RECENT DEVELOPMENTS OF A NEW CONCEPT OF VACCINES AND THEIR EFFECTS ON PROGRAMMES FOR CONTROLLING ANIMAL DISEASES

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Summary: Molecular biology and technological advances in DNA recombination have ushered in a new era in vaccinology.

Before the advent of molecular biology techniques, live vaccine strains were attenuated by multiple passages in various animal species or in tissue culture. Within the past few years it has been possible to develop serological marker vaccines, obtained by deleting a gene that codes for a glycoprotein, the function of which is not essential. Live recombinant vectored vaccines expressing an immunogenic glycoprotein of another infective agent have also been developed.

Using specific examples the author analyses the impact of the discovery of these new vaccines on control programmes for regulated diseases, such as Aujeszky’s disease, classical swine fever and rabies. The use of these deleted vaccines, allowing vaccinated animals to be distinguished from infected animals, has given fresh impetus to a number of control programmes, as screening for infection is now possible even when systematic vaccination has to be applied.

Nevertheless, in light of the experience gained in different countries, notably in the context of control programmes for Aujeszky’s disease and classical swine fever, it would not appear to be possible to achieve disease eradication by vaccination alone. The advantages and limitations of this approach are therefore discussed in detail.

Lastly, a new generation of DNA vaccines is being rapidly developed and will no doubt allow the immune response that they induce to be more easily targeted.

1. INTRODUCTION

Molecular biology and technological advances in DNA recombination have ushered in a new era in vaccinology.

In particular, 'deleted' vaccines, used in conjunction with an appropriate diagnostic kit, have emerged over the past ten years. The first such vaccines were used to protect pigs against Aujeszky’s disease. The same principles were subsequently applied to the development of vaccines against infectious bovine rhinotracheitis and classical swine fever, in the latter case re-launching the debate on whether to use sanitary or medical prophylactic treatments.

Furthermore, recombinant vaccines have been used to protect avian species against Newcastle disease and avian influenza and, for many years, another recombinant vaccine that can be administered orally has been successfully used to vaccinate wild animals against rabies.

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1 DNA: deoxyribonucleic acid.
2. GENERAL PRINCIPLES

2.1. Controlled attenuation

Prior to the development of molecular biology techniques, attenuated vaccine strains were obtained by multiple passage in tissue culture or an unnatural host. Later, the use of monoclonal antibodies capable of exercising selection pressure to favour the emergence of apathogenic but immunogenic mutants made it possible to obtain very effective vaccines, such as the 'SAG2' vaccine against rabies (8).

However, DNA recombination techniques now make it possible to directly remove (delete) the genes associated with the virulence of certain infective agents, or to remove the coding genes for enzymes that play a role in the way the virus replicates in the organism.

2.2. Serologic marker vaccines

When a gene is deleted that codes for a glycoprotein, the function of which is not essential but which is associated with the virulence of the infective agent, the nonexpression of this glycoprotein can be used to create a serological marker vaccine. In effect, the attenuated vaccine strain will not induce specific antibodies of the glycoprotein, as it is no longer expressed. This makes it possible to distinguish vaccinated animals from infected animals because, as this glycoprotein is systematically expressed in virulent field strains, any infected animal will produce antibodies against it (see Figure 1a).

2.3. Live recombinant vectored vaccines

There are many examples of live recombinant vectored vaccines expressing an immunogenic glycoprotein of another infective agent. One of the earliest, and certainly best-known, examples is the genetically modified vaccinia virus expressing the glycoprotein of rabies virus (see Figure 1b), which has been successfully used to control sylvatic rabies (2).

3. AN HISTORICAL EXAMPLE: VACCINES AGAINST AUJESZKY’S DISEASE

3.1. Development of new vaccines

During the early stage, advances in molecular biology contributed to better knowledge of the genome of existing vaccine strains. Later, by studying conventional vaccine strains, it was found that certain coding sequences of the single-sequence short section of the 'Bartha' strain of the Aujeszky’s disease virus had been deleted. These sequences, situated in enzymatic restriction fragment BamHI no. 7, code for two structural glycoproteins: gE and gI. Accordingly, the Bartha strain, when isolated under natural conditions, does not express Ge. This makes it possible to distinguish vaccinated pigs from infected pigs, provided, of course, that the corresponding ELISA2 kits are used. ELISA kits make it possible to detect anti-gE antibodies in the serum of pigs by using monoclonal antibodies that are very specific to certain antigenic determinants of gE, as described by Van Oirschot et al. (17).

