New approaches to the development of virus vaccines for veterinary use

K. Yamanouchi (1), T. Barrett (2) & C. Kai (3)

(1) Nippon Institute for Biological Science, 2221-1 Shin-machi, Ome, Tokyo 198, Japan
(2) Institute for Animal Health, Pirbright Laboratory, Ash Road, Woking, GU24 ONF, United Kingdom
(3) Department of Veterinary Microbiology, Faculty of Agriculture, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

Summary
The marked progress in recombinant deoxyribonucleic acid (DNA) technology during the past decade has led to the development of a variety of safe new vaccine vectors which are capable of efficiently expressing foreign immunogens. These have been based on a variety of virus types - poxviruses, herpesviruses and adenoviruses - and have led to the production of many new potential recombinant vaccines. Of these recombinant vaccines, the rabies vaccine, in which the rabies G protein is expressed in a vaccinia vector, has been widely used in the field to prevent the spread of rabies both in Europe and in the United States of America. A recombinant Newcastle disease virus vaccine, using fowlpox virus as the vector to express immunogenic proteins from the Newcastle disease virus, has been licensed as the first commercial recombinant vectored vaccine. Many other recombinant virus vaccines are still at the stage of laboratory or field testing.

The most recent breakthrough in vaccinology has been the success with the use of naked DNA as a means of vaccination. This approach has shown great promise in mouse model systems and has now become the most active field in new vaccine development. Molecular redesigning of conventional ribonucleic acid (RNA) viruses to obtain more stable attenuated vaccines was previously possible only for positive-strand RNA viruses, such as poliovirus. However, recent advances in molecular biological techniques have enabled the rescuing of negative-strand viruses from DNA copies of their genomes. This has made it possible to engineer specific changes in the genomes of Rhabdoviridae and Paramyxoviridae, both of which include several viruses of veterinary importance. The authors describe the current progress in the development of vector vaccines, DNA vaccines and vaccines based on engineered positive- and negative-strand RNA virus genomes, with special emphasis on their application to diseases of veterinary importance.

Keywords

Introduction
In 1796, Edward Jenner conducted the first smallpox vaccination using material obtained from a lesion on a cow suffering from cowpox. Subsequently, the vaccine was maintained by arm-to-arm passage until the mid-1840s, when the technique for the production of smallpox vaccine on the skin of calves or sheep was developed by Negri, a medical doctor in Naples (3). This was the first use of animals for large-scale vaccine production. Thereafter, numerous vaccines for both human and animal use were produced in animals.

The next breakthrough in technology came in the mid-1950s, when chicken embryos and tissue culture cells were adapted for the production of vaccines. However, the basic concept
remained unchanged, i.e. virus growth in animals or cells derived from animals. It is noteworthy that the global eradication of smallpox, a landmark in the history of microbiology, was achieved using only the oldest type of vaccine, produced in the skin of calves and sheep.

Advances achieved in recombinant deoxyribonucleic acid (DNA) technology in the 1970s opened a new era in vaccine development. Instead of growing viruses in animals or cells, individual protective antigens could be expressed in large quantities in mammalian or insect cells or in bacteria, the so-called second generation of subunit vaccines. Owing to an inadequate immune response to viral proteins produced in this way, very few successes have been achieved, in spite of great efforts over the past 20 years. However, recombinant DNA technology also made it possible to use safe virus vectors for the expression of protective antigens from dangerous pathogens, i.e. recombinant vector vaccines. Unlike the recombinant subunit vaccines, recombinant vector vaccines have been shown to result in highly immunogenic responses to the foreign protein. A recombinant rabies vaccine, which uses vaccinia virus as the vector, has already been widely used in areas of endemic rabies in Western Europe. Many other recombinant vector vaccines have proved their usefulness in laboratory trials, and great efforts are being made to improve their safety for field use.

Most recombinant vaccines have been based on DNA virus (poxvirus) vectors but another approach has been to use conventional ribonucleic acid (RNA) virus vaccines, such as the poliovirus vaccine, to express immunogenic epitopes from other viruses for which no effective live attenuated vaccines exist. This is a relatively easy task, since the virion RNA produced from the modified DNA copy of the genome of positive-strand virus is infectious when injected into susceptible cells. In the case of negative-strand viruses, the task is more difficult since the genome RNA is not infectious. However, recent advances in molecular techniques have enabled this difficulty to be overcome, and it is now possible to produce infectious virus from DNA copies of RNA viruses, such as rabies, Sendai virus, measles, rinderpest and respiratory syncytial virus (13). Extra reading frames have been incorporated into these genomes and it is now possible to produce genetically defined and marked vaccines and to use these viruses as vectors for other immunogenic proteins.

In 1990, a unique approach to vaccination was reported, i.e. the use of naked DNA encoding immunogenic proteins as vaccines. Over the past seven years, DNA vaccines have been produced for a variety of diseases and tested in the laboratory with considerable success. These advances in molecular technology have created new opportunities to produce safer, genetically defined vaccines for both human and veterinary use. In this paper the present situation regarding the development and testing of virus vector vaccines and DNA vaccines, as well as the potential usefulness of the rescue system of negative-strand RNA viruses, will be reviewed.

