for the development of viral vectors for delivery of veterinary vaccines there are several key points to consider: (1) Will the vectored vaccine give a delivery advantage compared to
### TABLE V

**Other Virus Vectors for the Delivery of Veterinary Vaccines**

| Vector       | Pathogen | Antigen      | Test animal | Reference               |
|--------------|----------|--------------|-------------|-------------------------|
| CPMV         | MEV      | VP2 epitope  | Mink        | Dalsgaard et al. (1997) |
| Poliovirus   | FMDV     | Epitopes     | Guinea pig  | Kitson et al. (1991)    |
| Retrovirus   | NDV      | HN           | Chicken     | Morrison et al. (1990)  |
| Retrovirus   | Influenza| HA           | Chicken     | Hunt et al. (1988)      |

### Other Alternative Viral Vectors That Have the Potential for Veterinary Vaccine Delivery

| Amplicons | VLPs         | SFV          | Sinbis      | Bacteriophage          |
|-----------|--------------|--------------|-------------|------------------------|
| Frenkel et al. (1994) | Jagadish et al. (1996) | Atkins et al. (1996) | Pugachev et al. (1995) | Bastien et al. (1997) |
| Smith et al. (1995) | Porter et al. (1996) | Mossman et al. (1996) |             |                        |
| Fink et al. (1996) | Roy (1996)    |               | Zhou et al. (1995) |                        |
| Pechan et al. (1996) | Schodel et al. (1994a) | Zhou et al. (1994) |             |                        |
| Starr et al. (1996) | Schodel et al. (1994b) |             |             |                        |

Key: CPMV, cowpea mosaic virus; MEV, mink enteritis virus.
what’s already available? (2) Will the vectored vaccine give a manufacturing advantage compared to what’s already available? (3) Will the vectored vaccine provide improved safety compared to what’s already available? (5) Will the vectored vaccine increase the duration of immunity compared to what’s already available? (6) Will the vectored vaccine be more convenient to store compared to what’s already available? (7) Is the vectored vaccine compatible with other vaccines? If there is no other alternative available then the answer to these questions is easy. However, if there are alternative vaccines available then the answers to these questions become very important because the answers will determine whether a vectored vaccine is merely a good laboratory idea or a successful vaccine.

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