HOW BIOTECHNOLOGIES CAN IMPROVE BOVINE VACCINOLOGY?

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1. INTRODUCTION

Infectious diseases, and especially viral diseases, can be prevented or controlled by several means. For many years, only hygienic measures based on animal culling were the methods of choice to control highly contagious diseases, like foot-and-mouth-disease (FMD) virus which can spread very quickly and very efficiently in a naïve population (Grubman & Baxt, 2004). Due to the development of new technologies, vaccination is now proposed to act in addition to other measures in order to control such epidemic diseases. Vaccination is also used to control several endemic diseases which can have profound economic effect in cattle production. Biotechnology is constantly applied to improve the quality of newly developed vaccines (Vannier & Martignat, 2005).

The current research is not only orientated by the vaccine research and development to the pathogen, but also to the host. Indeed, there are several trends to adapt the genetic background of animals for an increased resistance to infectious diseases. Immunogenetic is a growing field which is outside the scope of this paper (Whitelaw & Sang, 2005).

The purity of a vaccine is an essential quality. Vaccines must be free of adventitious agents, free of antibiotics or other preservatives and its chemical composition must be precisely determined. The national and international drug agencies are very concerned by the biological and chemical purity of a product. However, safety and efficacy remain the main qualities of a vaccine (Moulin, 2005). In addition, in Buiatrics, other practical factors must be taken into account, such as the cost and an easy use of the product.

This review will therefore focus on the improvement of safety and almost efficacy of bovine vaccines. Its aim is indeed to illustrate the current research on the improvement of vaccinology in Buiatrics by several examples.

2. VIRUS-HOST INTERACTIONS

Molecular virology has greatly expanded our knowledge of viruses over the last years. The virus-host interactions are studied both from the view of the virus and the view of the host. The

description of the cellular receptor of the virus and the viral ligand which attaches to the receptor helps to design more specific vaccines. Indeed, the antigenicity of FMD vaccines is mainly carried by a part of the capsid protein VP1 which consists in a highly variable loop responsible of the attachment to cellular integrins (Baranowski *et al.* 2001).

The fight against persistent infections, bovine viral diarrhoea - mucosal disease (BVD-MD) or infectious bovine rhinotracheitis (IBR) virus, is difficult. There is neither vaccine nor immune reaction able to cure a bovine from a persistent chronic or latent infection. The research mainly focuses on the prevention of persistent infections.

Most viruses have developed sophisticated strategies to evade the immune response. Herpesviruses can decrease the expression of major histocompatibility complex type 1 molecules, which, in association with viral peptides, make possible the recognition of the infected cell by specific T lymphocytes. The same viruses can attract large molecules, like C3b complement or immunoglobulins, on the surface of infected cells (Favoreel *et al.* 2003). These features make the cell less recognized by the immune effectors. Another strategy is the release of virokines, viral surrogates of lymphokines, by the infected cells. Fortunately, this escape is usually partial and bovines are able to mount an immune response against most pathogens. However they have to be studied in order to avoid as much as possible a negative interaction with the immune response when a vaccine is applied.

The pathogenesis of some diseases is driven by an unbalanced immune response. Bovine respiratory syncytial virus (BRSV) immunization can be orientated to a T helper 2 lymphocytes response (Antonis *et al.* 2003). A good knowledge of this phenomenon is essential for the development of safe and efficient vaccines.

Although the *in vitro* approach is already able to answer a lot of questions, safety and efficacy need to be supported by *in vivo* experiments. Especially, the vaccine efficacy is assessed by challenge experiments. These trials must be compliant with good clinical practices, good laboratory practices and animal welfare regulations. When a biotechnological product is evaluated, the experiments can only be carried out following the biosafety requirements, in contained facilities of the appropriate biosafety level 2, 3 or 4, depending on the infectious agent. This makes such experiments very expensive. Field trials can support the experimental data. They can be validated by using modern epidemiological and statistical tools. These *in vivo* experiments are needed to complement the *in vitro* study of virus-host interactions because they mimic the complexity of a natural infection.

3. THE BIOTECHNOLOGICAL TOOLS

Bovine vaccinology takes benefit of the advances in fast DNA sequencing, site-specific mutagenesis, bacterial artificial chromosome cloning, real time polymerase chain reaction (RT-PCR), DNA plasmidic immunisation, reverse genetics, genetic recombination and reassortment, and new viral vectors. In addition, the field of adjuvants, compounds which can be used both in inert and live vaccines, is continuously expanding (Bowersock & Martin, 1999).