## Vector vaccines based on deoxyribonucleic acid viruses

Following the demonstration in 1982 that foreign antigens could be expressed using vaccinia virus (26, 35), many attempts have been made to develop a variety of new vaccines using vaccinia virus as a vector, especially in the field of veterinary medicine. Table I summarises the examples of vector vaccines developed for veterinary use.

At first, the smallpox vaccine, which was used in the campaign for the global eradication of smallpox, was used as the vector. However, the low morbidity and mortality rate associated with smallpox vaccination was considered to pose too great a risk to make such recombinant vaccines acceptable for widespread human use. This is particularly the case in areas of the world where large sections of the population are immunocompromised due to high rates of human immunodeficiency virus (HIV) infection. More attenuated strains of the vaccine were investigated and, more recently, vaccinia virus attenuated by genetic manipulation was constructed, in the search for suitable vectors for the expression of immunogenic foreign proteins for veterinary and human use. The concept of vector attenuation has been developed still further by the use of poxviruses that do not replicate in the target host, i.e. avipox viruses in mammals. Other large DNA viruses, such as the herpesviruses, have been used as vectors for particular animal species. These include Aujeszky's disease (pseudorabies) virus for use in pigs, herpesvirus of turkeys and feline and canine herpesviruses.

Adenovirus, a smaller DNA virus, has also been used as an expression vector. However, due to technical difficulties, particularly the tight constraints on the size of DNA insert that can be accommodated in the genome, construction of recombinant adenoviruses has been limited. Adenoviruses expressing foreign proteins are considered suitable as vectors in 'gene therapy' and this has led to technical improvements in the vectors now available. In the future, adenovirus-recombinant vaccines may be more widely tested, particularly as they have the potential to elicit a strong mucosal immune response.

## Recombinant vaccines based on mammalian poxvirus vectors

Although the pioneering work on recombinant vaccine production was conducted using vaccinia virus, the only vaccinia-based recombinant virus licensed as a veterinary vaccine is the rabies-recombinant vaccine. This vaccine is used for the oral vaccination against rabies of foxes in Europe and raccoons and striped skunks in North America; these species being the most important natural reservoirs of rabies.
on their respective continents. The recombinant rabies vaccine was developed by inserting the glycoprotein G gene of rabies virus into the Copenhagen strain of vaccinia virus, one of the strains used as a smallpox vaccine (7). The greater resistance to environmental temperature fluctuations of vaccinia recombinants, in relation to the naturally attenuated rabies strain used in domestic dogs, makes them ideal vaccines for spiking baits, which are then distributed from the air. The vaccine maintains its potency for many months, even in conditions where it is frequently frozen and thawed. Previous attempts to control sylvatic rabies in Europe using attenuated rabies in baits for oral vaccination were not very effective, due to vaccine instability. The recombinant vaccine very effectively solved this problem.

The demonstration of successful oral vaccination of foxes with the rabies-recombinant vaccine in 1986 was followed by extensive investigations into the safety of this vaccine for domestic, laboratory and wild animals. Tests were first conducted under laboratory conditions. These were followed by carefully controlled and monitored field tests performed in Western Europe to vaccinate wild foxes. Similar tests, in which raccoons and coyotes were vaccinated, were later conducted in the United States of America (USA). Between 1989 and 1995, approximately 8.5 million doses were dispersed in these areas without any problems, demonstrating the effectiveness of wildlife vaccination using recombinant vaccines on a large scale. These field studies have been described in detail in a recent review (9). An important feature of the campaign was to persuade the public that the vaccine was safe and so gain public approval for the widespread use of vaccinia-based recombinant vaccines (36).

Other important veterinary diseases, for which the existing vaccine is unstable or safely attenuated strains of the pathogen are not available, could also benefit from the use of recombinant vaccines. Rinderpest is one of the most important contagious viral diseases of domestic animals, affecting mainly cattle and buffalo. A major obstacle to the campaign to eradicate rinderpest is the heat-labile nature of the current rinderpest vaccine, despite its very high efficacy. With the aim of producing a more heat-stable vaccine, three different recombinant rinderpest vaccines (RRVs) have been produced.

The first expresses the H protein gene of rinderpest virus, using the LC16mO attenuated smallpox vaccine strain as the vector (57). The second expresses both the H and F protein genes of rinderpest virus, using the Wyeth smallpox vaccine strain as the vector (19). The third is a mixture of two separate recombinants, which expresses either the H or the F protein gene of rinderpest virus, using an attenuated vaccine strain of capripox virus as a vector (32). The RRV (LC16mO) vaccine has been extensively tested under laboratory conditions to establish its efficacy in protecting against challenge with virulent rinderpest virus. Its safety in cattle, genetic stability through several passages in cattle and long-lasting immunity,

