

Québec/2004 Canada



23^e Congrès mondial de buiatrie • Québec, Canada, 11-16 juillet 2004
23 Congreso Mundial de Buiatria • Québec, Canada, 11-16 de Julio 2004

23rd World Buiatrics Congress • Québec, Canada, July 11-16, 2004
23. Welt-Kongress für Buiatrik • Québec, Canada, 11.-16. Juli 2004

Vaccines: Alternatives to Antimicrobials to Control Diseases of Cattle

Andrew A. Potter*, George Mutwiri, Pon Benjamin, Philip Griebel, Lorne A. Babiuk
Vaccine & Infectious Disease Organization, University of Saskatchewan, 120 Veterinary Road,
Saskatoon, SK, Canada S7N 5E3.

Introduction

Vaccination was introduced over 200 years ago in Western society by Jenner, who demonstrated that exposure to a pathogen resulted in protection against subsequent exposure and disease. Immunization has since proven to be the single-most effective means of preventing infectious diseases in both humans and animals, yet the true potential of the technology has not yet been reached. Most of the advances made in the field of vaccine development have been accomplished without an understanding of microbial pathogenesis or the host response to infection. However, advances made over the past two decades have provided a wealth of tools in the areas of molecular biology, immunology, vaccine formulation and delivery, and genomics which are revolutionizing the way we view the use of vaccines.

Current Vaccine Types and Vaccination Strategies

Current vaccines used by the industry contain either live or killed organisms (or components thereof). The live-attenuated products are conceptually similar to those used by Jenner, while the killed products are much the same as those introduced a century ago by Pasteur. Live vaccines are generally thought to be more effective than killed products since they have the potential to stimulate an immune response similar to a natural infection. However, in order to achieve this, the vaccine would need to be delivered to a mucosal surface for respiratory and enteric infections, something that is rarely done. This has been a major impediment to increasing the efficacy of live products.

Live viral vaccines produced by conventional technologies are generally made less virulent by laboratory passage, growth on non-target host cells, or by random mutagenesis, resulting in variable levels of attenuation and thus variable immune responses. Fortunately, over the past 15 years the fields of molecular biology and genomics have provided powerful tools for the precise attenuation of various viruses, making it possible to reproducibly make vaccine strains with known virulence and immunogenicity, thus increasing both safety and efficacy. For example, the E1 region of bovine adenovirus can be deleted, making the virus incapable of replicating in its host^{8,9}. However, it can be propagated in the laboratory using cell lines containing this region. Such viruses exhibit the safety of killed products while maintaining the ability to target and infect host cells. This technology can be extended to identify regions in viral genomes where genes coding for protective proteins from other pathogens can be inserted, resulting in protection against not only the vector but the antigen which is expressed

¹². Adenoviruses, herpesviruses and poxviruses have been used extensively for this purpose. No bacterial products are available for cattle, in part due to our ability to efficiently produce cost-effective killed products. In addition, the use of antibiotics would preclude the effectiveness of such vaccines.

Killed vaccines are generally thought to be safer than live products since there is no risk of reversion to a virulent phenotype, and can be used safely in pregnant animals. However, they are usually not as effective as live products for a number of reasons. These include a lack of protective antigens, inactivation of protective components¹ and the Th2-bias of the immune response. The latter may be acceptable for some bacterial diseases, cell-mediated responses are essential for providing protection against most viral infections as well as intracellular bacteria such as *Haemophilus somnus*. Fortunately, advances in molecular biology over the past two decades has made it possible to identify specific virulence factors of pathogenic organisms and to produce them in a cost effective manner using recombinant DNA technology. While only a handful of products are commercially available at present, it is likely that the majority of killed products of the future will be produced in this fashion.

All killed vaccines require an adjuvant to increase the magnitude of the immune response. Adjuvants have historically been oil- or alum-based and such formulations are delivered parenterally, a practice which limits their ability to induce mucosal immunity. Furthermore, they often induce injection site reactions, including granulomas and fibrosarcomas. Such reactions have been shown to cost approximately six dollars in losses per animal¹⁰. Thus, there is clearly room for improvement of killed vaccine formulations in order for their full potential to be realized.