4. TYPES OF VACCINES

Vaccines can be put into three main categories: inert, live and DNA vaccines (Pastoret *et al.* 1997).

The simplest inert vaccines are classical inactivated vaccines. These vaccines are microbiologically safe because inactivated. Infectious viruses could remain in the vaccine only in the case of failure in the inactivation process. Recently, subunit vaccines have been developed. They are devoid of nucleic acid so that no infectious particle is present in such products. These vaccines contain one or

more viral antigenic proteins usually produced in bioreactors. When these proteins are glycosylated, they need to be obtained from a eukaryotic system, which renders their production more expensive. The immunogenicity of subunit vaccines can be increased by the addition of adjuvants which can either facilitate the capture of these proteins by immune effector cells or stimulate the immune reaction around the injection site. The presence of adjuvant may facilitate hypersensitivity reactions in some animals. Pharmacovigilance reports are therefore essential to document the occurrence of these reactions.

Live vaccines can be made of viruses deriving from field viruses by serial passages in cell culture. In several instances, this procedure can attenuate the virulence of the virus which is now suitable as a vaccine strain. This classical approach is still used for many vaccines already licensed for cattle. However, the molecular knowledge of viruses allows a better definition of the genetic background of virulence. Specific attenuation can be therefore obtained by targeting these virulence genes and by deleting them. Vectored vaccines find also their place in this category. A virus is used as a vector to carry a foreign gene expressing the antigen to which immunisation is aimed. All these live vaccines are continuously tested for the absence of extraneous agents. The potential of such live vaccines to revert to virulence is documented in the license application. This can be considered as a very rare event. The use of vectors prepared from already attenuated or avirulent viruses is an advantage. Live vaccines behave like live micro-organisms in the animal. They stimulate a larger panel of immune response than classical inert vaccines. Indeed, live viruses penetrate into susceptible cells and their antigens are expressed both on these cells and in the extracellular space. Vector viruses can be designed to deliver the antigen in specific tissues or organs. Moreover, live vaccines can be injected on mucosal tissues and also stimulate a mucosal immune response. These vaccines are usually not combined with an adjuvant.

DNA vaccines have been recently developed. The principle of these vaccines is the injection of naked DNA made by a plasmid containing the gene of the antigen of interest. The DNA is naturally transfected into body cells where the gene will be expressed. The antigen is therefore processed in the cell and exposed to the immune system. The fact that the antigen is expressed in the cell makes these DNA vaccines close to live vaccines with regard to the stimulation of both humoral and cellular immune response. In veterinary medicine, only one DNA vaccine is licensed. Indeed, a DNA vaccine against West Nile fever in horses was marketed in USA in 2006. There is no DNA vaccine in cattle. Although DNA vaccines are very stable, several pitfalls must be overcome as the need for repeated injections and for high doses of plasmid DNA. Numerous laboratories try to improve DNA vaccines by modifying the promoters and enhancers, by developing special delivery systems or by adding sequences coding for cytokines.

5. MAJOR EFFICACY ISSUES

5.1 Immunisation of the neonate and the young calf

Although its immune system is not fully mature, neonatal calf is immune competent and is able to react against an antigenic stimulation (Goddeeris, 1998). However, maternally derived antibodies can interfere with the development of an active immune response after vaccination in young calves. Intranasal vaccination can partly overcome this interference (Lemaire *et al.* 2001). Such vaccination requires the use of live attenuated vaccine. Current developments involve vectored vaccines; they are based on a vector virus which has been modified by the insertion of a transgene coding for the antigen of interest in a genomic region non essential for the virus replication. There are several examples of live virus vectors like herpesviruses, poxviruses or adenoviruses (Gogev *et al.* 2003). The best vectors are attenuated, do not persist in the vaccinated host and deliver the transgene allowing its optimal expression in a sufficient number of cells in the organism. They are supposed to induce a full range of immune response.

