ISSN 1466-2523

Animal Health Research Reviews **10**(2); 155–158
doi:10.1017/S1466252309990211

Vaccination for respiratory immunity: latest developments

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Received 12 August 2009; Accepted 21 October 2009

Abstract

Advances over the last 20 years in immunology and molecular biology have provided many new tools for identifying the important antigens and new ways to achieve the appropriate immune responses to these antigens. These provide many more options to achieve the best immune response from deletion mutations, subunit antigens, vectors or DNA immunization. These tools are being adopted to screen, discover and produce the appropriate antigens and to deliver them by the optimal method and with novel adjuvants to achieve the appropriate immune response. These developments will result in vaccines for respiratory disease that are safer and more efficacious, and provide greater flexibility for use and administration.

Keywords: conserved antigens, protective antigens, reverse vaccinology, DNA vaccines, DIVA vaccines

Introduction

There has been a long association of vaccines and cattle starting with the beginning of vaccine era in 1796 when Dr Edward Jenner discovered that immunity to smallpox could be produced by inoculating a person with material from a cowpox lesion. Jenner called the material used for inoculation vaccine, from the root word *vacca* (cow). Vaccines continue to be an essential tool in the control of bovine respiratory disease (BRD) and new technologies play an important role in improving our control programs.

The basic techniques for developing most of the bovine respiratory vaccines currently in use have not changed dramatically since the early 20th century for bacterins and extracts, and since 40 years ago for the development of attenuated live virus vaccines. However, in the last 20 years, there have been tremendous advancements in immunology and biotechnology which are now being utilized to develop novel cattle respiratory vaccines. The ideal vaccine focuses the appropriate immune response against only the essential antigens, is not distracted by non-essential antigens, is not reactive, acts quickly and has an immune response that is distinguishable from a post infection immune response. The adaptation of these new technologies has the advantage of offering vaccines with better efficacy, greater safety, flexibility of use and even the potential of eradication of some diseases.

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New technologies

Antigen discovery and delivery

Pathogenic bacteria and viruses have evolved to survive by evading the immune systems of the host animals. Many of the antigens of a pathogen do not necessarily generate a beneficial immune response and some can act to avoid and to divert the immune system and protect the pathogen (Hornef *et al.*, 2002). Some pathogens have multiple serotypes and only a few protective antigens might be conserved between isolates from the same species of pathogen. The first and obvious critical part of vaccine development is to identify the conserved and protective antigens.

One approach to identifying antigens is to focus on suspected or known essential virulence or metabolic functions of the pathogen. Examples of these types of antigens are those associated with pathogen attachment, phagocytosis, pathology (i.e. toxins) or essential nutrient acquisition. Knowledge of a pathogen's virulence and metabolic needs has helped us search for specific

candidate antigens, such as exotoxins (Chang *et al*., 1987), proteins associated with viral entry into cells such as the glycoprotein gD for bovine herpes virus 1 (Van Drunnen Little-Van Den Hurk *et al*., 1993) and nutrient transport proteins such as iron acquisition proteins for *Pasteurella mulocida* (Prado *et al*., 2005). SDS-PAGE, western blot, protein sequencing and gene sequencing, and immunohistochemistry techniques have been used to identify and purify these antigens. The antigens can be concentrated, or the genes cloned and sub-unit recombinant antigens expressed to make antigens for vaccines.

With new developments in both molecular biology and bioinformatics there is a new approach to antigen discovery. Instead of starting and searching for specific pre-conceived antigens, this process uses a mass screening approach, wherein multiple isolates of a pathogen can be genetically sequenced and then bioinformatics can be used to identify potential antigen characteristics and screen out the antigens that are likely to make good vaccine targets (such as highly conserved outer membrane proteins). Those proteins are then screened using healthy animals and laboratory testing for appropriate immune responses. The name given to this approach is reverse vaccinology (Rappuoli, 2000). The major advantage for reverse vaccinology is its ability to find many vaccine targets that might not have been considered *a priori*. The downside is that only protein antigens can be targeted using this process. There are no reports of these techniques being used for BRD; however, they have been used with success to identify new antigens against human pathogens such as *Neisseria meningitidis* (Pizza *et al*., 2000).