Tools for Antigen Identification

While viruses produce a relatively small number of antigens, most of which have been well characterized, bacteria and parasites are capable of producing several thousand different proteins of which only a handful are capable of inducing protective immunity. It is now well recognized that many microbial proteins associated with virulence, and hence immunity, are produced only in their animal host and not in the laboratory, making their identification and characterization more difficult. An example of this is bacterial proteins involved in the acquisition of iron. *M. haemolytica*, *H. somnus*, *P. multocida* and other related bacteria produce a novel set of proteins in their host whose sole function is to acquire iron from host transferrin^{2,6,7}. These antigens have proven to be effective vaccine components in every bacterial species tested to date as is illustrated in Figure 1 for *M. haemolytica*, *H. somnus* and *A. pleuropneumoniae*, and thus are an example of a “platform technology” in their own right. However, it is still a time-consuming process to identify such virulence factors. A number of technologies have been recently developed which now allow the rapid identification of these important components. Perhaps the single most important tool has been provided by the field of genomics, in which the entire DNA sequence of pathogenic microbes is being elucidated. At present, over 60 bacterial genomes have been sequenced and the first parasite genome, *Cryptosporidium parvum*, is now complete as well¹¹. The sequences of over 54 animal viruses are also known. Genomic data is useful in that it provides a database of all potential proteins made by an organism, and *in silico* tools have been developed to predict which components

might be useful vaccine antigens, based on homology to other proteins in publicly-accessible databases as well as the presence of specific “virulence signatures”.

Vaccine Formulation & Delivery

While our ability to identify microbial antigens capable of conferring protective immunity is now moving forward at an unparalleled rate, these represent only a part of an effective vaccine. The true potential of these antigens or live vaccines will not be realized until new adjuvants and immunomodulators are developed to enhance the quality and quantity of the immune responses, and until new methods for the delivery of these vaccines to mucosal surfaces are developed. This is especially challenging in the veterinary field where management practices dictate the acceptability of vaccination methods. As was mentioned above, live attenuated vaccines are certainly the most attractive types of vaccines for mucosal delivery, since they will ultimately not only result in protection against disease, but also against infection. However, there are problems with stability of live viral vaccines delivered to the gastrointestinal tract which will need to be overcome, and intranasal delivery has not been as attractive for bovine vaccines compared to other animal species. Interestingly, live attenuated bacterial vaccines have been shown to be effective in inducing immunity in the gastrointestinal tract of cattle when delivered orally, but their use has been limited for the reasons described above.

Numerous adjuvants and immunomodulators are currently under development for enhancing responses against subunit vaccines. These include novel polymers capable of improving both the magnitude and quality of the immune response (Figure 2) as well as immunomodulators which have the same effect. An excellent example of the latter is CpG-containing molecules which have been demonstrated to not only improve immune responses⁵, but also to stimulate the innate immune system to provide non-specific protection against infection. This approach to improving conventional products has also been demonstrated with CpG-containing formulations⁵.

There has been increasing interest in using plants for production of vaccine components as well as feed-based delivery vehicles. This is attractive from both a cost perspective as well as for the induction of immunity in the gut, especially for cattle. One such system is based on fusing antigens to canola oil bodies, thus resulting in an adjuvanted subunit vaccine which could be delivered in feed or by injection. Alfalfa and clover⁴ have also been used for the expression of *M. haemolytica* antigens. While there have been a number of reports describing immune responses in animals following feeding of transgenic plants expressing heterologous antigens^{3,13}, no specific vaccines are on the horizon for use in cattle.

Abstract

Infectious diseases of livestock continue to be a significant cause of economic losses to producers. Although vaccines have been available for the common infectious diseases of cattle for decades, most have not lived up to their true potential in terms of reducing the incidence of disease. However, this is now changing due to a number of novel technologies which permit the rapid identification of microbial antigens for use in vaccines, as well as the selection of new adjuvants and delivery methods. Ultimately, these platform technologies will permit the rapid, cost-effective development of vaccines which will live up to their potential for reducing animal disease.