We have developed a human adenovirus type 5 based vector expressing glycoprotein D of bovine herpesvirus 1 (BoHV-1) administered intranasally which was proven to be efficacious in the prevention of experimentally induced infectious bovine rhinotracheitis (IBR) (Gogev *et al.* 2002). However, although protection was reached, only few cells were infected by the vector, because it is replication defective (Gogev *et al.* 2004b). This approach was improved by the addition of an adjuvant which can increase the uptake of viruses by cells (Gogev *et al.* 2004a). In our case, we develop the use of chitosans, biological polymers of glucosamines. A major drawback of the industrial development of replication defective vectors as vaccines remains the high titres of the vector needed to reach protection. Indeed, because it is replication defective, there will be only one round of cellular infection requiring high doses of the vector for a successful infection and immunisation. Species-specific adenovirus vectors, bovine adenoviruses e.g., are further developed (Babiuk & Tikoo, 2000). The presence of passive or active antibodies naturally acquired by calves has not to interfere with the vector. The intranasal administration of a vector minimises the risk of such interference.

DNA vaccination can be able to overcome the interference mediated by maternally derived antibodies. Although such vaccination is not yet fully operational in bovines, requiring repeated injections, good results have been obtained in other production species, like lambs, in passively immunised neonates (van Drunen Littel-van den Hurk *et al.* 1999). The efficacy of DNA vaccines can be improved by the addition of suitable adjuvants, as CpG, or by a prime boost strategy (Toussaint *et al.* 2005).

5.2 Duration of immunity

The assessment of duration of immunity is a sensitive issue. In human medicine, booster vaccinations are performed at long intervals. In comparison, in bovine medicine, revaccination is usually on a yearly basis, and sometimes every six months (in the case of repeated IBR vaccination, e.g.). There is a need for vaccines providing a long lasting immunity. Even if such vaccines are available, the companies must have enough resources to perform challenge experiments in cattle vaccinated a long time before inoculation. These experiments are extremely expensive but are required in most instances, at least if the European Union regulations have to be fulfilled.

The definition of the best antigens, the best presentation of these antigens to the immune response, the best orientation of the immune response are indeed required. Vaccinology can take profit of the knowledge of the molecular architecture of the antigens and of the last advances of immunology. In particular, the choice of adjuvant is likely to be crucial to improve the post-vaccinal immune response. Another way is to develop special devices which can progressively deliver either the antigens or the DNA in the case of a DNA vaccine, providing repeated exposure to the antigens. Nanotechnologies are developing such “smart” delivery systems (Scott, 2005).

5.3 Fitness of the antigenic content

RNA viruses are known to create quasi-species and to be able of very quick evolution and adaptation to new situations. These RNA viruses are responsible for several major cattle diseases, for example: FMD, BVD, parainfluenza 3 virus infection, BRSV infection. The case of FMD virus evolution is well documented and efforts are made to continuously adapt the antigenic content of inactivated vaccines to fit with the current circulating strains. There is a parallel between the temporal evolution of BRSV glycoprotein G sequence and the intensive use of vaccination in several European countries, although a direct relationship has not been evidenced yet (Valarcher *et al.* 2000).

In this case, the biotechnology can help bovine vaccinology by the development of fast tools of molecular epidemiology able to type representative circulating strains and to alert as soon as an antigenic drift can be suspected from the sequencing of appropriate parts of the genome. Furthermore, computer tools should be available to give a prediction that this drift is significantly related to an absence of response by the current vaccines, requiring therefore the introduction of new antigens in the vaccines.

5.4 Differentiation of vaccinated from infected animals

The development of marker vaccines is a major advance in the control of diseases. These vaccines have been named DIVA vaccines (van Oirschot, 1999).

In cattle, two different kinds of markers have been proposed. In the case of IBR vaccines licensed in Europe, the vaccine marker is a deletion in the glycoprotein E gene (van Oirschot *et al.* 1996). In the case of FMD vaccines, conventional vaccines, provided a further step of vaccine purification in the industrial process, can be used. The biotechnological advance relies on refined diagnostic tests able to identify antibodies directed against non structural (NS) viral proteins. These NS proteins are only expressed in infected cells. Therefore naturally infected animals develop a serological response to these NS proteins, whereas animals vaccinated with inert vaccines do not raise any immune response to these NS proteins. The difference between infected and vaccinated animals can therefore be theoretically made (Clavijo *et al.* 2004).

This concept of marker vaccine has been adapted to non ruminant diseases, such as classical swine fever subunit and avian influenza vaccines (Vannier & Martignat, 2005).