Both inactivated and attenuated respiratory viral vaccines have been available for many years (Schwarz *et al*., 1957); however, inactivated viruses have not always provided the appropriate immunity (usually better for antibody response and poorer for cellular immunity) and the attenuated viruses can have safety issues such as shedding and abortion. Viruses have much smaller genomes than bacteria and although many of the major protective antigens have been identified, the delivery of these antigens in a safe and effective manner can be difficult. A number of strategies have been devised to deliver the required antigens in a manner that is safe and generates the appropriate immune response. With some viruses, it has been possible to make subunit antigens by cloning the genes for the antigens and expressing them in cell lines (Van Drunnen Little-Van Den Hurk *et al*., 1993) and vaccinating with the subunit antigens. Another method is to identify virulence antigens and then make specific gene deletions in the virus, resulting in better and safer attenuated viruses (van Engelenburg *et al*., 1994). An alternative approach is to clone the genes from the major protective antigen(s) and insert the genes into a viral vector (Hammond and Johnson, 2005). A viral vector is a virus that has been modified to be safe but able to deliver the genes into the host where the genes can be expressed and produce the antigens in the host cell. This produces the viral antigens in a manner similar to natural infection and the antigens are presented to the host's immune system in a natural way that can elicit the appropriate immune response. Some vectors are designed so that they are incomplete and cannot continue to replicate in the host and will only undergo one cycle of antigen production (Dudek and Knipe, 2005). One additional method of delivering antigens to a host is the use of plasmids delivered directly to the host as DNA vaccines (Salonius *et al*., 2007). The impetus for developing gene-based or DNA vaccines is the desire to induce the potent cellular and humoral immune responses attainable by live organism vaccines with a simple, highly purified subunit vaccine based on plasmid DNA. While this technology has seemed to be very attractive, it has been technically difficult to translate into practice with only a few examples of licensed products.

Any of these vaccine technologies that have only some of the antigens of the whole pathogen have the potential advantage of being marker or DIVA vaccines (Pasick, 2004). Vaccinated animals can be differentiated from infected animals by the lack of response to the deleted antigens and therefore these vaccines can be useful in eradication programs.

Adjuvants

The field of adjuvant technology has also advanced. Early adjuvants were based on trying to achieve a balance of antigen depot, inflammation and safety to improve the vaccine response. With our better knowledge of immunology we can now target specific innate immune responses to stimulate the appropriate response for the vaccine (Harandi *et al*., 2009). Recognition of the role of different segments of the innate immune system allows the targeting of specific responses. By targeting specific Toll-like receptors (TLRs), we can stimulate specific responses. Small molecule immune potentiators (SMIPs) are specific TLR agonists that can be used to stimulate the specific and desired immune responses (O'Hagan and De Gregorio, 2009).

New diseases

One important area of vaccine research is to identify emerging diseases and move quickly to provide vaccines to provide protection. We have not recognized any significant new emerging respiratory diseases in cattle. In swine, there have been two major emerging viral diseases in the last 20 years porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV-2). It would seem likely that with our cattle production methods and mingling of cattle from many sources, we should anticipate that there could be

https://doi.org/10.1017/S1466252309990211 Published online by Cambridge University Press

an emerging disease at any time. In humans, they have also been able to use molecular techniques to identify a number of previously unrecognized pathogens (Osterhaus, 2008). It seems possible that the use of similar techniques would lead to the discovery of novel respiratory pathogens in cattle. New vaccine technologies could be used to identify protective immune responses and quickly make vaccines for any new or newly identified bovine respiratory pathogens.

Proof of concept

One area that still is a major bottleneck in the research and development of new vaccines is the use of animal models to test and demonstrate vaccine efficacy. In fact, this is often a rate limiting stage in vaccine research. As our understanding of the immune system and pathology improves we can use more *in vitro* and *ex vivo* immunological measures to assess efficacy. This will reduce the use of disease models to evaluate vaccine efficacy. To register novel vaccines, we still need to complete challenge models in the host animal. One major issue in the design and interpretation of studies is the variation in response to vaccination and to challenge. This variability in the response to challenge and vaccination requires the use of increased numbers of animals. The chance allocation of all poor vaccine responders or good responders to one group can obviously lead to variations in trial results or even incorrect conclusions. As we develop more knowledge of the association between immune responses to vaccines and disease susceptibility, we can use these tools to pre-screen animals to optimize trial designs. *In vivo* and *in vitro* testing of immune responses has been correlated with disease susceptibility (Benga *et al*., 2009) and genotyping has also been used to examine the immune responsiveness of individual animals (Hernandez *et al*., 2002). We should be able to identify, allocate and randomize the appropriate animals for research trials using these techniques and overcome much of the variability in trials.

Practical constraints

A final issue for vaccine developers is the desire by the marketplace to have the convenience of the largest possible combinations of antigens. It is unlikely that one technology will deliver all the appropriate attributes for all the various combinations of antigens. Many of these technologies are unlikely to work effectively in combinations with other technologies. Either compromises in efficacy, safety or convenience may have to be made to achieve the large combinations desired by cattle producers.

Conclusion

Novel vaccine technologies should improve the efficacy, safety and flexibility of the respiratory vaccines. By targeting specific antigens with specific immune responses, we should be able to improve the level of protection. These novel technologies should also give us greater flexibility in the timing of the administration of vaccines allowing animals to be properly immunized prior to exposure. The use of more purified antigens, new adjuvants, deletion mutants, vectors and DNA immunization will improve the safety of our vaccines reducing both local and systemic adverse events. Vaccination is already an important tool for controlling respiratory disease in cattle and with the new technologies and vaccines available they will likely become even more important.

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https://doi.org/10.1017/S1466252309990211 Published online by Cambridge University Press