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Vector</th>
<th>Inserted gene</th>
<th>Remarks</th>
<th>Reference</th>
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<td>Rabies</td>
<td>Vaccinia</td>
<td>G</td>
<td>Widely used in the field</td>
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<td>Rinderpest</td>
<td>Vaccinia (LC16mO)</td>
<td>H</td>
<td>Ready for field testing</td>
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<tr>
<td></td>
<td>Vaccinia (Wyeth)</td>
<td>H, F</td>
<td>Also effective on lumpy skin disease</td>
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<tr>
<td>Bovine leukaemia</td>
<td>Vaccinia (LC15mO)</td>
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<td>(34)</td>
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<td>Vaccinia (NYVAC)</td>
<td>gD</td>
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<tr>
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<td>Swinepox</td>
<td>gp50, gp53</td>
<td>Partial protection</td>
<td>(50)</td>
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<tr>
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<td>Fowlpox</td>
<td>HA</td>
<td>Also effective on fowlpox. Licensed</td>
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<td>Fowlpox</td>
<td>HN</td>
<td>Also effective on fowlpox</td>
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<td>Infectious bursal disease (Gumboro disease)</td>
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<td>VP2</td>
<td>Also effective on fowlpox</td>
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<td>Canarypox</td>
<td>G</td>
<td>Non-replicative</td>
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<td>Viral haemorrhagic disease of rabbits</td>
<td>Canarypox</td>
<td>Capsid</td>
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<td>gpE1</td>
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<td>(60)</td>
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<tr>
<td>Feline leukaemia</td>
<td>Feline herpes 1</td>
<td>gp70/p15</td>
<td>Also effective on Marek’s disease</td>
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<td>Newcastle disease</td>
<td>Herpesvirus of turkeys</td>
<td>HN</td>
<td></td>
<td>(31)</td>
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<tr>
<td>Viral haemorrhagic disease of rabbits</td>
<td>(Marek’s disease vaccine)</td>
<td>Capsid</td>
<td>Also effective on myxomatosis</td>
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<td>Adeno 5</td>
<td>G</td>
<td>Oral immunisation</td>
<td>(58)</td>
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greater than one year, were confirmed (22). This vaccine (LC16mO) was also shown to be highly heat-stable when lyophilised - a stability comparable to that of the original smallpox vaccine - and is now ready for testing under field conditions. The RRV (Wyeth) has similarly been shown to protect cattle from challenge with virulent rinderpest virus in preliminary short-term trials (19). The RRV (capripox) has the added advantages that it can be used to protect cattle against both rinderpest and lumpy skin disease and is non-pathogenic for humans, making it extremely safe to handle. One disadvantage associated with the RRV (capripox) vaccine is the interference with the efficacy of the vaccine caused by pre-existing anti-capripox antibodies, a factor which was not a problem in the case of RRV (LC16mO) (32, 56).

Swinepox, another mammalian pox virus, has been considered as a vector for use in pigs. The disease caused by swinepox virus in pigs is relatively benign, its natural host range is restricted to this species and it is found worldwide. Based on these ideal vector characteristics, a recombinant Aujeszky's disease virus was developed. The glycoproteins (gp) gp50 and gp63 of Aujeszky's disease virus were inserted into the thymidine kinase gene of swinepox virus, under the control of the vaccinia p7.5K promoter. However, only partial protection of pigs against challenge with virulent Aujeszky's disease virus was conferred by this recombinant vaccine (50).

**Further attenuation of the vaccinia virus vector**

Generalised vaccinia and encephalitis were complications of vaccination in one out of 300,000 people vaccinated during the global smallpox eradication campaign conducted in the 1970s, and the risks may be greater in immune-compromised people. Since the end of mass smallpox vaccination, the number of people susceptible to infection with vaccinia virus is increasing and the possibility exists of cross-infection from animals vaccinated with vaccinia-recombinant vaccines. During the smallpox eradication campaign, four highly attenuated strains of smallpox virus were developed for use as vaccines. These were the LC16mO and LC16m8 strains (21), the modified Ankara (MVA) strain (29) and the CV-1 strain (24).

Both LC16mO and LC16m8 were derived from the Lister strain by selecting a temperature-sensitive mutant, which was then grown in rabbit kidney (RK) 13 cells. These strains were shown to be attenuated in human trials but only the latter was licensed as an attenuated smallpox vaccine.

The attenuation of the MVA strain was also confirmed in large-scale human trials. Since this virus was passaged over 570 times in chicken embryo fibroblasts, it does not productively infect mammalian cells and must be grown in avian cells (42), making it an ideal vaccinia strain for use as a recombinant vaccine vector.

The CV-1 strain was passaged in chicken embryo organ culture and on the chorioallantoic membranes of chicken embryos more than 70 times in total. In a comparative study of subcutaneous inoculation in humans, however, this virus was found to be less likely to induce antibody, compared with the Lister and other strains (12). As a result of the high immunogenicity of the LC16mO strain in rabbits and cattle, the recombinant rinderpest vaccine was developed using this vaccinia strain as the vector, and its efficacy and safety were confirmed, as described in the previous section (see 'Recombinant vaccines based on mammalian poxvirus vectors', above). The MVA strain was used to make a recombinant influenza vaccine expressing the haemagglutinin and nucleoprotein genes of influenza virus, and protective immunity was demonstrated in mice (45).