Subsequently, knowledge about the molecular biology of mutants of the Aujeszky’s disease virus led to a better understanding of the functions of the glycoproteins of the virus. The first factor of virulence that was identified in the herpes virus was the thymidine-kinase enzyme, which allows the virus to replicate itself in the central nervous system. Later, the virulence of strains of the Aujeszky’s disease virus not expressing the glycoprotein membrane gE was seen to have diminished considerably compared with that of field viruses (1). This gE would therefore appear to play a major role in the spread of the virus within the nervous system, with the infection spreading both through the olfactory tract and trigeminal cavity (10). This knowledge has made it possible to develop new vaccines by means of genetic recombination. The genome of the vaccine strains has therefore been modified using genetic recombination techniques in order to excise, remove or delete certain sequences that code for glycoproteins not then expressed. These proteins do not induce antibodies in vaccinated animals and so are used as serological markers. The functions of these same proteins are often partially responsible for the virulence of field strains (such as gE); their nonexpression helps to reduce or eliminate the pathogenicity of these vaccine strains, which always express the major glycoproteins (gB, gC, gD), inducing protective immune responses in vaccinated or infected pigs.

2 ELISA : enzyme-linked immunosorbent assay.
Another generation of vaccines, not yet on the market, has appeared, which uses live vaccine strains of the genetically modified Aujeszky’s disease virus as an expression vector of the gene coding for the immunogenic proteins of other viruses, such as classical swine fever (18). These 'hybrid' viruses protect the vaccinated animal against both Aujeszky’s disease and classical swine fever. Moreover, in-depth knowledge of the molecular biology of the Aujeszky’s disease virus has led to the creation of recombinants that cannot be shed by the vaccinated animal in an infectious form. Such recombinants can, however, spread from one cell to another of the infected organism, as do conventional vaccine strains, but in rather limited sites (6). The membrane glycoprotein gD (gp50) of the Aujeszky’s disease virus is essential to allow the virus to enter the cells, but it is not essential for the subsequent stages of viral replication. As a result, mutants that do not express this glycoprotein but are phenotypically complemented, are able to infect cells by spreading from one to the other. However, the virions that descend from these clones released from the infected cells are not infectious, as they are incapable of expressing gD (13).

Finally, the considerable progress that has been made with immunological adjuvant technology should not be overlooked, even though this is not directly linked with molecular biology. We have seen the emergence of vaccines against Aujeszky’s disease that are used by tank-mixing a live attenuated strain with an adjuvant composed of mineral oils. At the same time, the nature of the oils used in adjuvant composition has evolved, as has the emulsion technology, making the vaccines increasingly immunogenic, whilst at the same time reducing considerably local reactions at the site of injection.

3.2. Impact of these new vaccines on programmes for controlling Aujeszky’s disease

The use of deleted serological marker vaccines has meant a considerable advance in programmes to control Aujeszky’s disease.

Firstly, these vaccines have made mass vaccination possible, whilst retaining the means for serological diagnosis. This has enabled vaccinated and infected herds to be pinpointed and the necessary measures to be applied to prevent the field virus from spreading outside these herds.

Secondly, it has become possible to implement gradual sanitation measures in vaccinated and infected herds, by culling the infected sows, as required. These infected sows were detected through serological screening using the ELISA technique, which enables vaccinated pigs to be distinguished from those that have been vaccinated and are infected.

This means that vaccination has a combined effect that allows a programme of prophylactic treatment to be carried out in total safety. Mass vaccination, conducted several years in succession, limits the quantity of virus shed into the air by the infected pigs (see Figure 2), thereby considerably reducing the probability and scale of the airborne spread of contagion between herds (14, 15). Furthermore, systematic vaccination avoids economic losses due to a poorly controlled infection. Consequently, after several years of vaccination in a country or region, the prevalence of infection gradually diminishes by introducing sanitation measures into the infected herds and continually culling the oldest infected sows; also, the incidence of infection remains very low and is kept under control. However, the cost of vaccination must be taken into account when calculating the total cost of a prophylactic treatment.

