REVIEW

Recombinant Viral Vector Vaccines for the Veterinary Use

Naoaki YOKOYAMA, Ken MAEDA¹⁾, and Takeshi MIKAMI*

Department of Veterinary Microbiology, Faculty of Agriculture, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113 and

¹⁾Department of Veterinary Microbiology, Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753, Japan

(Received 20 November 1996/Accepted 5 February 1997)

ABSTRACT. Recently, genetically engineering using recombinant DNA techniques has been applied to design new viral vaccines in order to reduce some problems which present viral vaccines have. Up to now, many viruses have been investigated for development of recombinant attenuated vaccines or live viral vectors for delivery of foreign immunogenic antigens. In this review, we introduced three kind of viruses; herpesviruses, vaccinia viruses, and adenoviruses, which have best widely been studied as recombinant vaccines or delivery vaccines for the veterinary use. — KEY WORDS: recombinant polyvalent vaccine, recombinant vaccine, viral vector.

J. Vet. Med. Sci. 59(5): 311–322, 1997

More than 200 years of research and development of effective vaccines, since vaccinia virus was used as the first vaccine against smallpox, make it possible to protect animals against natural infections with many microbial pathogens [80]. Vaccines are the most effective and cheap prophylactic tools in veterinary medicine. Vaccines include attenuated live vaccines, subunit vaccines which are derived from parts of the microbial pathogen, and inactivated vaccines. Ideally, vaccines should induce a lifelong protective immunity against the target pathogen but not cause clinical or pathological signs of diseases in the vaccinated animals. Vaccines are used not only to control clinical signs following infection with field-type pathogens, but also to reduce transmission of the pathogen in the host species, eventually with the purpose of eliminating the pathogen. However, such ideal vaccines are rare in the veterinary field. Many vaccines are either of limited effectiveness or have harmful side effects. In addition, there are still severe diseases with no effective vaccines.

Recently, genetic engineering using recombinant DNA techniques has been applied to design new vaccines in order to reduce some problems which present vaccines have. A recombinant vaccine for delivery of a foreign antigen is also a type of genetically engineered vaccine. The delivery vaccines contain an attenuated live vector in which foreign genetic information derived from one or more other microbial pathogens has been incorporated. The recombinant vaccines are applied to induce protective immunity against the pathogen(s) from which the heterologous genetic information has been derived, in combination with or without immunity against field-type variants of the vector. Today, some viruses have been used as live viral vectors. These include poxviruses, herpesviruses, and adenoviruses. The choice of a viral

vector for development of recombinant polyvalent or delivery vaccines is determined by many factors, of which host range of the vector, replication in the target animal, expression of foreign antigen, induction of protective immunity, duration of the immunity, costs of production, safety, and stability of the recombinant virus are among the most important.

RECOMBINANT HERPESVIRUSES

1 HERPESVIRUSES AND PRESENT VACCINES

Herpesviruses are widely disseminated in nature, and almost all species of mammals and birds have herpesvirus infections [93]. The most characteristic feature of herpesviruses is that they establish latency after primary infection and can be periodically reactivated in their natural hosts [37]. Reactivation of latent virus resulting in virus spread is assumed to be a major mechanism whereby the virus remains endemic. The success of this viral strategy is evidenced by the widespread incidence of herpesvirus infections. Some herpesviruses cause asymptomatic primary infection early in life and disease upon reactivation, while others produce disease after acute infection and are shed asymptotically after reactivation. Some of these viruses are host specific or have a narrow host range (feline herpesvirus type 1 (FHV-1), canine herpesvirus, and others), while others have a broad host range (pseudorabies (or Aujeszky's disease) virus (PRV), B virus, and others).

Because the importance of herpesvirus infections in domestic and companion animals has been recognized for a long time, there are many commercial vaccines available for controlling these diseases. Live vaccines are more commonly used, because inactivated vaccines, although they are much safer, have been less effective. Live herpesvirus vaccine requires attenuation of the virus which must induce a protective immunity. The attenuation used to be a blind occurrence during cultivation of the virus. For example,

* CORRESPONDENCE TO: Dr. MIKAMI, T., Department of Veterinary Microbiology, Faculty of Agriculture, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan.

attenuation has been achieved by multiple passages in animals, eggs, or cell cultures originating from the natural or a non-natural host, and has sometimes been stimulated by culturing at non-optimal temperatures or in the presence of mitogens. Live vaccines produced by these conventional procedures carry usually unknown genetic changes and the attenuation is mostly due to the loss of viral virulence factors by the genetic changes. However, when the changes are small, such as point mutations, there is a danger of reversion to virulence.

Some of these conventional vaccines have sometimes caused several diseases, for example, abortion in cattle after vaccination for bovine herpesvirus type 1 (BHV-1) infection [102]. When such a case follows vaccination, it is important to differentiate the vaccine strain from field-type virus in order to exclude the possibility that natural infection may have occurred at the time of vaccination. In control programs, it is also advantageous to distinguish vaccinated animals from naturally infected animals by serological means. However, most of the present vaccines do not allow either of these possibilities.

2 GENETICALLY ENGINEERED HERPESVIRUS VACCINES

Attenuation of alphaherpesvirus can be achieved by deletion of the gene which contributes to viral virulence. Gene deletion by a recombinant DNA technique may make it possible to generate a safer and more practical vaccine than conventionally attenuated vaccines. In particular, genetically engineered recombinant vaccines with large and multiple deletions are expected to create strains that are less likely to revert to virulence.

For development of such a recombinant herpesvirus vaccine, it is important to search for non-essential genes that contribute to viral virulence. Viral proteins known to be involved in the virulence of herpesviruses are a class of enzymes that function at the level of DNA synthesis; the thymidine kinase (TK), ribonuclease reductase, and dUTPase [14, 25, 91]. A protein kinase encoded in the unique short (Us) region also appears to affect virulence at least in PRV [47, 48]. Deletion of these genes results in a recombinant virus with a reduced replication efficiency and attenuated phenotype. Another well-characterized group is glycoproteins. Glycoproteins, which present at the surface of the virion and the virus-infected cells, are involved in the interaction between herpesvirus and its host, but several glycoproteins are non-essential for virus replication in cell cultures and affect pathogenesis to a certain extent. Successive deletion of virulence genes might, however, result in a reduced ability to protect against challenge infection [48]. A delicate balance exists between achieving sufficient attenuation to provide safety and maintaining immunogenicity for the efficacy of a vaccine.

Here, we introduce some examples of genetically engineered herpesvirus vaccines and the application of these recombinant vaccines in the veterinary field.

1) TK gene

TK, encoded in the genome of many herpesviruses, is not essential for virus growth in actively dividing tissue culture cells [25]. TK synthesized by alphaherpesviruses appears to facilitate the growth of virus in non-dividing cells [25]. Many recent investigations have demonstrated that TK deficient mutants of herpes simplex virus type 1 (HSV-1) [25], PRV [49, 112], BHV-1 [50], equine herpesvirus type 1 (EHV-1) [101], and FHV-1 [131] are remarkably attenuated for their natural hosts or mice and appear to be less readily reactivated from neurons [21]. Kit *et al.* reported the loss of ability of BHV-1 TK deficient mutant to infect the fetus and to cause abortions [51] and the reduction of ability of the mutant to develop latent infections [50]. Although the mechanisms of TK gene are unknown fully, such mutants have been proposed as the basis for vaccine development. In particular, TK deficient mutants of PRV, which have shown a reduced virulence for pigs and other animals but still to produce a good protection, are now available as commercial vaccines for control of Aujeszky's disease in Europe, North America, and Japan. Avian herpesviruses also encode TK and the construction of a TK deficient mutant of infectious laryngotracheitis virus has been reported [85]. Relatively little information is available for the TK of Marek's disease virus. TK gene of non-pathogenic herpesvirus of turkeys (HVT) was proposed as an insertion site for expression of foreign genes [98].

Such genetically engineered vaccines allow the possibility of identification of vaccine virus by probes for an inserted sequence or by PCR amplification across the deleted region [49, 102, 130]. However, it is still impossible to differentiate vaccinated animals from naturally infected animals. The development of herpesvirus vaccines with deletions of structural proteins, such as non-essential glycoproteins, allows serological differentiation between vaccinated and naturally infected animals.

2) Non-essential glycoprotein genes

In better studied viruses, such as PRV or BHV-1, three glycoproteins (gE, gI, and gC) have been reported to be non-essential for viral replication in cell cultures, but implicated in important viral function such as target cell recognition and virulence [23, 42, 47, 113]. For example, after intranasal inoculation in pigs, PRV gE deletion mutants had a reduced capability to propagate in olfactory and trigeminal pathways [56, 79] and showed a reduced virulence in pigs [23, 42, 47, 48]. A PRV vaccine carrying deletions in both gE and TK was shown to be innocuous but still highly immunogenic for pigs [72, 120]. No specific function has been assigned to gG and the PRV gG deficient mutants behave like field-type PRV in animal models as well as in cell cultures [3, 47]. Herpesviruses with major deletions of these non-essential glycoprotein genes have been used as live attenuated vaccines, for example, the Norden and Bartha strains of PRV [88] and the Kentucky A

strain of EHV-1 [27].