Attenuation of a conventional smallpox vaccine (Copenhagen strain) has been attempted by deleting virulence-associated and host-range genes. In total, 18 genes were serially deleted from the genome to produce a virus which was designated the NYVAC strain. The attenuated nature of the resultant virus was demonstrated by the following criteria:

a) no detectable induration or ulceration at the inoculation site on rabbit skin

b) rapid clearance of infectious virus from the intradermal site of inoculation on rabbit skin

c) absence of testicular inflammation in nude mice

d) reduced virulence, as demonstrated by intracerebral inoculation into newborn mice

e) reduced ability to replicate in nude mice or immunosuppressed mice

f) reduced ability to replicate in human cells in vitro (46).

However, possible adverse reactions in humans have not yet been examined. A recombinant Aujeszky's disease vaccine expressing Aujeszky's disease virus glycoproteins was developed using this strain of vaccinia virus which, in contrast to the swinepox-recombinant mentioned in the previous section (see 'Recombinant vaccines based on mammalian poxvirus vectors', above) (50), gave significant protection when tested in pigs (10).

**Recombinant vaccines based on avipoxvirus vectors**

Live attenuated fowlpox vaccine has been widely used to produce recombinant vaccines for chickens. Fowlpox virus, an avipoxvirus, belongs to the same family as vaccinia virus (Chordopoxvirinae) and essentially the same approach can be used to produce the recombinant vaccines. Like the capripox-recombinant viruses described above, these fowlpox vaccines are dual vaccines, which can be used to protect chickens against both the disease specific to the inserted gene and also against virulent fowlpox. The other application of avipoxvirus vectors is their potential for use as highly safe vaccines for mammalian species, including humans, since...
they do not produce infectious virus in non-avian cells. Although the avipoxviruses can efficiently infect mammalian cells, complete virus replication does not occur. The blockage probably occurs at the stage of late gene expression and no progeny virus is produced (43).

A recombinant vaccine against avian influenza, in which the HA gene of avian influenza virus was inserted into an attenuated fowlpox virus, was shown to protect chickens against challenge with virulent avian influenza virus (47). Other useful avian vaccines have been produced using the same fowlpox vector. Recombinant Newcastle disease virus (NDV) vaccine expressing the F protein gene was shown to confer protective immunity in chickens (48). A recombinant infectious bursal disease (IBD, or Gumboro disease) was also produced by inserting the VP2 gene of IBD virus into the fowlpox vector. This was shown to induce partial protective immunity in chickens against IBD (4). A second recombinant NDV vaccine expressing both the HN and F protein genes of NDV was licensed in the USA in 1994 and became the first commercially available vector vaccine (27).

A very active area of research is the application of avipoxvirus vectors for the production of recombinant vaccines for use in mammals. Although recombinant vaccines based on vaccinia virus vectors have been shown to be highly effective in generating protective immunity, vaccinia virus may cause adverse reactions in some people (see "Further attenuation of the vaccinia virus vector", above). Although this risk is small, it has effectively delayed the use of recombinant vaccines, particularly as there are an increasing number of immunosuppressed people, due to HIV infection. A potential solution to this problem is the substitution of avipoxvirus as the vector (17). Despite the incomplete replication cycle, recombinant avipoxviruses expressing foreign genes have been shown to elicit protective immune responses in mammals. In preliminary experiments, two avipoxviruses, fowlpox and canarypox, were used as vectors to develop recombinant rabies vaccines expressing the G protein gene of the virus. By some as yet unknown mechanism, the canarypox vector induced a protective immunity against rabies more than 100 times higher, in terms of immunising dose, than that induced by the fowlpox vector. Canarypox-recombinant vaccines have been shown to confer protective immunity against rabies in mice, cats and dogs (17, 49), against viral haemorrhagic disease in rabbits (15) and against canine distemper in ferrets (44).

**Recombinant vaccines based on herpesvirus vectors**

Herpesviruses are also considered to be useful vectors for vaccines. The herpesvirus genome is a linear DNA molecule of approximately 150 kilobase pairs, into which foreign DNA can be inserted in a similar way to the treatment of poxviruses. However, due to the lack of suitably attenuated strains, attempts at developing vector vaccines have been limited. Live attenuated Aujeszky's disease virus expressing the envelope glycoprotein E1 of classical swine fever virus has been constructed, and was shown to protect pigs against both classical swine fever and Aujeszky's disease viruses (51).