![Virus titre (log 10)](image-url)
Although available information was limited to a single medical prophylactic treatment, a recent publication sheds some very interesting light on the cumulative costs of the various prophylactic strategies (19). The authors effectively demonstrate that the risk of viral spread in the fattening pen diminishes greatly after setting up an effective vaccination programme. However, they also show that, as the risk—regression curve is asymptotic, this risk remains at a low, but persistent level (see Figure 3).

Furthermore, the same authors compared the cumulative costs, over 10 years, of various measures for controlling Aujeszky’s disease in northern Germany, following the introduction of prophylactic treatment (Table 1). Of the five possible strategies, the most economical is the 'G4' strategy, based on systematic vaccination, followed by screening of infected herds, associated with the slaughter of sows presenting antibodies. Of course, this is a cumulative cost which takes all costs into account: those of the State, those of trade organisations and those of breeders. The authors note that the prevalence of infection diminishes during the first 2 years, but that vaccination alone is not enough to eliminate infection; during the final years of the programme, the Aujeszky’s disease virus persisted in a small number of herds. After 42 months of vaccination, few herds remained that still harboured infected breeding animals. The detection and elimination of these breeding animals leads to a sharp drop in the prevalence of infection in breeding herds, whilst the risk of infection in fattening farms (or of fattened animals in other farms) becomes zero (19).

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Alternative measures</th>
<th>Cumulated cost over 10 years (in thousands of euros)</th>
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<tbody>
<tr>
<td>G1</td>
<td>Vaccination of sows twice per year</td>
<td>18 085</td>
</tr>
<tr>
<td>G2</td>
<td>Vaccination of sows three times per year</td>
<td>18 143</td>
</tr>
<tr>
<td>G3</td>
<td>Vaccination of sows three times per year, and of pigs for consumption once per year</td>
<td>13 534</td>
</tr>
<tr>
<td>G4</td>
<td>Vaccination of sows three times per year, and of pigs for consumption once per year</td>
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<td></td>
<td>Serological controls and slaughter of pigs presenting infectious antibodies</td>
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<td></td>
<td>(where prevalence is &lt; 10%)</td>
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<tr>
<td>G5</td>
<td>Control and slaughter of pigs with infectious antibodies</td>
<td>9 907</td>
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<td>19 342</td>
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In the light of past and present experience, it has therefore become possible to develop a strategy for using vaccines to control Aujeszky’s disease.
In countries that have sufficient economic resources to envisage eradication of the infection, there are two possible options:

- Where the prevalence of infection in a given territory is high, or there is a high density of pig herds, mass vaccination with effective deleted vaccines is the only means of reducing prevalence; however, although these measures are necessary, they are not in themselves sufficient to eradicate the infection. Identification, screening and culling of the infected breeding animals appear to be essential to successful eradication all the while continuing to systematically vaccinate animals at least 2 years after elimination of the last infected pig. In the latter case, it is advisable to control the movements of piglets, pigs for consumption and breeding animals as much as possible.

- By contrast, in regions with a low herd density and low prevalence of Aujeszky’s disease, serological screening and the culling of infected breeders or total slaughter of certain herds, appear to be the most effective, and in some cases the most economical, measures for achieving eradication. Such measures have been successfully introduced in Denmark, the United Kingdom, Sweden and several regions of France.

Countries that do not have sufficient economic resources, in particular developing countries, can use deleted vaccines to limit the economic losses due to the Aujeszky’s disease virus, whilst at the same time gradually sanitising the production chain. These countries must, moreover, introduce control measures, by vaccination, in breeding and multiplication farms, as well as in artificial insemination centres.

4. NEW GENERATION VACCINES AGAINST INFECTIOUS BOVINE RHINOTRACHEITIS

The concept of deleted serological marker vaccines has been applied to the production of new generation vaccines against infectious bovine rhinotracheitis. Various types of serological marker vaccines have been developed to make it possible to differentiate vaccinated cattle from those that are infected: live vaccine deleted in gE, killed virus vaccine deleted in gE, and sub-unit vaccine composed of the viral glycoprotein gD (16).