Animals vaccinated with the recombinant virus with deletions of these non-essential glycoproteins will not develop specific antibodies against the glycoprotein, whereas animals infected with field-type virus will produce antibodies against all proteins. An enzyme-linked immunosorbent assay (ELISA) which can specifically detect antibody against the particular glycoprotein will therefore allow vaccinated animals to be differentiated from infected animals [26, 42, 54, 117, 120]. However, animals which have been exposed to both viruses cannot be differentiated from infected animals. This might be achieved by the insertion of a marker protein expression cassette, such as *E. coli* β -galactosidase (lacZ) or firefly luciferase, into the gene encoding these non-essential glycoproteins.

3) Essential glycoprotein D gene

In addition to concerns about the ability of genetically engineered viruses to establish latency, another major concern is that they may be shed into the environment and infect other, non-target animals [135]. One way to overcome both of these concerns is to construct a vaccine which has only limited capacity for replication. Peeters *et al.* [87] described a replication defective vaccine based on PRV. The gD of PRV is essential for virus entry but is not required for subsequent steps in virus replication cycle. PRV mutant lacking gD can be grown in complementing cells, which express gD, and the progeny virus released from the cells contains gD. Therefore, the recombinant virus can infect the cells of inoculated animals and spread by direct cell-to-cell transmission. However, progeny virions released from the infected cells are non-infectious and further replication cycle does not proceed, because they lack gD. Such a replication defective virus may constitute a new generation of safe, non-transmissible live vaccines.

3 RECOMBINANT POLYVALENT VACCINES USING A HERPESVIRUS VECTOR

Recombinant herpesvirus vaccines not only are used to induce a protective immune response against the herpesvirus infection, but also are proposed as suitable candidates for vector vaccines to induce protective immune responses against other pathogens. Herpesviruses have a large genome and appear to contain a number of non-essential genes which can be replaced by foreign genes. In this way, it is possible to provide a recombinant polyvalent vaccine for control of multiple disease conditions with only one dose.

Since, in 1984, the first recombinant vaccine based on HSV-1 was constructed for the delivery of a foreign antigen from hepatitis B virus for human medicine [99], development of a recombinant polyvalent vaccine based on alphaherpesvirus has been applied for the veterinary field and some of the recombinant viruses have been readily examined *in vivo* to assess the immune response against the foreign antigen. Here, we introduce some examples of the

recombinant polyvalent vaccine. Recombinant polyvalent vaccines using an FHV-1 vector are especially described in next section.

1) PRV vector

Several groups reported the constructions of recombinant PRV expressing the immunogenic antigen of classical swine fever virus (CSFV) [38, 78, 118]. Van Zijl *et al.* [118] showed that the recombinant PRV, in which the gene encoding the E1 glycoprotein of CSFV was exchanged with most of the gG locus of PRV, protected pigs against both Aujeszky's disease and classical swine fever. Hoof van Iddekinge *et al.* [38] reported that the gE locus of PRV is also a suitable insertion site for expression of the E1 gene and the immediately early gene promoter of human cytomegalovirus (pHCMV) is specifically useful for induction of the protective immunity.

2) BHV-1 vector

BHV-1 has also been proposed as a viral vector for development of a recombinant polyvalent vaccine for cattle [52, 53]. Kit *et al.* [52] reported the construction of a recombinant BHV-1 expressing the capsid protein (VP1) epitope of foot-and-mouth disease virus (FMDV) as fusion proteins with BHV-1 gC. The VP1 epitope was expressed on the surface of virus-infected cells and virus particles. Vaccination with the recombinant BHV-1 induced protective levels of anti-FMDV antibodies in calves and protected them against virulent BHV-1 challenge. The gC gene of PRV has been inserted into the genome of BHV-1 with its own promoter or with the BHV-1 gC promoter, and in both cases the PRV gC was expressed efficiently [55]. The PRV gC protein produced by the recombinant BHV-1 has been employed as the antigen in an ELISA diagnostic test [55].

3) HVT vector

HVT, which is non-pathogenic for chickens, has been used extensively as a vaccine against Marek's disease [86]. Some groups showed that HVT can be used as a viral vector for expression of antigen from other avian pathogens [20, 73, 74, 98, 103]. The recombinant HVT, expressing the fusion (F) antigen of Newcastle disease virus (NDV), protected chickens against challenge infection with virulent NDV and retained the capacity of HVT to protect against Marek's disease [73]. Darteil *et al.* [20] constructed two recombinant HVT expressing the VP2 protein of infectious bursal disease virus and reported that a recombinant HVT, in which the VP2 gene was inserted at HVT gI locus with the pHCMV, induces a full protection against IBDV challenge.

4 GENETICALLY ENGINEERED FHV-1 VACCINES AND RECOMBINANT POLYVALENT VACCINES USING AN FHV-1 VECTOR

4.1 Genetically engineered FHV-1 vaccines

To develop genetically engineered FHV-1 vaccine, it is necessary to characterize some non-essential genes of FHV-1. The mutant, in which the gene is deleted, should preserve a full capacity of the parent FHV-1 to replicate at the primary sites of infection (ocular, nasal, and oral mucosae) and yet be completely attenuated. The search for virulence factors in FHV-1 offered some advantages; FHV-1 causes a well defined syndrome (upper respiratory tract disease) and genetically engineered FHV-1 can be readily studied in the natural host, instead of using other animal models. Since cats are one of the smallest animals which can be naturally infected with alphaherpesvirus and are easy to deal with, FHV-1 infection can also be useful for a possible model in *in vivo* study of other alphaherpesvirus infections in the veterinary field.

LacZ expression cassette is available for identification of virulence genes of FHV-1 [106, 124–126]. The expression cassette can be inserted at a specific region, which is non-essential for virus propagation, in subfragments of the FHV-1 genome. Susceptible cells, such as Crandell feline kidney cells [19], are infected with parent FHV-1 strain and then transfected with the cloning plasmid (transfer vector), or alternatively, cotransfected with parent viral DNA and the transfer vector. In the cells, viral sequences of the transfer vector recombine with identical regions in the parent viral genome. The resulting recombined FHV-1 carries the expression cassette at the specific site and can be detected by staining with Blueo-gal. If the target gene of FHV-1 is TK gene, the expression cassette is not needed, because FHV-1 TK deficient mutants are easily selected by thymidine arabinoside [83, 130, 132]. After characterization of the *in vitro* biological properties of the recombinant virus, these recombinant FHV-1 are examined in cats to identify the effect of mutation on FHV-1 virulence gene.

During last five years, several groups have reported the nucleotide sequences of FHV-1 and some of the genes have been characterized [44–46, 61–63, 66–68, 70, 83, 104, 105, 124, 125, 130]. However, there are relatively few reports that non-essential genes have been identified in the genome of FHV-1, compared with the other animal alphaherpesviruses.

1) FHV-1 TK gene

FHV-1 TK gene was first identified by Nunberg *et al.* [83], using American FHV-1 strain. DNA sequence analysis of the identified TK gene revealed an open reading frame (ORF) of 343 amino acids. They also constructed a recombinant FHV-1 deleted an *EcoRV-HindIII* fragment within the TK gene and reported that the recombinant FHV-1 was deficient in TK enzymatic activity [83].

Yokoyama *et al.* [130] also determined the TK gene of a Japanese FHV-1 strain and constructed another recombinant FHV-1 deleted an *EcoRV-SmaI* fragment within the gene. The nucleotide sequence of the TK gene of both strains was

exactly the same. Growth kinetics of the recombinant FHV-1 in cell cultures was similar to that of the parent virus, but plaques produced by the recombinant FHV-1 were significantly smaller than those of the parent virus [130]. Next, Yokoyama *et al.* [131] carried out two experiments to assess the pathogenicity and vaccine efficacy of the recombinant FHV-1 in cats. The first experiment made it obvious that, following multiple inoculations of the recombinant FHV-1 by the intraocular, intranasal, and oral routes, the virus was found to be sufficiently attenuated in cats, although a high titer of the virus was recovered from target organs (eye, nose, and mouth). In the second experiment, two intramuscular vaccinations with the recombinant FHV-1 could prevent to a significant degree clinical responses in cats against subsequent virulent FHV-1 challenge [131].

2) FHV-1 ORF2 gene downstream of the gC

Willemse *et al.* [124] identified a novel virulence factor in the unique long (UL) region of the FHV-1 genome. This locus is the positional homologue of HSV-1 UL45 and is located downstream of the gene encoding FHV-1 gC. The gene was designated as ORF2, since no significant sequence homology was observed with UL45. They constructed two recombinant FHV-1, in which a lacZ expression cassette was integrated into the ORF2, and reported that these recombinant FHV-1 were able to grow *in vitro* and *in vivo* as parent virus did, but oronasal administration of the recombinant viruses to cats demonstrated a reduced virulence when compared with that of the parent virus.

3) FHV-1 Us 8.5 gene

Us 8.5, which encodes 100 amino acids, is found to be located at the Us region and unique to FHV-1 by analysis of the putative polypeptide with other herpesviruses [125]. Although no significant homology in the sequence was found, Us 8.5 might be functionally related to Us 8.5 of HSV-1 and gene 75 of EHV-1, judging from its size, position, and orientation. A recombinant FHV-1 containing a lacZ expression cassette at the Us 8.5 locus was able to grow in cell cultures as the parent virus did, indicating that the Us 8.5 is non-essential for FHV-1 replication. However, pathogenicity of the recombinant FHV-1 is unknown.