Herpesvirus of turkeys (HVT) has been widely used as a live vaccine to protect against Marek's disease. A recombinant HVT was constructed by inserting either the fusion protein gene or the haemagglutinin-neuraminidase gene of NDV virus into a non-essential gene of HVT. Again, the resulting recombinant vaccine was shown to confer partial protective immunity against NDV in chickens, without affecting protective immunity against HVT (31). Feline herpesvirus (FHV) type 1 was used to develop recombinant vaccines against feline leukaemia virus (FeLV) and feline calicivirus infections. In the case of the feline calicivirus vaccine, the thymidine kinase gene was used for the insertion of the capsid protein gene. The recombinant vaccine was shown to induce neutralising antibody in cats following intranasal and oral immunisation, although protection against the virus challenge has not yet been tested (60). A recombinant vaccine expressing the glycoprotein gene of FeLV was reconstituted using FHV-1 as a vector. The weakly conserved gene (ORF 2) located downstream of the glycoprotein gC homologue in FHV was used as the insertion site for the FeLV glycoprotein gene, instead of the more usual thymidine kinase gene, which has been used in the case of the other alpha-herpesviruses. The recombinant vaccine was shown to protect cats from FeLV-induced viraemia by oronasal vaccination, indicating successful mucosal immunisation (52). Recently, the rabies virus G protein was expressed in canine herpesvirus and shown to protect dogs against challenge with the rabies virus (53).

**Recombinant vaccines based on adenovirus vectors**

Adenoviruses are being developed as gene delivery systems for gene therapy and, as a result, have also been investigated as vectors for the production of recombinant vaccines. An adenovirus-recombinant containing measles virus nucleoprotein gene has been shown to protect mice from virus challenge (16). Adenovirus has also been used as an alternative to poxvirus vectors for the production of recombinant vaccines for oral vaccination of wildlife against rabies (11, 58). The rabies virus G gene was inserted in place of the deleted E3 transcription unit of human adenovirus type 5, under the control of exogenous promoters or endogenous adenovirus promoters. Oral administration of the rabies adenovirus-recombinant vaccine protected skunks and foxes against rabies. Only transient faecal excretion of the virus was observed, indicating little or no replication of the virus in the intestine, and suggesting that minimal or no transmission would occur among animals under field conditions. The widespread presence of adenovirus type 5 in the human population was taken as evidence that this vector was safe for use in combating rabies in wildlife. However, the relatively heat-labile nature of adenovirus means that more
Recombinant vaccines based on positive-strand ribonucleic acid viruses

In the case of positive-strand RNA viruses, advances in molecular techniques have enabled scientists to modify individual genes, as in the case of DNA viruses. Moreover, because of their small size, whole virus genomes can be copied into DNA and manipulated at will. This not only allows known attenuating mutations to be incorporated into the genome to produce genetically defined and marked vaccines but also means that extra coding sequences for foreign proteins can be incorporated to produce vector vaccines. The RNA transcribed from a DNA clone in vitro is infectious if injected into a cell. To obtain infectious virus, injecting the DNA is sufficient since the RNA can be transcribed from this DNA in the cell, if it has a suitable promoter (38). This approach has been applied successfully to many plant and animal viruses (8).

Some unusual approaches to vaccination have been attempted using Picornaviridae, small positive-strand RNA viruses. The size and very ordered structure of these viruses do not allow insertion of significant amounts of foreign genetic material, but small pieces of DNA coding for known protective epitopes can be inserted in particular regions of the capsid proteins. Poliovirus is one of the safest and most widely used of the human vaccines, and DNA copies of its genome can be used to obtain live virus from transfected cells. In addition, the antigenic regions on the virus capsid protein have been identified and these have been replaced by protective epitopes from other viruses, most notably foot and mouth disease virus (FMDV) (25). The antigenic sites on FMDV capsid proteins have been extensively studied and mapped very accurately and, since the two viruses are similar in structure, it is possible to exchange antigenic sites without fatally disrupting the virus. These chimeric viruses have been shown to elicit immune responses specific to FMDV in guinea-pigs but have not been tested in the target animals for the vaccine: cattle, pigs, sheep and goats.

Other epitopes derived from HIV have also been inserted into the poliovirus genome. Plant picornaviruses, e.g. cowpea mosaic virus, have also had FMDV-protective epitopes inserted into their capsid proteins, with the hope that infecting plants with these virus chimeras will eventually lead to oral vaccination of the animals which eat them (37). The same authors also inserted epitopes from human rhinovirus 14 (HRV-14) and HIV into cowpea mosaic virus but only the HRV-14 chimera was tested for immunogenicity. There is no safely attenuated FMDV vaccine, and the protective response to purified inactivated virus antigen preparations is very short-lived. Vaccination, which is very costly, must be conducted every six months if it is to be effective, and so any improvement on the existing inactivated FMDV vaccines would be of great economic benefit. Such colourful approaches to vaccination may not, in the end, lead to useful vaccines, but they will provide insights into the immune responses to protective epitopes expressed in unusual ways.

Recombinant vaccines based on negative-strand ribonucleic acid viruses

The recent success in rescuing live negative-strand viruses from DNA copies of their genomes has made it possible to consider the use of these viruses as vectors for the delivery of foreign genes. Unlike the genomes of positive-strand RNA viruses, these genomes are non-infectious and the replicating unit is the negative-sense RNA genome, encapsidated by the nucleocapsid protein and associated with the polymerase and phosphoproteins which are needed to copy the messenger RNA. These accessory proteins must be provided in the cell together with the genome RNA to enable virus transcription and replication to occur (13). Since these viruses are pleiomorphic, there is less constraint on the size of the foreign DNA that can be inserted. The envelope glycoprotein 120 gene of HIV has been inserted into paramyxovirus genome (61).