5. VACCINES AGAINST CLASSICAL SWINE FEVER

5.1. Development of new vaccines

New generation vaccines against classical swine fever have recently emerged but are not yet on the market.

The E2 glycoprotein of the virus was expressed from a baculovirus that grows on the cells of insects. With this preparation, to which has been added an adjuvant composed of water in oil emulsion, pigs received two intramuscular vaccinations at the age of 10-12 weeks. All of these vaccinated pigs were totally protected in a test with the virulent strain 'Brescia' (7).

Another sub-unit vaccine with serological markers was developed, using the same principle, that expressed the glycoprotein E2 of the classical swine fever virus in baculovirus. The effectiveness of this sub-unit vaccine was evaluated taking two criteria into account: protection against a virulent test and the degree to which transmission of a virulent field virus from pig to pig was reduced. It was demonstrated that a single dose of vaccine protected pigs against a virulent test, two weeks later, with a strain titrating 100 lethal doses (LD)₅₀ per inoculum. However, during this assay, the test virus was transmitted from a vaccinated pig to a control pig. Nevertheless, between three weeks and six months following vaccination, the pigs presented no clinical signs of the disease and had not transmitted the test virus to pigs brought into contact with them (11).

Moreover, 'strain 783', a vaccine strain of the Aujeszky’s disease virus, was used as the expression vector for the gene coding for glycoprotein E2 of the classical swine fever virus (12, 18).

Finally, the Chinese strain ('C strain') effectively protects pigs against classical swine fever. Used for decades as a vaccine, it is considered to be the most effective and safest of the live vaccine strains. However, as with other conventional products, this vaccine does not allow vaccinated pigs to be serologically differentiated from infected pigs with a field strain. A serological marker vaccine has therefore been developed, based on the Chinese C strain. A hybrid virus has also been created using genetic engineering. To achieve this, the gene that codes for the antigenic N-terminal portion of glycoprotein E2 of the C strain was replaced by the sequences of the Brescia strain coding for the equivalent portion of glycoprotein E2 of this strain. Such gene substitution makes it possible to
differentiate this hybrid virus from the Brescia strain and from the parental C strain, through the combined use of specific monoclonal antibodies from the Brescia strain and from the C strain C (12).

All of these vaccines are still at the experimental stage, but some are under investigation with a view to obtaining authorisation to market them. In principle, they can all be used to differentiate vaccinated pigs from pigs infected by a virulent strain.

5.2. The impact of these new vaccines on programmes to eradicate and control classical swine fever

Following the serious epizootic that hit several European countries in 1997, many people believe that the use of these new generation serological marker vaccines could prevent a further animal health catastrophe.

While there is no doubt as to the benefit of such vaccines, it is nevertheless necessary to rationalise their use and to assess their limitations. For example, an analysis of the situation that existed when the first outbreaks appeared in the Netherlands, revealed that more than 22 herds were already infected when the primary outbreak was identified in the region of Venhorst on 4 February 1997. The situation rapidly became dramatic for the region because breeders had already sold piglets before the veterinary administration could isolate the infected zone. This led to rapid spread of the infection in the south of the country. However, at the start of an epizootic, the success of control measures depends on their being rapidly implemented after the appearance of the first outbreak. Vaccination is no substitute for basic measures to control contagious diseases.

Under such circumstances, the use of a serological marker vaccine would not radically alter the basic nature of the problem, as it does not obviate the need for intervention on potentially infected animals, to identify them, to take a sample of serum before any animals are transported, in other words, to strictly control the movement of pigs. At the start of an epizootic in regions with a high density of pig herds, zonal vaccination could, nevertheless, be envisaged in order to prevent the virus from replicating too rapidly and to limit the cost of preventive slaughter. However, in this case, transmission of the virus must be limited and control measures must be properly applied and effective.

In any case, if a country, region or zone has undergone sanitary prophylaxis, all of the vaccinated herds should be slaughtered before the pigs leave the country, region or zone.