4) FHV-1 gI and gE genes

FHV-1 gI and gE genes are also located at the Us region [105, 125]. By expression with a recombinant vaccinia virus, the immature gI form showed a molecular weight (MW) of 67 kDa and the immature gE form did a MW of 83 kDa. In the case of single expression of each gene, neither was found on the cell surface, suggesting that gE-gI interaction is required for transport from the endoplasmic reticulum [70].

Willemse *et al.* [126] constructed a recombinant FHV-1

which has an insertion of lacZ expression cassette at the gI gene. The recombinant FHV-1 displayed a decreased replication not only in cell cultures but also in the respiratory tract of infected cats. Oronasal administration of the recombinant virus caused less severe clinical signs than that of the parent FHV-1 strain. In addition, oronasally vaccinated cats were better protected from the clinical signs of a challenge infection than subcutaneously vaccinated cats.

Sussman *et al.* [106] reported the construction of another recombinant FHV-1 which lacked part of the gI gene and both the 5'-transcriptional control and 5'-encoding regions of the gE gene. The recombinant virus showed a decreased replication in one step growth kinetics and produced very small plaques in cell cultures, but cats inoculated intranasally or subcutaneously with the recombinant FHV-1 responded with only mild clinical signs and induced a strong protective immunity against subsequent virulent FHV-1 challenge [57, 106].

4.2 Recombinant polyvalent vaccines using an FHV-1 vector

The non-essential genes involved in FHV-1 virulence can also be used as insertion sites of heterologous genes encoding immunogenic antigens of other pathogens for cats. Pathogens causing upper respiratory diseases similar to FHV-1, such as feline calicivirus (FCV), *Chlamydia psittaci* or *Bordetella bronchiseptica*, would be suitable donor organisms in terms of the FHV-1 replicative tropism. Since conventional FHV-1 vaccine is presently used as a combination with FCV and feline panleukopenia virus (FPLV) vaccines [30], it might also be useful for development of a recombinant polyvalent vaccine which could induce protective immunities against the three pathogens at the same time. Other antigens expressed by the FHV-1 vector may be derived from feline leukaemia virus (FeLV), feline immunodeficiency virus (FIV), and feline infectious peritonitis virus, because the development of conventional vaccines for these infections has not been fully successful.

FHV-1 appears to have some merit in respect of safety when used as a live vector for cat vaccination; 1) Strain homogeneity of FHV-1 isolates suggested stability of genomic DNA [64, 65]. It is likely that recombinant viruses inserted with other foreign genes are expected to be stable. 2) Because FHV-1 has a very narrow host range restricted to feline species, recombinant viruses would not infect other animals. 3) Household cats are usually not kept in large numbers sharing a limited amount of space. 4) Infection with FHV-1 normally is not lethal and the use of live vaccine strains is already generally accepted.

1) Expression of FeLV antigens

Cole *et al.* [17] reported the constructions of two recombinant FHV-1 expressing the envelope (*env*) or *gag* protein of FeLV, in which these expression cassettes, utilizing the phCMV, have been inserted within the FHV-1

TK gene. Wardley *et al.* [122] vaccinated cats first intranasally and orally with the recombinant FHV-1 expressing *env* and *gag* proteins and later intramuscularly with *gag/env* particles purified from insect cells that were coinfecting with two recombinant baculoviruses expressing *env* or *gag* protein, and achieved 100% protection against FeLV challenge. However, these recombinant FHV-1 displayed a limited efficacy in terms of protection unless followed by the booster vaccination.

Willemse *et al.* [127] constructed another recombinant FHV-1. To express the *env* protein of FeLV, the expression cassette, containing the long terminal repeat (LTR) promoter from Rous sarcoma virus (RSV), was integrated at the ORF2 locus of FHV-1. Oronasal vaccination with the recombinant FHV-1 engendered a significant protection against FeLV infection. They considered that the ultimate goal for future FeLV vaccines ideally would be that they protect not only against a persistent or transient viraemia but should also be capable of preventing the primary infection with FeLV at the epithelial tissues of the upper respiratory tract.

2) Expression of FCV antigens

Recently, the construction of a recombinant FHV-1, which contains an entire ORF encoding the capsid protein of FCV at the TK deletion locus of FHV-1, has been described [132]. FCV immunogenic antigens were expressed mainly as a fusion capsid protein with the N-terminal part of TK under the control of the FHV-1 TK promoter by the recombinant FHV-1 [132]. *In vivo* experiments to assess the vaccine efficacy of the recombinant FHV-1 against FCV and FHV-1 infections in cats revealed that two vaccinations of the recombinant FHV-1 by the intraocular, intranasal, and oral routes protected cats to a significant degree against subsequent virulent challenges with both parent FCV and FHV-1 strains [133].

3) Expression of FIV antigens

Verschoor *et al.* [119] constructed a recombinant FHV-1 expressing the FIV *env* protein, in which the expression cassette containing the LTR promoter from RSV was inserted at the ORF2 locus of FHV-1. Although they vaccinated cats first oronasally and subcutaneously with the recombinant FHV-1 and later twice intramuscularly or subcutaneously with bacterial expression product containing the V3 region of the FIV *env* gene, FIV-specific antibody responses were weak and the vaccination did not withstand challenge with a low dose of homologous FIV.

RECOMBINANT VACCINIA VIRUSES

1 VACCINIA VIRUS VECTOR

Vaccinia virus is a member of the poxvirus family and has a wide host range that includes human beings, monkeys, cattle, horses, swine, sheep, goats, and mice. During this

past century, vaccination with the vaccinia virus has protected hundreds of millions of people worldwide from smallpox. At present, vaccinia virus is also known as the best widely studied viral vector for expression of foreign antigens [18, 77, 129]. Vaccinia virus can be grown to high titers *in vitro* and is stable under storage conditions that may lead to loss of infectivity of other live viral vaccines. The vaccinia virus has a large number of genes which can be deleted without preventing replication in cell cultures and, in many cases, the deletion mutant has reduced virulence in animal models [18, 77]. These genes have been applied as insertion sites for expression of foreign antigens and genes derived from a variety of pathogens have been stably introduced into the virus, providing potential recombinant vaccines for both veterinary and human applications, for example, genes from hepatitis B virus [76], human immunodeficiency virus (HIV) [18, 129], HSV-1 [18], simian immunodeficiency virus [40], FIV [100], rabies virus [8], rinderpest virus [32], PRV [12], bovine ephemeral fever virus [36], and rabbit haemorrhagic disease virus [7]. Both humoral and cellular immune responses to the expression products from these recombinant vaccinia viruses have been demonstrated in animals and humans and, in many cases, their protective immunities have been induced in challenge studies. For example, a recombinant vaccinia virus expressing the glycoprotein G of rabies virus has been practically applied as an oral bait vaccine in a 2,200 km² region of Belgium [11]. Recombinant vaccinia viruses are especially available for using in developing countries. Lyophilized vaccinia virus is heat-stable and can be reconstituted easily. Vaccinia virus can also be grown outside the laboratory. For example, scarification of the abdomen of a single calf will provide material for more than 200,000 vaccine doses [129].

The broad host range of vaccinia virus makes it suitable for veterinary use and vaccinia virus has become one of the most commonly used viral vectors. However, it is capable of infecting many different species in addition to the target animals for vaccination against specific disease. Additionally, vaccinia virus has the potential, although slight, to cause serious adverse effects in some immunocompromised persons or adverse reactions in a small number of normal recipients, and it can also be transmitted by contacts. These properties are of particular concern in view of the worldwide epidemic of HIV infection and the consequent large pool of asymptomatic but potentially immunocompromised individuals among vaccination target populations and their contacts. Because of these considerations, there has been reluctance to pursue further development of vaccinia as a vaccine vector. As a result, attention has shifted to the use of host-restricted poxviruses, such as raccoonpox, fowlpox, canarypox, capripox, and swinepox viruses.

2 OTHER POXVIRUS VECTORS

1) Orthopox viruses

Although vaccinia virus belongs to the Orthopoxvirus genus, raccoon poxvirus, another member of this genus, has also been developed as an expression viral vector primarily for use as a wild-life rabies vaccine [24, 59]. Recently, Hu *et al.* [41] reported the construction of a recombinant raccoon poxvirus containing the VP2 gene of FPLV into the TK locus of the virus. Two subcutaneous vaccinations with the recombinant raccoon poxvirus were shown to protect cats against FPLV challenge.

2) Avipox viruses

Productive replication of avipoxviruses is naturally restricted to avian species and they have been used as ideal vectors for developing recombinant vaccines for commercial poultry. Fowlpox virus (FPV) is the best characterized virus and the most frequently used as a viral vector in the avipox genus. Protective efficacy of chickens against avian influenza virus [6, 107], NDV [9, 84, 109], infectious bursal disease virus [5, 35], avian leukosis virus [82], avian reticuloendotheliosis virus [13], and Marek's disease virus [81] has recently been reported. Canarypox [110] and pigeonpox [58] vectors have also been described. Both vaccinia and avipoxvirus promoters were used for expression.