The great advantage of such systems is the generally very long-lasting immunity generated following infection or vaccination with paramyxoviruses (e.g. measles and rinderpest viruses induce life-long immunity), and if such long-lasting immunity could be transferred to other diseases, such as FMD, the advantages would be considerable. A synthetic gene incorporating B- and T-cell stimulating epitopes from FMDV has been successfully inserted and expressed in the rinderpest virus genome and the protective immunity generated against FMDV is now being studied (2). The other advantage of this system is the ability to place marker genes into the genomes of paramyxovirus vaccine strains, with the consequent ability to distinguish vaccination from natural infection easily, using serological techniques (see 'Role of recombinant vaccines in vaccination campaigns', below).

Deoxyribonucleic acid vaccines

The idea of using naked DNA as a vaccine was proposed by Wolff and colleagues in 1990 when they found that genes such as chloramphenicol acetyltransferase, luciferase and beta-galactosidase were expressed when plasmid DNA
means of delivery of the vaccine and greatly reduces the bombardment. In the latter case, microscopic gold beads are used in the delivery of DNA constructs into plants to produce transgenic plants. The gene gun is a much more efficient way, either by direct intramuscular injection or by particle bombardment. A DNA vaccine consists only of Escherichia coli-derived plasmid DNA into which a DNA fragment coding for vaccine antigen is inserted. The vaccine gene is placed under the control of a strong mammalian promoter sequence and is taken up by and expressed in host cells when injected intramuscularly into animals. Thus, the DNA plasmid serves as an amplification system to produce sufficient DNA coding for the vaccine antigen gene for injection into the animal and is not an essential component of the vaccine. Unlike virus vectors, the plasmid is incapable of replicating in mammalian cells. Expression of the vaccine antigen under these conditions leads not only to the development of neutralising antibody but also to cytotoxic T lymphocytes (CTLs) in most cases. Owing to the simplicity of the manufacturing process and the inoculation procedure, and the efficiency with which both arms of the immune system are stimulated, DNA vaccination is now considered to be a highly promising approach to combating diseases against which more conventional vaccines have been ineffective.

Advantages of deoxyribonucleic acid vaccines

The production of DNA vaccines is based on the growth of bacterial plasmid DNA, so that large-scale production at high purity can be conducted inexpensively. As the bacterial plasmids apparently do not replicate in mammals, DNA vaccines are considered to be safe compared with the other live virus or bacterial vaccines, which may retain some pathogenic potential. In addition, since DNA is not absorbed by a receptor-dependent mechanism, there are no antibodies that can interfere with the uptake of DNA into the cell and it may be possible to immunise young animals with residual maternal antibody. As DNA vaccines consist only of plasmid DNA and should contain no contaminating proteins, multiple vaccinations would seem to be possible without generating immune responses to the vector DNA. The genetically stable nature of DNA would eliminate problems such as reversion to virulence of the vaccine, and it would be easier to construct DNA vaccines against pathogens which are either difficult to culture or dangerous to handle. Another advantage of DNA vaccines is that the high stability of DNA preparations, which means that a cold chain for storage and transport of the vaccine is unnecessary.

Delivery of the DNA vaccine has been conducted in two basic ways, either by direct intramuscular injection or by particle bombardment. In the latter case, microscopic gold beads are coated with the vaccine DNA and shot into the skin cells using a special particle gun ('gene gun'), which has been widely used in the delivery of DNA constructs into plants to produce transgenic plants. The gene gun is a much more efficient means of delivery of the vaccine and greatly reduces the effective dose required. As these particle guns are very expensive at present, their veterinary application may have to await the development of more inexpensive apparatus.

One of the important features of DNA vaccines is their ability to induce a cell-mediated immune response through the production of CTLs, which are considered to be essential in protection against several virus infections (59). To induce CTL activity, the antigen must be produced in the cytoplasm of the host cell and then transported to the endoplasmic reticulum, where the antigen is processed and its peptides associated with a major histocompatibility complex (MHC) class I molecule, before being transported to the surface of the cell. There the peptide/MHC I complex comes into contact with the antigen-presenting cells. This contact results in the induction of CTLs which will recognise and kill cells expressing the complex on the surface. These antigen-processing and presentation processes apparently occur in muscle cells for antigens expressed from DNA vaccines. However, precise experimental evidence for this has not yet been reported.