5.5 One shot vaccination

A drawback of inert vaccines is the usual need for two vaccinations repeated at an interval of 3 to 5 weeks in order to stimulate the active immune response. In practice, it is easier to vaccinate cattle with one single injection.

A newly developed rinderpest vaccine is based on vaccinia virus as a vector. A single vaccination is efficient because the virus replicates at a low rate in the organism providing the same immune response as a conventional vaccine. A further advantage of this vaccine is its thermostability, facilitating its use in tropical regions (Ohishi *et al.* 2000).

5.6 Needle-free vaccination

Intramuscular and subcutaneous vaccine injections lead to tissue injuries due to the use of a needle. These injuries provoke economic losses to the beef industry. To avoid these tissue damages, alternative routes of injection can be tried. Intranasal vaccination is used for several live-attenuated vaccines, especially against BoHV-1. Their administration is however time-consuming. The use of needle-free delivery system makes possible intradermal, subcutaneous or intramuscular injections without skin injury. The intradermal injection also prevents muscle damages. Using these devices, intradermal injection of adjuvanted DNA plasmids allows calves to be efficiently immunised against BoHV-1 (van Drunen Littel-van den Hurk *et al.* 2006).

6. CONCLUSIONS

Vaccines are very sophisticated biological products. Their current development takes benefit from the most recent advances in biotechnology. Biotechnologically engineered vaccines begin to be used in bovine medicine. Slowly, they will replace most of the current products provided they offer

better safety and efficacy and reduced costs of production. However, biotechnological tools are also extremely useful to improve the knowledge of the pathogen, like its diversity and evolution, of the host, like its immune response, and the interactions between host and pathogen.

7. SUMMARY

Biotechnologies provide new tools which can improve the quality of bovine vaccines. Molecular virology has greatly expanded our knowledge of viruses and is the basis for the elucidation of complex virus-host interactions. Molecular and cellular immunology gives a lot of information on the immune response and how it should be driven to afford protection. DNA technology already has profound implications in the design of the future vaccines. Furthermore, a new science, the science of adjuvants will help to define the best immunogenic vaccine formulation.
Vaccines can be put into three main categories: inert, live and DNA vaccines. Subunit vaccines take part of the inert vaccines. Vectored vaccines belong to the category of live vaccine. The recent development of DNA vaccines has not been achieved yet by the marketing of a bovine product.

Several major efficacy issues are addressed to new vaccines: immunisation of the nenonate and the young calf, duration of immunity, fitness of the antigenic content of the vaccine, differentiation of vaccinated from infected animals, one shot vaccination and needle free vaccination, a.o.

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8. KEY WORDS

Vaccine, bovine, biotechnology, DNA, virus.

9. RESUME

Les biotechnologies apportent de nouveaux outils qui peuvent améliorer la qualité des vaccins bovins. La virologie moléculaire a grandement contribué à élargir notre connaissance des virus et elle est la base de la compréhension des interactions complexes entre les virus et leurs hôtes. L'immunologie cellulaire et moléculaire donne beaucoup d'informations sur la réponse immune et les mécanismes à induire pour obtenir une protection. La technologie de l'ADN a déjà des implications profondes dans le développement des vaccins du futur. De plus, une nouvelle science, celle des adjuvants, sera très utile pour définir la meilleure formulation immunogénique des futurs vaccins.

Les vaccins rentrent dans trois catégories : les vaccines inertes, vivants et les vaccins à ADN. Les vaccins sous-unitaires font partie des vaccins inertes. Les vaccins vectorisés rentrent dans celle des vaccins vivants. Les développements récents des vaccins à ADN n'ont pas encore mené à la mise sur le marché de produits destinés aux bovins.

Les nouveaux vaccins doivent répondre à de nombreux besoins : l'immunisation du nouveau-né et du jeune veau, la durée de l'immunité, l'adéquation du contenu antigénique des vaccins, la différenciation entre animaux vaccinés et infectés, la vaccination en une seule injection et la vaccination sans injection, entre autres.

Les vaccins sont des produits biologiques très sophistiqués. Leur développement actuel tire profit des avancées récentes en biotechnologie. Les vaccins produits par ingénierie biotechnologique

commencent à être utilisés en médecine bovine. Ils remplaceront progressivement la plupart des produits actuels pour autant qu'ils offrent une meilleure innocuité et efficacité et des coûts réduits de production.

10. MOTS CLES

Vaccin, bovin, biotechnologie, ADN, virus.

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