The novel idea of using avipoxviruses as non-replicating vectors in mammals has been advocated primarily for safety reasons [4]. The viruses undergo abortive replication and fail to produce infectious progeny virus in mammalian cell lines and animals. But they can achieve expression of foreign gene products and function effectively as an immunization vector for both animals and humans [29, 108, 110, 111, 123]. For example, sufficient expression of the rabies virus glycoprotein was obtained with a recombinant FPV to protect in immunized mice, cats, and dogs [108] and the recombinant vaccine based on canarypox virus was shown to be more effective than that based on FPV [110]. In other reports, both FPV [123] and canarypox virus [111] vectors expressing measles virus (MV) antigens induced a protective immunity in animals against MV or the related canine distemper virus challenge.

3) Capripox viruses

Capripox virus (CPV) does not infect man and has a restricted host range, which includes cattle, sheep, goats, and buffalo. Therefore it would make a more suitable viral vector for expressing immunogenic antigens of veterinary importance in their species. Romero *et al.* [94–97] reported the constructions of recombinant CPV, containing the fusion (F) or haemagglutinin (H) gene of rinderpest virus, and their vaccine efficacy in goats or cattle against rinderpest, peste des petits, or lumpy skin diseases. Wade-Evans *et al.* [121] constructed a recombinant CPV expressing the major core protein VP7 of bluetongue virus (BTV). Vaccination with the recombinant virus provided a partial protection against virulent BTV challenge in sheep.

4) Suipox virus

Swinepox virus is the only known member of the Suipoxvirus genus and is naturally restricted to pigs [69]. Van der Leek *et al.* [116] constructed a recombinant swinepox virus, in which genes encoding for gD and gI of PRV were inserted into the TK gene of swinepox virus with a vaccinia virus promoter for expression. When vaccinated by scarification or intramuscular injection with the recombinant virus, significantly fewer vaccinated pigs developed clinical Aujeszky's disease against PRV challenge.

RECOMBINANT ADENOVIRUSES

Human adenoviruses have been shown to be suitable recombinant vaccine vectors for expression of foreign antigens [34]. The regions that have been used for inserting foreign genes include the early (E) region 1 (E1), E3, and a site upstream of E4 [34]. The E3 region is not required for viral replication in any normally permissive cell cultures and many foreign proteins have been successfully expressed, when inserted into the E3 region, including proteins encoded by genes from HSV-1 [43], hepatitis B virus [16, 75], respiratory syncytial virus [39], HIV [60], simian immunodeficiency virus [15], vesicular stomatitis virus [89], rabies virus [90], bovine parainfluenza virus type 3 [10], BHV-1 [71], bovine coronavirus [134], and swine transmissible gastroenteritis virus [114, 115]. Inserts in this region have been shown to be expressed in non-permissive as well as permissive cell lines [89] and have also been found to elicit humoral, mucosal, and cellular immune responses to antigens encoded by the inserted foreign gene in a variety of different animals [10, 16, 39, 60, 71, 75, 89, 90, 114, 115, 134]. Most of these replication competent adenoviruses demonstrated a good efficacy for vaccination purposes, but biosafety of such constructions remains to be assessed.

On the other hand, replication incompetent adenoviruses can be constructed by deleting the E1 gene. Since the E1 gene is required for initiation of viral replication, replication-incompetent recombinant viruses containing foreign genes in the E1 region can be produced only in a complementing cell line, such as 293 cells that constitutively expresses E1 proteins [33]. However, complete replication is not required for the expression of foreign genes in E1 deleted viruses in cells which do not provide complementary E1 functions [2]. Such replication-incompetent adenovirus vectors are currently being studied as attractive candidates mainly for gene therapy purposes. Immunization of experimental animals with the recombinant adenovirus containing genes in the E1 region can induce a systemic immune response to the foreign gene product even in the absence of viral replication and provide a protection against subsequent challenge [1, 22, 28, 71, 92, 128]. Despite the safety advantages of replication incompetent viruses, the drawback of using such mutants is that high titers of virus must be

injected into animals to elicit a strong immune response.

Some properties of these viruses are very promising [31]; 1) Adenoviruses have the ability to express high levels of the foreign antigen in most cells including muscular, epithelial, neuronal, and macrophage cells. 2) Viral DNA is maintained mainly as an episome for several months, the transduced gene being expressed during this time in such a way that a long lasting immune response can be expected. 3) All mammal species which have been tested to date, including chimpanzee, tamarin, cattle, pig, dog, ferret, rabbit, mice, hamster, and rat, develop an immune response against foreign genes carried by recombinant adenoviruses [1, 10, 16, 22, 39, 60, 71, 75, 89, 90, 114, 115, 134].

CONCLUDING REMARKS

Up to now, many viruses have been used as viral vectors for delivery of foreign antigens. The development of these recombinant polyvalent or delivery vaccine may be of value in the veterinary field, for example, when attenuation of the target pathogen is not possible, when immunity to one or more pathogens must be raised at the same time, or when satisfactory quantities of the target pathogen or the live vaccine cannot be produced easily or safely. On the other hand, for their practical use, there might be still some potential problems that must be overcome, for example, safety issues such as reversion of virulence or recombination with field-type viruses, spread in the environment, genetic stability, and interference with pre-existing immunity to the vaccine vector.

To construct ideal recombinant viral vaccines, it is first necessary to select a good viral vector. The choice for such a viral vector should be determined by the host range of the vector, safety considerations, its replication properties, its stability, existing immunity that could diminish replication of the vector, the amount of foreign DNA which can be inserted without affecting infectivity, and the place where the primary immune reaction is induced by antigens expressed by the vector. Next, a suitable insertion site for expression of foreign antigen should be identified. As for replication competent vectors, the vector virus in which the expression cassette is inserted should preserve the full capacity of the parent virus to replicate at the primary site of infection and yet be completely attenuated. For expression of the foreign antigen, the selection of the promoter is also important. Finally, these recombinant virus should be readily examined in the target animal to assess the vaccine efficacy and safety.

Recent advances in molecular biology and immunology will allow the further strategies in development of next generation vaccines. The genesis of cell-mediated immune responses requires presentation of antigens by major histocompatibility complex (MHC) class I molecules, which is accomplished by processing of endogenously expressed antigens. Extracellular antigens produced by viral vectors can be targeted for presentation by MHC class I molecules and lead to the genesis of cytotoxic T lymphocyte responses.

By modifying the foreign gene inserted in viral vectors, it might be possible to enhance the affinity to MHC class I molecules and the immunogenicity of antigens. In addition, development of recombinant vaccines by insertion of homologous cytokine genes is also interesting as a new approach to induce more effective and lifelong protective immunity. In future, further development of recombinant polyvalent or delivery vaccine using better ideal vectors will be expected for veterinary use.

ACKNOWLEDGMENTS. We are grateful to Dr. L. N. Payne (Institute for Animal Health, Compton, Newbury, Berkshire) for critical reading of this manuscript and suggestions. These studies cited from this laboratory were supported by grants from the Ministry of Education, Science, Sports, and Culture, and from the Ministry of Agriculture, Forestry and Fisheries of Japan.