Deoxyribonucleic acid vaccines for veterinary use

Owing to the advantages of DNA vaccines detailed above, they have been widely studied as candidate vaccines for both humans and animals and are considered to hold great potential for future vaccine development. Table II summarises the literature on DNA vaccines developed for veterinary use thus far. In most cases, the production of neutralising antibody as well as protection against virulent challenge has been shown to occur. In addition, CTL activity has been demonstrated for certain viruses, including bovine herpesvirus (14), rabies virus (54), influenza virus (18) and

### Table II

<table>
<thead>
<tr>
<th>Vaccine</th>
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<th>Animals tested</th>
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<td>Glycoprotein</td>
<td>Cattle, mice</td>
<td>(4)</td>
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<tr>
<td>Avian influenza</td>
<td>HA</td>
<td>Chickens</td>
<td>(18)</td>
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<td></td>
<td>M protein</td>
<td>Ferrets</td>
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<td></td>
<td>Nucleoprotein</td>
<td>Mice, monkeys</td>
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<tr>
<td>Lymphocytic</td>
<td>Glycoprotein</td>
<td>Mice</td>
<td>(28)</td>
</tr>
<tr>
<td>choriomeningitis</td>
<td>Nucleoprotein</td>
<td></td>
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<tr>
<td>Mycoplasma pulmonis</td>
<td>M.p. DNA</td>
<td>Mice</td>
<td>(3)</td>
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<td></td>
<td>M.p. DNA library</td>
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<tr>
<td>Rabies</td>
<td>Glycoprotein</td>
<td>Mice</td>
<td>(54)</td>
</tr>
<tr>
<td>Bovine viral diarrhoea</td>
<td>gp53 (E2)</td>
<td>Mice</td>
<td>(20)</td>
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<tr>
<td>Feline immuno deficiency virus</td>
<td>Entire feline immunodeficiency virus genome</td>
<td>Cats</td>
<td>(36)</td>
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<tr>
<td>Newcastle disease</td>
<td>Fusion protein</td>
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<td>(40)</td>
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<td>Aujeszky's disease</td>
<td>Glycoprotein O</td>
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M : matrix
M.p. : Mycoplasma pulmonis
DNA : deoxyribonucleic acid
gp : glycoprotein
lymphocytic choriomeningitis (LCM) virus (59). Although the vaccine against LCM virus has no veterinary relevance, it can serve as a model for the effectiveness of DNA vaccines in cases of persistent viral infection (28).

Most DNA vaccines have been produced against virus diseases for which the protective antigens have been identified and well studied. A unique approach was used for Mycoplasma pulmonis, a mouse pathogen for which protective antigens have not yet been identified. Genomic DNA was cloned into a plasmid expression vector and the resulting library of gene fragments was used in mouse immunisation studies to screen for protective antigens. Protection was demonstrated in mice vaccinated with different expression libraries: so-called expression library immunisation. This method produces a multipartite vaccine which can be further screened to identify the essential protective epitopes of proteins. This approach may prove useful for pathogens which are difficult to grow or attenuate or in cases where the genes responsible for the protective immune response have not been identified (3).

Problems which remain to be solved

Although the effectiveness of DNA vaccine has been shown for a variety of viruses and other micro-organisms, some basic questions concerning the nature of the protection mechanisms remain unanswered. The DNA introduced into mouse muscle cells has been shown to persist episomally for up to 18 months. It is speculated that the DNA is continuously expressed and the antigen is processed for MHC class I presentation in the cytoplasm of muscle cells, but the type of cell in the muscle which presents the antigen for CTL induction is not known. Plasmid vectors expressing large amounts of gene product do not necessarily induce immune response to the encoded antigen. Short immunostimulatory DNA sequences, which contain a CpG dinucleotide in a particular base context, have been identified (41). These induce pro-inflammatory cytokines and improve the immune response to the plasmid-expressed foreign DNA.

Mechanisms to increase the immunogenicity of DNA vaccines are an important area for future study. A better understanding of the mechanisms that induce CTL activity will enhance the ability to design more effective conventional and DNA vaccines in future. As DNA vaccines rely on very strong viral promoters to drive expression of the foreign genes, accidental integration of DNA into the genome of those vaccinated is of major concern when contemplating use of these vaccines in humans. This kind of risk, however, may be trivial in most farm animals with short lifespans, but may have to be considered when using DNA vaccines for animals with long lifespans.

Role of recombinant vaccines in vaccination campaigns

In the future, recombinant vaccines may serve an additional purpose as marked vaccines. Since animals vaccinated with recombinant viruses can be distinguished serologically from naturally infected animals, the use of these vaccines will not interfere with serological studies to detect the presence of any wild virus type.

The potential usefulness of the recombinant vaccine approach was demonstrated in the case of avian influenza, as the US Department of Agriculture has prohibited the use of conventional vaccines in chickens because the vaccine complicates the serological screening programme to eliminate the disease. Rinderpest seromonitoring is currently based on the detection of antibody against the H protein (1). Animals which have been naturally infected will also have antibodies directed against the nucleoprotein (NP) of rinderpest virus, unlike the vaccinated animals. Testing sera using an enzyme-linked immunosorbent assay based on the presence of anti-NP antibodies will allow detection of continuing disease (23).

Countries wishing to declare freedom from rinderpest must follow the strict guidelines laid down by the Office International des Epizooties (OIE): the so-called 'OIE Pathway'. According to this protocol, provisional freedom from the disease can only be declared when mass vaccination has been ended and replaced by active serosurveillance to look for the presence of rinderpest virus (33).