Finally, as with Aujeszky’s disease, the new generation vaccines can be of great service to developing countries, which are unable to envisage eradication, but which nevertheless wish to control certain sectors of the production chain and to limit the spread of the virus whilst practising vaccination.

6. NEW GENERATION VACCINES USING POXVIRUSES AS EXPRESSION VECTORS

6.1. Vaccine against rabies

The only vaccine for veterinary use that is sold using the recombinant vaccinia poxvirus as the expression vector is an orally administered anti-rabies vaccine (20). This vaccine is administered to foxes in Europe, as well as to racoons, skunks and coyotes in North America, these species being the main reservoirs of the rabies virus on both continents. This recombinant vaccine was developed by inserting the gene coding for glycoprotein G of the rabies virus into the genome of the 'Copenhagen' strain of the vaccinia virus, one of the strains used for vaccinating humans against smallpox (3). The greater resistance of the recombinant poxvirus to heat fluctuations encountered in the natural environment, compared with that of vaccine strains naturally attenuated from the rabies virus, make it an ideal vaccine for inclusion in the bait, which is dispersed throughout the countryside. The immunogenicity of the vaccine remains stable for several months, even after several cycles of being frozen and defrosted.

Numerous tests were conducted in an experimental station to demonstrate the total inocuousness of this vaccine in domestic, wild and laboratory animals. These tests were followed by vaccine trials on wild animals in Europe and the United States of America. Between 1989 and 1995, around 8.5 million doses of this vaccine were dispersed in the field without any problem, thereby demonstrating the effectiveness of large-scale vaccination of wild animals using a recombinant vaccine. Furthermore, in Western Europe, the use of new generation vaccines (including vaccine SAG2) has led to the disappearance of sylvatic rabies from vast areas in several countries (5). The prolonged absence of rabies cases in these regions is unequivocal proof of the fact that the rabies virus has been eliminated from wild animal populations (4).

6.2. Vaccine against Newcastle disease and avian influenza
A recombinant vaccine against Newcastle disease has been developed using as the expression vector an attenuated vaccine against fowl pox. Genes coding for proteins HN and F of the Newcastle disease virus were inserted into this vector. The vaccine has been marketed in the United States of America since 1994 (9). Furthermore, the haemagglutinin gene of the avian influenza virus was inserted into an attenuated vaccine vector of fowl pox and the resultant recombinant proved to be capable of protecting the chicks against a virulent test with an avian influenza virus. Moreover, the USDA\(^3\) bans the use of conventional vaccines against avian influenza so as not to jeopardise the serological screening programmes aimed at eradicating the infection, with the recombinant vaccines being used as serological marker vaccines (20).

7. OTHER VACCINES THAT COULD BE MARKETED IN THE NEAR FUTURE

Rinderpest is one of the most serious contagious viral diseases of cattle and buffalo. One of the obstacles to campaigns for eradicating this disease is the relative thermostability of the vaccines that are currently available. In a bid to produce a vaccine with better thermostability, three different recombinant vaccines have been produced (20). The first expresses the gene coding for protein H of the rinderpest virus, using as the vector the attenuated strain of vaccinia 'LC16mO'. The second expresses the two genes coding for proteins H and F of the rinderpest virus, using the attenuated 'Wyeth' strain of the vaccinia as the vector. Finally, the third combines two recombinants that each express protein H or F of the rinderpest virus, using as the vector an attenuated caprine poxvirus. The first of these vaccines has been subjected to numerous trials, under confined conditions, in order to verify its effectiveness in relation to a virulent test. Its innocuousness in cattle, its trueness to type after several passages in cattle, and the duration of induced immunity (more than 1 year) have been confirmed. The thermostability of this vaccine, when freeze-dried, is excellent (20).

Finally, numerous DNA vaccines are being developed and could usher in a new era in vaccinology: plasmids containing the haemagglutinin gene of the avian influenza virus; plasmids containing the gene coding for glycoprotein gB of bovine herpes virus 1; plasmids containing the gene coding for glycoprotein GP5 of the porcine reproductive and respiratory syndrome virus, etc.

In the majority of cases, these recombinant vaccines will be used as serological marker vaccines to make it possible to distinguish vaccinated animals from infected animals.

REFERENCES


\(^3\) USDA : United States Department of Agriculture.