REFERENCES

- Adam, M., Lepottier, M. F., and Eloit, M. 1994. Vaccination of pigs with replication-defective adenovirus vectored vaccines: the example of pseudorabies. *Vet. Microbiol.* 42: 205–215.
- Alkhatib, G. and Briedis, D. J. 1988. High-level eucaryotic *in vivo* expression of biologically active measles virus hemagglutinin by using an adenovirus type 5 helper-free vector system. *J. Virol.* 62: 2718–2727.
- Babic, N., Mettenleiter, T. C., Ugolini, G., Flamand, A., and Coulon, P. 1994. Propagation of pseudorabies virus in the nervous system of the mouse after intranasal inoculation. *Virology* 204: 616–625.
- Baxy, D. and Paoletti, E. 1992. Potential use of non-replicating vectors as recombinant vaccines. *Vaccine* 10: 8–9.
- Bayliss, C. D., Peters, R. W., Cook, J. K. A., Reece, R. L., Howes, K., Binns, M. M., and Bournsnel, M. E. G. 1991. A recombinant fowlpox virus that expresses the VP2 antigen of infectious bursal disease virus induces protection against mortality caused by the virus. *Arch. Virol.* 120: 193–205.
- Beard, C. W., Schnitzlein, W. M., and Tripathy, D. N. 1991. Protection of chickens against highly pathogenic avian influenza virus (H5N2) by recombinant fowlpox viruses. *Avian Dis.* 35: 356–359.
- Bertagnoli, S., Gelfi, J., Petit, F., Vautherot, J. F., Rasschaert, D., Laurent, S., Le Gall, G., Boilletot, E., Chantal, J., and Boucraut-Baralon, C. 1996. Protection of rabbits against rabbit viral haemorrhagic disease with a vaccinia-RHDV recombinant virus. *Vaccine* 14: 506–510.
- Blancou, J., Kieny, M. P., Lathe, R., Lecocq, J. P., Pastoret, P. P., Soulebot, J. P., and Desmettre, P. 1986. Oral vaccination of the fox against rabies using a live recombinant vaccinia virus. *Nature (Lond.)* 322: 373–375.
- Bournsnel, M. E. G., Green, P. F., Campbell, J. I. A., Deuter, A., Peters, R. W., Tomley, F. M., Samson, A. C. R., Chambers, P., Emmerson, P. T., and Binns, M. M. 1990. Insertion of the fusion gene from Newcastle disease virus into a non-essential region in the terminal repeats of fowlpox virus and demonstration of protective immunity induced by the recombinant. *J. Gen. Virol.* 71: 621–628.
- Breker-Klassen, M. M., Yoo, D., Mittal, S. K., Sorden, S. D., Haines, D. M., and Babiuk, L. A. 1995. Recombinant type 5 adenoviruses expressing bovine parainfluenza virus type 3 glycoproteins protect *Sigmodon hispidus* cotton rats from bovine parainfluenza virus type 3 infection. *J. Virol.* 69: 4308–4315.
- Brochier, B., Kieny, M. P., Costy, F., Coppens, P., Bauduin, B., Lecocq, J. P., Languet, B., Chappuis, G., Desmettre, P., Afiademanyo, K., Libois, R., and Pastoret, P.-P. 1991. Largescale eradication of rabies using recombined vaccinia-rabies vaccine. *Nature (Lond.)* 354: 520–522.
- Brockmeier, S. L., Lager, K. M., Tartaglia, J., Riviere, M., Paoletti, E., and Mengeling, W. L. 1993. Vaccination of pigs against pseudorabies with highly attenuated vaccinia (NYVAC) recombinant viruses. *Vet. Microbiol.* 38: 41–58.
- Calvert, J. G., Nazerian, K., Witter, R. L., and Yanagida, N. 1993. Fowlpox virus recombinants expressing the envelope glycoprotein of an avian reticuloendotheliosis retrovirus induce neutralizing antibodies and reduce viremia in chickens. *J. Virol.* 67: 3069–3076.
- Cameron, J. M., McDougall, I., Marsden, H. S., Preston, V. G., Ryan, D. M., and Subak-Sharpe, J. H. 1988. Ribonucleotide reductase encoded by herpes simplex virus is a determinant of pathogenicity of the virus in mice and a valid antiviral target. *J. Gen. Virol.* 69: 2607–2612.
- Caravokyri, C., Pringle, C. R., and Leppard, K. N. 1993. Human adenovirus type 5 recombinants expressing simian immunodeficiency virus macaque strain *gag* antigens. *J. Gen. Virol.* 74: 2819–2824.
- Chengalvala, M. V., Bhat, B. M., Bhat, R., Lubeck, M. D., Mizutani, S., Davis, A. R., and Hung, P. P. 1994. Immunogenicity of high expression adenovirus-hepatitis B virus recombinant vaccines in dogs. *J. Gen. Virol.* 75: 125–131.
- Cole, G. E., Stacy-Phipps, S., and Nunberg, J. H. 1990. Recombinant feline herpesviruses expressing feline leukemia virus envelope and *gag* proteins. *J. Virol.* 64: 4930–4938.
- Cox, W. I., Tartaglia, J., and Paoletti, E. 1992. Poxvirus recombinants as live vaccines. pp. 123–162. *In: Recombinant Poxviruses* (Binns, M. M. and Smith, G. L. eds.), CRC Press, Boca Raton.
- Crandell, R. A., Fabricant, C. G., and Nelson-Rees, W. A. 1973. Development, characterization, and viral susceptibility of a feline (*Felis catrus*) renal cell line (CRFK). *In Vitro* 9: 176–185.
- Darteil, R., Bublot, M., Laplace, E., Bouquet, J.-F., Audonnet, J.-C., and Riviere, M. 1995. Herpesvirus of turkey recombinant viruses expressing infectious bursal disease virus (IBDV) VP2 immunogen induce protection against an IBDV virulent challenge in chickens. *Virology* 211: 481–490.
- Efstathiou, S., Kemp, S., Darby, G., and Minson, A. C. 1989. The role of herpes simplex virus type 1 thymidine kinase in pathogenesis. *J. Gen. Virol.* 70: 869–879.
- Eloit, M., Gilardi-Hebenstreit, P., Toma, B., and Perricaudet, M. 1990. Construction of a defective adenovirus vector expressing the pseudorabies virus glycoprotein gp50 and its use as a live vaccine. *J. Gen. Virol.* 71: 2425–2431.
- Enquist, L. W. 1994. Infection of the mammalian nervous system by pseudorabies virus (PRV). *Semin. Virol.* 5: 221–231.
- Esposito, J. J., Knight, J. C., Shaddock, J. H., Novembre, F. J., and Baer, G. M. 1988. Successful oral rabies vaccination of raccoons with raccoon poxvirus recombinants expressing rabies virus glycoprotein. *Virology* 165: 313–316.
- Field, H. J. and Wildy, P. 1978. The pathogenicity of thymidine kinase-deficient mutants of herpes simplex virus. *J. Hyg.*

- 81: 267–277.
26. Flores, E. F., Osorio, F. A., Zanella, E. L., Kit, S., and Kit, M. 1993. Efficacy of a deletion mutant bovine herpesvirus-1 (BHV-1) vaccine that allows serologic differentiation of vaccinated from naturally infected animals. *J. Vet. Diagn. Invest.* 5: 534–540.
27. Flowers, C. C. and O'Callaghan, D. J. 1992. The equine herpesvirus type 1 (EHV-1) homolog of herpes simplex virus type 1 US9 and the nature of a major deletion within the unique short segment of the EHV-1 KyA strain genome. *Virology* 190: 307–315.
28. Fooks, A. R., Schadeck, E., Liebert, U. G., Dowsett, A. B., Rima, B. K., Steward, M., Stephenson, J. R., and Wilkinson, G. W. G. 1995. High-level expression of the measles virus nucleocapsid protein by using a replication-deficient adenovirus vector: Induction of an MHC-1-restricted CTL response and protection in a murine model. *Virology* 210: 456–465.
29. Fries, L. F., Tartaglia, J., Taylor, J., Kauffman, E. K., Meignier, B., Paoletti, E., and Plotkin, S. 1996. Human safety and immunogenicity of a canarypox-rabies glycoprotein recombinant vaccine: An alternative poxvirus vector system. *Vaccine* 14: 428–434.
30. Gaskell, R. M. 1989. Vaccination of the young kitten. *J. Small Anim. Pract.* 30: 618–624.
31. Gerard, R. D. and Meidell, R. S. 1993. Adenovirus-mediated gene transfer. *Trends. Cardiovasc. Med.* 3: 171–177.
32. Giavedoni, L., Jones, L., Mebus, C., and Yilma, T. 1991. A vaccinia virus double recombinant expressing the F and H genes of rinderpest virus protects cattle against rinderpest and causes no pock lesions. *Proc. Natl. Acad. Sci. U.S.A.* 88: 8011–8015.
33. Graham, F. L., Smiley, J., Russel, W. C., and Nairn, R. 1977. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J. Gen. Virol.* 36: 59–72.
34. Grunhaus, A. and Horwitz, M. S. 1992. Adenoviruses as cloning vectors. *Semin. Virol.* 3: 237–252.
35. Heine, H.-G. and Boyle, D. B. 1993. Infectious bursal disease virus structural protein VP2 expressed by a fowlpox virus recombinant confers protection against disease in chickens. *Arch. Virol.* 131: 277–292.
36. Hertig, C., Pye, A. D., Hyatt, A. D., Davis, S. S., McWilliam, S. M., Heine, H. G., Walker, P. J., and Boyle, D. B. 1995. Vaccinia virus-expressed bovine ephemeral fever virus G but not G_{NS} glycoprotein induces neutralizing antibodies and protects against experimental infection. *J. Gen. Virol.* 77: 631–640.
37. Hill, T. G. 1985. Herpes simplex viruses latency. pp. 175–225. In: *The Herpesviruses* (Roizman, B. ed.), Plenum Press, New York and London.
38. Hooft van Iddekinge, B. J. L., De Wild, N., Wensvoort, G., Kimman, T. G., Gielkens, A. L. J., and Moormann, R. J. M. 1996. Comparison of the protective efficacy of recombinant pseudorabies viruses against pseudorabies and classical swine fever in pigs: Influence of different promoters on gene expression and on protection. *Vaccine* 14: 6–12.
39. Hsu, K.-H., Lubeck, M. D., Bhat, B. M., Bhat, R. A., Kostek, B., Selling, B. H., Mizutani, S., Davis, A. R., and Hung, P. P. 1994. Efficacy of adenovirus-vectored respiratory syncytial virus vaccines in a new ferret model. *Vaccine* 12: 607–612.
40. Hu, S.-L., Abrams, K., Barber, G. N., Moran, P., Zarling, J. M., Langlois, A. L., Kuller, L., Morton, W. R., and Benveniste, R. E. 1992. Protection of macaques against SIV infection by subunit vaccines of SIV envelope glycoprotein gp160. *Science* 255: 456–459.
41. Hu, L., Esposito, J. J., and Scott, F. W. 1996. Raccoon poxvirus feline panleukopenia virus VP2 recombinant protects cats against FPV challenge. *Virology* 218: 248–252.
42. Jacobs, L. 1994. Glycoprotein E of pseudorabies virus and homologous proteins in other *alphaherpesvirinae*. [Review]. *Arch. Virol.* 137: 209–228.
43. Johnson, D. C., Ghosh-Choudhury, G., Smiley, J. R., Fallis, L., and Graham, F. L. 1988. Abundant expression of herpes simplex virus glycoprotein gB using an adenovirus vector. *Virology* 164: 1–14.
44. Kawaguchi, Y., Maeda, K., Miyazawa, T., Ono, M., Kai, C., and Mikami, T. 1994. Nucleotide sequence and characterization of the feline herpesvirus type 1 immediate early gene. *Virology* 204: 430–435.
45. Kawaguchi, Y., Maeda, K., Ono, M., Yokoyama, N., Tsubota, K., Fujita, K., Tohya, Y., Kai, C., and Mikami, T. 1996. Characterization of the feline herpesvirus type 1 immediate early gene expression. *J. Vet. Med. Sci.* 58: 715–721.
46. Kawaguchi, Y., Maeda, K., Yokoyama, N., Fujita, K., Ono, M., Tohya, Y., and Mikami, T. 1997. Expression of the feline herpesvirus type 1 ICP4 gene is controlled by two alternative promoters. *Arch. Virol.* 142: 239–253.
47. Kimman, T. G., De Wind, N., Oei-Lei, N., Pol, J. M. A., Berns, A. J. M., and Gielkens, A. L. J. 1992. Contribution of single genes within the unique short region of Aujeszky's disease virus (suid herpesvirus type 1) to virulence, pathogenesis and immunogenicity. *J. Gen. Virol.* 73: 243–251.
48. Kimman, T. G., De Wind, N., De Bruin, T., De Visser, Y., and Voermans, J. 1994. Inactivation of glycoprotein gE and thymidine kinase or the US3-encoded protein kinase synergistically decreases *in vivo* replication of pseudorabies virus and the induction of protective immunity. *Virology* 205: 511–518.
49. Kit, S., Kit, M., and Pirtle, E. C. 1985. Attenuated properties of thymidine kinase-negative deletion mutant of pseudorabies virus. *Am. J. Vet. Res.* 46: 1359–1367.
50. Kit, S., Qavi, H., Gaines, J. D., Billingsley, P., and McConnell, S. 1985. Thymidine kinase-negative bovine herpesvirus type 1 mutant is stable and highly attenuated in calves. *Arch. Virol.* 86: 63–83.
51. Kit, S., Kit, M., and McConnell, S. 1986. Intramuscular and intravaginal vaccination of pregnant cows with thymidine kinase-negative, temperature-resistant infectious bovine rhinotracheitis virus (bovine herpesvirus 1). *Vaccine* 4: 55–61.
52. Kit, M., Kit, S., Little, S. P., Di Marchi, R. D., and Gale, C. 1991. Bovine herpesvirus-1 (infectious bovine rhinotracheitis virus)-based viral vector which expresses foot-and-mouth disease epitopes. *Vaccine* 9: 564–572.
53. Kit, S., Kit, M., Di Marchi, R. D., Little, S. P., and Gale, C. 1991. Modified-live infectious bovine rhinotracheitis virus vaccine expressing monomer and dimer forms of foot-and-mouth disease capsid protein epitopes on surface of hybrid virus particles. *Arch. Virol.* 120: 1–17.
54. Kit, M. and Kit, S. 1991. Sensitive glycoprotein gIII blocking ELISA to distinguish between pseudorabies (Aujeszky's disease)-infected and vaccinated pigs. *Vet. Microbiol.* 28: 141–155.
55. Kit, S., Otsuka, H., and Kit, M. 1992. Expression of porcine pseudorabies virus genes by a bovine herpesvirus-1 (infectious bovine rhinotracheitis virus) vector. *Arch. Virol.* 124:

- 1–20.
56. Kritas, S. K., Pensaert, M. B., and Mettenleiter, T. C. 1994. Role of envelope glycoproteins gI, gp63 and gIII in the invasion and spread of Aujeszky's disease virus in the olfactory nervous pathway of the pig. *J. Gen. Virol.* 75: 2319–2327.
57. Kruger, J. M., Sussman, M. D., and Maes, R. K. 1996. Glycoproteins gI and gE of feline herpesvirus-1 are virulence genes: Safety and efficacy of a gI-gE deletion mutant in the natural host. *Virology* 220: 299–308.
58. Letellier, C., Burny, A., and Meulemans, G. 1991. Construction of a pigeonpox virus recombinant: Expression of the Newcastle disease virus (NDV) fusion glycoprotein and protection of chickens against NDV challenge. *Arch. Virol.* 118: 43–56.
59. Lodmell, D. L., Summer, J. W., Esposito, J. J., Bellini, W. J., and Ewalt, L. C. 1991. Raccoon poxvirus recombinants expressing the rabies virus nucleoprotein protect mice against lethal rabies virus infection. *J. Virol.* 65:3400–3405.
60. Lubeck, M. D., Natuk, R. J., Chengalvala, M., Chanda, P. K., Murthy, K. K., Murthy, S., Mizutani, S., Lee, S.-G., Wada, M. S., Bhat, B. M., Bhat, R., Dheer, S. K., Eichberg, J. W., Davis, A. R., and Hung, P. P. 1994. Immunogenicity of recombinant adenovirus-human immunodeficiency virus vaccines in chimpanzees following intranasal administration. *AIDS Res. Hum. Retroviruses* 10: 1443–1449.
61. Maeda, K., Horimoto, T., Norimine, J., Kawaguchi, Y., Tomonaga, K., Niikura, M., Kai, C., Takahashi, E., and Mikami, T. 1992. Identification and nucleotide sequence of a gene in feline herpesvirus type 1 homologous to the herpes simplex virus gene encoding the glycoprotein B. *Arch. Virol.* 127: 387–397.
62. Maeda, K., Kawaguchi, Y., Kamiya, N., Ono, M., Tohya, Y., Kai, C., and Mikami, T. 1993. Identification and nucleotide sequence of a gene in feline herpesvirus type 1 homologous to the herpes simplex virus gene encoding the glycoprotein H. *Arch. Virol.* 132: 183–191.
63. Maeda, K., Kawaguchi, Y., Ono, M., Inoshima, Y., Miyazawa, T., Tohya, Y., Kai, C., and Mikami, T. 1994. A gD homologous gene of feline herpesvirus type 1 encodes a hemagglutinin (gp60). *Virology* 202: 1034–1038.
64. Maeda, K., Kawaguchi, Y., Ono, M., Tajima, T., and Mikami, T. 1995. Restriction endonuclease analysis of field isolates of feline herpesvirus type 1 and identification of the heterogeneous regions. *J. Clin. Microbiol.* 33: 217–221.
65. Maeda, K., Kawaguchi, Y., Ono, M., Tajima, T., and Mikami, T. 1995. Comparisons among feline herpesvirus type 1 isolates by immunoblot analysis. *J. Vet. Med. Sci.* 57: 147–150.
66. Maeda, K., Hirasawa, K., Kawaguchi, Y., Ono, M., Mori, T., Gemma, T., Yokoyama, N., Doi, K., and Mikami, T. 1995. Expression and identification of the feline herpesvirus type 1 glycoprotein B (gp143/108). *Virus Res.* 39: 55–61.
67. Maeda, K., Ono, M., Kawaguchi, Y., Okazaki, M., Yokoyama, N., Tohya, Y., and Mikami, T. 1997. Adhesion of insect cells expressing the feline herpesvirus type 1 hemagglutinin (gD) to feline cell lines. *J. Vet. Med. Sci.* 59: 217–219.
68. Maeda, K., Yokoyama, N., Fujita, K., and Mikami, T. 1996. Identification and characterization of the feline herpesvirus type 1 glycoprotein C gene. *Virus Genes*: (in press).
69. Mahnel, H. 1989. Swinepox, pp. 5–15. In: *Virus Infections of Porcines* (Pensaert, ed.), Elsevier Science, Amsterdam.
70. Mijnes, J. D. F., Van der Horst, L. M., Van Anken, E., Horzinek, M. C., Rottier, P. J. M., and De Groot, R. J. 1996. Biosynthesis of glycoproteins E and I of feline herpesvirus: gE-gI interaction is required for intracellular transport. *J. Virol.* 70: 5466–5475.
71. Mittal, S. K., Papp, Z., Tikoo, S. K., Baca-Estrada, M. E., Yoo, D., Benko, M., and Babiuk, L. A. 1996. Induction of systemic and mucosal immune responses in cotton rats immunized with human adenovirus type 5 recombinants expressing the full and truncated forms of bovine herpesvirus type 1 glycoprotein gD. *Virology* 222: 299–309.
72. Moormann, R. J. M., De Rover, T., Briaire, J., Peeters, B. P. H., Gielkens, A. L. J., and Van Oirschot, J. T. 1990. Inactivation of the thymidine kinase gene of a gI deletion mutant of pseudorabies virus generates a safe but still highly immunogenic vaccine strain. *J. Gen. Virol.* 71: 1591–1595.
73. Morgan, R. W., Gelb, J. J., Schreurs, C. S., Lütticken, D., Rosenberger, J. K., and Sondermeijer, P. J. A. 1992. Protection of chickens from Newcastle and Marek's diseases with a recombinant herpesvirus of turkeys vaccine expressing the Newcastle disease virus fusion protein. *Avian Dis.* 36: 858–870.
74. Morgan, R. W., Gelb, J. J., Pope, C. R., and Sondermeijer, P. J. A. 1993. Efficacy in chickens of a herpesvirus of turkeys recombinant vaccine containing the fusion gene of Newcastle disease virus: Onset of protection and effect of maternal antibodies. *Avian Dis.* 37: 1032–1040.
75. Morin, J. E., Lubeck, M. D., Barton, J. E., Conley, A. J., Davis, A. R., and Hung, P. P. 1987. Recombinant adenovirus induces antibody response to hepatitis B virus surface antigen in hamsters. *Proc. Natl. Acad. Sci. U.S.A.* 84: 4626–4630.
76. Moss, B., Smith, G. L., Gerin, J. L., and Purcell, R. H. 1984. Live recombinant vaccinia virus protects chimpanzees against hepatitis B. *Nature (Lond.)* 311: 67–69.
77. Moss, B. 1993. Poxvirus vectors: cytoplasmic expression of transferred genes. *Curr. Opin. Genet. Dev.* 3: 86–90.
78. Mulder, W. A. M., Priem, J., Glazenburg, K. L., Wagenaar, F., Gruys, E., Gielkens, A. L. J., Pol, J. M. A., and Kimman, T. G. 1994. Virulence and pathogenesis of non-virulent and virulent strains of pseudorabies virus expressing envelope glycoprotein E1 of hog cholera virus. *J. Gen. Virol.* 75: 117–124.
79. Mulder, W. A. M., Jacobs, L., Priem, J., Kok, G. L., Wagenaar, F., Kimman, T. G., and Pol, J. M. A. 1994. Glycoprotein gE-negative pseudorabies virus has a reduced capability to infect second- and third-order neurons of the olfactory and trigeminal routes in the porcine central nervous system. *J. Gen. Virol.* 75: 3095–3106.
80. Murphy, B. R. and Chanock, R. M. 1985. Immunization against viruses. pp. 349–370. In: *Virology* (Fields, B. N. ed.), Raven, New York.
81. Nazerian, K., Lee, L. F., Yanagida, N., and Ogawa, R. 1992. Protection against Marek's disease by a fowlpox virus recombinant expressing the glycoprotein B of Marek's disease virus. *J. Virol.* 66: 1409–1413.
82. Nazerian, K. and Yanagida, N. 1995. A recombinant fowlpox virus expressing the envelope antigen of subgroup A avian leukosis/sarcoma virus. *Avian Dis.* 39: 514–520.
83. Nunberg, J. H., Wright, D. K., Cole, G. E., Petrovskis, E. A., Post, L. E., Compton, T., and Gilbert, J. H. 1989. Identification of the thymidine kinase gene of feline herpesvirus: Use of degenerate oligonucleotides in the polymerase chain reaction to isolate herpesvirus gene homologs. *J. Virol.* 63: 3240–3249.
84. Ogawa, R., Yanagida, N., Saeki, S., Saito, S., Ohkawa, S.,