In some areas of the world, the decision to end the use of conventional vaccine is often difficult, as neighbouring countries may still be infected and cessation of vaccination will result in a large number of susceptible animals. This might have devastating consequences if the disease is present but undetected. As a first step, recombinant vaccines could be used, as these will not interfere with serosurveillance. It would then be possible to detect hidden disease without the risk of creating a large population of susceptible animals.
Nouvelles méthodes de production de vaccins antiviraux à usage vétérinaire

K. Yamanouchi, T. Barrett & C. Kai

Résumé
Les progrès sensibles enregistrés depuis dix ans dans le domaine des technologies utilisant l'acide désoxyribonucléique (ADN) recombinant ont conduit à l'élaboration de plusieurs vecteurs de vaccins nouveaux et sûrs, capables d'exprimer efficacement des immunogènes étrangers. Ceux-ci, qui font appel à divers types de virus – poxvirus, herpès-virus et adénovirus –, ont abouti à la production de nombreux vaccins recombinants potentiels. Parmi ces vaccins recombinants, le vaccin de la rage, dans lequel la protéine G de la rage s'exprime dans un vecteur de la vaccine, a été largement utilisé sur le terrain pour prévenir la propagation de cette maladie en Europe ainsi qu’aux États-Unis d’Amérique. Un vaccin à virus recombinant de la maladie de Newcastle, utilisant le virus de la variole aviaire comme vecteur pour exprimer des protéines immunogènes du virus, a été le premier vaccin commercial autorisé à vecteur recombinant. Nombre d'autres vaccins à virus recombinants sont actuellement à l'étude en laboratoire ou sur le terrain.

L'avancée la plus récente en vaccinologie a été la réussite de la vaccination avec de l'ADN nu. Cette méthode, qui semble très prometteuse sur souris, joue un rôle de premier plan dans le développement de nouveaux vaccins. Jusqu'à une période récente, la modification moléculaire des virus classiques à acide ribonucléique (ARN) en vue d'obtenir des vaccins à virus atténué plus stables n'était possible que pour les virus ARN à brin positif, tels que les poliovirus. Toutefois, les récents progrès accomplis par certaines techniques de biologie moléculaire ont permis de récupérer des virus à brin négatif à partir de répliques d’ADN de leurs génomes. On a ainsi pu procéder à des changements spécifiques dans les génomes des Rhabdoviridae et des Paramyxoviridae, qui incluent dans les deux cas plusieurs virus importants en médecine vétérinaire. Les auteurs décrivent dans cette étude le stade actuel du développement des vaccins à vecteur, des vaccins à ADN et des vaccins basés sur les génomes de virus ARN à brin positif et négatif obtenus par génie génétique, en mettant l'accent sur leur application à des maladies importantes en médecine vétérinaire.

Mots-clés

Nuevas soluciones para el desarrollo de vacunas víricas de uso veterinario

K. Yamanouchi, T. Barrett & C. Kai

Resumen
El notable progreso que durante el pasado decenio experimentó la tecnología del ADN (ácido desoxirribonucleico) recombinante ha llevado a la obtención de un amplio surtido de vectores de vacuna nuevos y seguros, capaces de expresar con eficiencia inmunógenos ajenos. Pertenecientes a diversos tipos víricos –poxvirus, herpès-virus y adénovirus–, dichos vectores ofrecen la posibilidad de fabricar gran número de nuevas vacunas recombinantes. De ellas, la vacuna
contra el virus de la rabia, obtenida por expresión de la proteína G de este virus en un vector vacíncia, se ha utilizado de forma generalizada sobre el terreno, tanto en Europa como en Estados Unidos de América, para prevenir la propagación de la enfermedad. La primera vacuna recombinante vehiculada por un vector que obtuvo licencia de comercialización es una vacuna contra el virus de la enfermedad de Newcastle, que utiliza el virus de la viruela aviar como vector donde se expresan proteínas inmunógenas del primero. Hay muchas otras vacunas de virus recombinantes que se encuentran aún en fase de experimentación, ya sea en laboratorio o sobre el terreno.

La novedad más reciente en vacunología es el éxito obtenido con el uso de ADN desnudo como medio de vacunación. Este sistema ha deparado resultados prometedores en modelos con ratones, hasta convertirse hoy en día en el campo donde más activamente se trabaja para crear nuevas vacunas. Hasta hace poco tiempo, la modificación molecular de virus ARN (ácido ribonucleico) convencionales para obtener vacunas atenuadas más estables sólo era posible con virus ARN de cadena positiva, como los poliovirus. Sin embargo, recientes progresos en las técnicas de biología molecular hacen posible rescatar virus de cadena negativa a partir de copias ADN de su genoma. Eso, a su vez, ha permitido introducir cambios específicos en el genoma de Rhabdoviridae y Paramyxoviridae, familias ambas que incluyen diversos virus de importancia veterinaria. Los autores describen los últimos avances en la fabricación de vacunas vehiculadas por vectores, vacunas de ADN y vacunas basadas en la modificación del genoma de virus ARN de cadena positiva o negativa, haciendo especial hincapié en sus aplicaciones a enfermedades de importancia veterinaria.

**Palabras clave**

**References**