- Gotoh, H., Kodama, K., Kamogawa, K., Sawaguchi, K., and Iritani, Y. 1990. Recombinant fowlpox virus inducing protective immunity against Newcastle disease and fowlpox virus. *Vaccine* 8: 486–490.
85. Okamura, H., Sakaguchi, M., Honda, T., Taneno, A., Matsuo, K., and Yamada, S. 1994. Construction of recombinant infectious laryngotracheitis virus expressing the lacZ gene of *E. coli* with thymidine kinase gene. *J. Vet. Med. Sci.* 56: 799–801.
 86. Okazaki, W., Purchase, H. G., and Burmester, B. R. 1970. Protection against Marek's disease by vaccination with a herpesvirus of turkeys. *Avian Dis.* 14: 413–429.
 87. Peeters, B., Bouma, A., De Bruin, T., Moormann, R., Gielkens, A., and Kimman, T. 1994. Non-transmissible pseudorabies virus gp50 mutants: A new generation of safe live vaccines. *Vaccine* 12: 375–380.
 88. Petrovskis, E. A., Timmins, J. G., Gierman, T. M., and Post, L. E. 1986. Deletions in vaccine strains of pseudorabies virus and their effect on synthesis of glycoprotein gp63. *J. Virol.* 60: 1166–1169.
 89. Prevec, L., Schneider, M., Rosenthal, K. L., Belbeck, L. W., Derbyshire, J. B., and Graham, F. L. 1989. Use of human adenovirus-based vectors for antigen expression in animals. *J. Gen. Virol.* 70: 429–434.
 90. Prevec, L., Campbell, J. B., Christie, B. S., Belbeck, L., and Graham, F. L. 1990. A recombinant human adenovirus vaccine against rabies. *J. Infect. Dis.* 161: 27–30.
 91. Pyles, R. B., Sawtell, N. M., and Thompson, R. L. 1992. Herpes simplex virus type 1 dUTPase mutants are attenuated for neurovirulence, neuroinvasiveness, and reactivation from latency. *J. Virol.* 66: 6706–6713.
 92. Ragot, T., Finerty, S., Watkins, P. E., Perricaudet, M., and Morgan, A. J. 1993. Replication-defective recombinant adenovirus expressing the Epstein-Barr virus (EBV) envelope glycoprotein gp340/220 induces protective immunity against EBV-induced lymphomas in the cottontop tamarin. *J. Gen. Virol.* 74: 501–507.
 93. Roizman, B. 1991. Herpesviridae. pp. 103–110. In: Classification and Nomenclature of Viruses. Arch. Virol. [Suppl. 2] (Francki, R. I. B., Fauquet, C. M., Knudson, D. L., and Brown, F. eds.), Springer-Verlag, Wien, New York.
 94. Romero, C. H., Barrett, T., Evans, S. A., Kitching, R. P., Gershon, P. D., Bostock, C., and Black, D. N. 1993. Single capripoxvirus recombinant vaccine for the protection of cattle against rinderpest and lumpy skin disease. *Vaccine* 11: 737–742.
 95. Romero, C. H., Barrett, T., Chamberlain, R. W., Kitching, R. P., Fleming, M., and Black, D. N. 1994. Recombinant capripoxvirus expressing the hemagglutinin protein gene of rinderpest virus: Protection of cattle against rinderpest and lumpy skin disease virus. *Virology* 204: 425–429.
 96. Romero, C. H., Barrett, T., Kitching, R. P., Carn, V. M., and Black, D. N. 1994. Protection of cattle against rinderpest and lumpy skin disease with a recombinant capripoxvirus expressing the fusion protein gene of rinderpest virus. *Vet. Rec.* 135: 152–154.
 97. Romero, C. H., Barrett, T., Kitching, R. P., Bostock, C., and Black, D. N. 1995. Protection of goats against peste des petits ruminants with recombinant capripoxviruses expressing the fusion and haemagglutinin protein genes of rinderpest virus. *Vaccine* 13: 36–40.
 98. Ross, L. J. N., Binns, M. M., Tyers, P., Pastorek, J., Zelnik, V., and Scott, S. 1993. Construction and properties of a turkey herpesvirus recombinant expressing the Marek's disease virus homologue of glycoprotein B of herpes simplex virus. *J. Gen. Virol.* 74: 371–377.
 99. Shih, M.-F., Arsenakis, M., Tiollais, P., and Roizman, B. 1984. Expression of hepatitis B virus S gene by herpes simplex virus type 1 vectors carrying α - and β -regulated gene chimeras. *Proc. Natl. Acad. Sci. U.S.A.* 81: 5867–5870.
 100. Siebelink, K. H. J., Tijhaar, E., Huisman, R. C., Huisman, W., De Ronde, A., Darby, I. H., Francis, M. J., Rimmelzwaan, G. F., and Osterhaus, A. D. M. E. 1995. Enhancement of feline immunodeficiency virus infection after immunization with envelope glycoprotein subunit vaccines. *J. Virol.* 69: 3704–3711.
 101. Slater, J. D., Gibson, J. S., and Field, H. J. 1993. Pathogenicity of a thymidine kinase-deficient mutant of equine herpesvirus 1 in mice and specific pathogen-free foals. *J. Gen. Virol.* 74: 819–828.
 102. Smith, G. A., Young, P. L., Rodwell, B. J., Kelly, M. A., Storie, G. J., Farrah, C. A., and Mattick, J. S. 1994. Development and trial of a bovine herpesvirus 1-thymidine kinase deletion virus as a vaccine. *Aust. Vet. J.* 71: 65–70.
 103. Sondermeijer, P. J. A., Claessens, J. A. J., Jenniskens, P. E., Mockett, A. P. A., Thijssen, R. A. J., Willemse, M. J., and Morgan, R. W. 1993. Avian herpesvirus as a live viral vector for the expression of heterologous antigens. *Vaccine* 11: 349–357.
 104. Spatz, S. J. and Maes, R. K. 1993. Immunological characterization of the feline herpesvirus-1 glycoprotein B and analysis of its deduced amino acid sequence. *Virology* 197: 125–136.
 105. Spatz, S. J., Rota, P. A., and Maes, R. K. 1994. Identification of the feline herpesvirus type 1 (FHV-1) genes encoding glycoproteins G, D, I and E: Expression of FHV-1 glycoprotein D in vaccinia and raccoon poxviruses. *J. Gen. Virol.* 75: 1235–1244.
 106. Sussman, M. D., Maes, R. K., Kruger, J. M., Spatz, S. J., and Venta, P. J. 1995. A feline herpesvirus-1 recombinant with a deletion in the genes for glycoproteins gI and gE is effective as a vaccine for feline rhinotracheitis. *Virology* 214: 12–20.
 107. Taylor, J., Weinberg, R., Kawaoka, Y., Webster, R. G., and Paoletti, E. 1988. Protective immunity against avian influenza induced by a fowlpox virus recombinant. *Vaccine* 6: 504–508.
 108. Taylor, J., Weinberg, R., Languet, B., Desmettre, P., and Paoletti, E. 1988. Recombinant fowlpox virus inducing protective immunity in non-avian species. *Vaccine* 6: 497–503.
 109. Taylor, J., Ebdauer, C., Rey-Senelongue, A., Bouquet, J.-F., Norton, E., Goebel, S., Desmettre, P., and Paoletti, E. 1990. Newcastle disease virus fusion protein expressed in a fowlpox virus recombinant confers protection in chickens. *J. Virol.* 64: 1441–1450.
 110. Taylor, J., Trimarchi, C., Weinberg, R., Languet, B., Guillemin, F., Desmettre, P., and Paoletti, E. 1991. Efficacy studies on a canarypox-rabies recombinant virus. *Vaccine* 9: 190–193.
 111. Taylor, J., Weinberg, R., Tartaglia, J., Richardson, C., Alkhatib, G., Briedis, D., Appel, M., Norton, E., and Paoletti, E. 1992. Nonreplicating viral vectors as potential vaccines: Recombinant canarypox virus expressing measles virus fusion (F) and hemagglutinin (HA) glycoproteins. *Virology* 187: 321–328.
 112. Tenser, R. B., Ressel, S. J., Fralish, F. A., and Jones, J. C. 1983. The role of pseudorabies virus thymidine kinase expression in trigeminal ganglion infection. *J. Gen. Virol.* 64:

- 1369–1373.
113. Tikoo, S. K., Campos, M., and Babiuk, L. A. 1995. Bovine herpesvirus 1: Biology, pathogenesis, and control. *Adv. Virus. Res.* 45: 191–223.
114. Torres, J. M., Sánchez, C., Suñe, C., Smerdou, C., Prevec, L., Graham, F., and Enjuanes, L. 1995. Induction of antibodies protecting against transmissible gastroenteritis coronavirus (TGEV) by recombinant adenovirus expressing TGEV spike protein. *Virology* 213: 503–516.
115. Torres, J. M., Alonso, C., Ortega, A., Mittal, S., Graham, F., and Enjuanes, L. 1996. Tropism of human adenovirus type 5-based vectors in swine and their ability to protect against transmissible gastroenteritis coronavirus. *J. Virol.* 70: 3770–3780.
116. Van der Leek, M. L., Feller, J. A., Sorensen, G., Isaacson, W., Adams, C. L., Rorde, D. J., Pfeiffer, N., Tran, T., Moyer, R. W., and Gibbs, E. P. J. 1994. Evaluation of swinepox virus as a vaccine vector in pigs using an Aujeszky's disease (pseudorabies) virus gene insert coding for glycoproteins gp50 and gp63. *Vet. Rec.* 134: 13–18.
117. Van Oirschot, J. T., Gielkens, A. L. J., Moormann, R. J. M., and Berns, A. J. M. 1990. Marker vaccines, virus protein-specific antibody assays and the control of Aujeszky's disease. [Review]. *Vet. Microbiol.* 23: 85–101.
118. Van Zijl, M., Wensvoort, G., De Kluyver, E., Hulst, M., Van der Gulden, H., Gielkens, A., Berns, A., and Moormann, R. 1991. Live attenuated pseudorabies virus expressing envelope glycoprotein E1 of hog cholera virus protects swine against both pseudorabies and hog cholera. *J. Virol.* 65: 2761–2765.
119. Verschoor, E. J., Willemse, M. J., Stam, J. G., Van Vliet, A. L. W., Pouwels, H., Chalmers, S. K., Horzinek, M. C., Sondermeijer, P. J. A., Hesselink, W., and De Ronde, A. 1996. Evaluation of subunit vaccines against feline immunodeficiency virus infection. *Vaccine* 14: 285–289.
120. Visser, N. and Lütticken, D. 1989. Experiences with a gI/TK⁻ modified live pseudorabies virus vaccine: strain Begona. pp. 37–44. *In: Vaccination and Control of Aujeszky's Disease* (Van Oirschot, J. T. ed.), Kluwer Academic Publishers, Dordrecht.
121. Wade-Evans, A. M., Romero, C. H., Mellor, P., Takamatsu, H., Anderson, J., Thevasagayam, J., Fleming, M. J., Mertens, P. P. C., and Black, D. N. 1996. Expression of the major core structural protein (VP7) of bluetongue virus, by a recombinant capripox virus, provides partial protection of sheep against a virulent heterotypic bluetongue virus challenge. *Virology* 220: 227–231.
122. Wardley, R. C., Berlinski, P. J., Thomsen, D. R., Meyer, A. L., and Post, L. E. 1992. The use of feline herpesvirus and baculovirus as vaccine vectors for the *gag* and *env* genes of feline leukaemia virus. *J. Gen. Virol.* 73: 1811–1818.
123. Wild, F., Giraudon, P., Spehner, D., Drillien, R., and Lecoq, J. P. 1990. Fowlpox virus recombinant encoding the measles virus fusion protein: Protection of mice against fatal measles encephalitis. *Vaccine* 8: 441–442.
124. Willemse, M. J., Chalmers, W. S. K., Cronenberg, A. M., Pfundt, R., Strijde, I. G. L., and Sondermeijer, P. J. A. 1994. The gene downstream of the gC homologue in feline herpesvirus type 1 is involved in the expression of virulence. *J. Gen. Virol.* 75: 3107–3116.
125. Willemse, M. J., Strijde, I. G. L., Van Schooneveld, S. H. B., Van den Berg, M. C., and Sondermeijer, P. J. A. 1995. Transcriptional analysis of the short segment of the feline herpesvirus type 1 genome and insertional mutagenesis of a unique reading frame. *Virology* 208: 704–711.
126. Willemse, M. J., Chalmers, W. S. K., and Sondermeijer, P. J. A. 1996. *In vivo* properties of a feline herpesvirus type 1 mutant carrying a lacZ insertion at the gI locus of the unique short segment. *Vaccine* 14: 1–5.
127. Willemse, M. J., Van Schooneveld, S. H. B., Chalmers, W. S. K., and Sondermeijer, P. J. A. 1996. Chapter 6: Vaccination against feline leukaemia using a new feline herpesvirus type 1 vector. pp. 75–86. *In: Genetically Modified Feline Herpesvirus for Oronasal Vaccination of Cats* (Willemse, M. J. ed.), Universiteit Utrecht, Utrecht.
128. Xiang, Z. Q., Yang, Y., Wilson, J. M., and Ertl, H. C. J. 1996. A replication-defective human adenovirus recombinant serves as a highly efficacious vaccine carrier. *Virology* 219: 220–227.
129. Yilma, T. 1994. Genetically engineered vaccines for animal viral diseases. *J. Am. Vet. Med. Assoc.* 204: 1606–1615.
130. Yokoyama, N., Maeda, K., Kawaguchi, Y., Ono, M., Tohya, Y., and Mikami, T. 1995. Construction of the recombinant feline herpesvirus type 1 deleted thymidine kinase gene. *J. Vet. Med. Sci.* 57: 709–714.
131. Yokoyama, N., Maeda, K., Tohya, Y., Kawaguchi, Y., Shin, Y.-S., Ono, M., Ishiguro, S., Fujikawa, Y., and Mikami, T. 1996. Pathogenicity and vaccine efficacy of a thymidine kinase-deficient mutant of feline herpesvirus type 1 in cats. *Arch. Virol.* 141: 481–494.
132. Yokoyama, N., Maeda, K., Tohya, Y., Kawaguchi, Y., Fijita, K., and Mikami, T. 1996. Recombinant feline herpesvirus type 1 expressing immunogenic proteins inducible virus neutralizing antibody against feline calicivirus in cats. *Vaccine* 14: 1657–1663.
133. Yokoyama, N., Maeda, K., Fujita, K., Ishiguro, S., Sagawa, T., Mochizuki, M., Tohya, Y., and Mikami, T. 1996. Vaccine efficacy of recombinant feline herpesvirus type 1 expressing immunogenic proteins of feline calicivirus in cats. *Arch. Virol.* 141: 2339–2351.
134. Yoo, D., Graham, F. L., Prevec, L., Parker, M. D., Benko, M., Zamb, T., and Babiuk, L. A. 1992. Synthesis and processing of the haemagglutinin-esterase glycoprotein of bovine coronavirus encoded in the E3 region of adenovirus. *J. Gen. Virol.* 73: 2591–2600.
135. Young, P. L. and Smith, G. A. 1995. Genetically altered herpesviruses as vaccines. *Vet. Microbiol.* 46: 175–179.