Résumé

Les maladies infectieuses engendrent encore aujourd'hui de lourdes pertes économiques. Bien que des vaccins aient été disponibles pour plusieurs maladies, plusieurs n'ont pas donné les résultats escomptés. Toutefois, de nouvelles technologies permettant une identification rapide des antigènes ainsi que la mise au point de nouveaux adjuvants et méthodes de livraison changent les données. À terme, ces nouvelles technologies vont permettre aux vaccins d'atteindre leur plein potentiel à des coûts raisonnables.

References

1. Duque H, *et al.* Effects of formalin inactivation on bovine herpes virus-1 glycoproteins and antibody response elicited by formalin-inactivated vaccines in rabbits. *Vaccine* 1989;7(6):513-20.
2. Gray-Owen SD, Schryvers AB. Bacterial transferrin and lactoferrin receptors. *Trends Microbiol* 1996;4(5):185-91.
3. Koprowski H, Yusibov V. The green revolution: plants as heterologous expression vectors. *Vaccine* 2001;19(17-19):2735-41.
4. Lee RW, *et al.* Towards development of an edible vaccine against bovine pneumonic pasteurellosis using transgenic white clover expressing a *Mannheimia haemolytica* A1 leukotoxin fusion protein. *Infect Immun* 2001;69(9):5786-93.
5. Mutwiri G, *et al.* Biological activity of immunostimulatory CpG DNA motifs in domestic animals. *Vet Immunol Immunopathol* 2003;91(2):89-103.
6. Ogunnariwo JA, Schryvers AB. Characterization of a novel transferrin receptor in bovine strains of *Pasteurella multocida*. *J Bacteriol* 2001;183(3):890-6.
7. Potter AA, *et al.* Protective capacity of the *Pasteurella haemolytica* transferrin-binding proteins TbpA and TbpB in cattle. *Microb Pathog* 1999;27(4):197-206.
8. Reddy PS, *et al.* Replication-defective bovine adenovirus type 3 as an expression vector. *J Virol* 1999;73(11):9137-44.
9. Reddy PS, *et al.* Nucleotide sequence, genome organization, and transcription map of bovine adenovirus type 3. *J Virol* 1998;72(2):1394-402.
10. Van Donkersgoed J, *et al.* The effect of vaccines and antimicrobials on the formation of injection site lesions in subprimals of experimentally injected beef calves. *Can Vet J* 1999;40(4):245-51.
11. Widmer G, *et al.* Genomics and genetics of *Cryptosporidium parvum*: the key to understanding cryptosporidiosis. *Microbes Infect* 2002;4(10):1081-90.
12. Yilma T, *et al.* Protection of cattle against rinderpest with vaccinia virus recombinants expressing the HA or F gene. *Science* 1988;242(4881):1058-61.
13. Yusibov V, *et al.* Expression in plants and immunogenicity of plant virus-based experimental rabies vaccine. *Vaccine* 2002;20(25-26):3155-64.

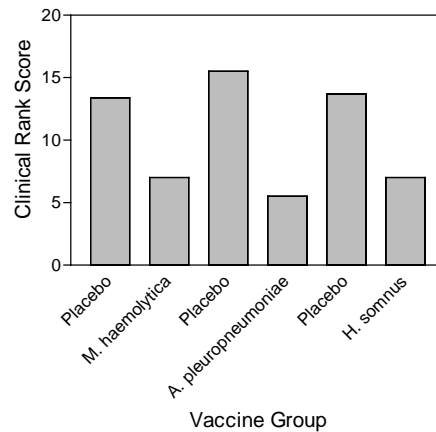


Figure 1: Comparison of the protective capacity of the transferrin-binding protein TfbB from *M. haemolytica*, *A. pleuropneumoniae* and *H. somnus*. Results are shown as pairs of placebo and vaccine groups and are expressed as clinical rank scores for comparative purposes. A score of approximately 5 indicates complete protection against experimental infection.

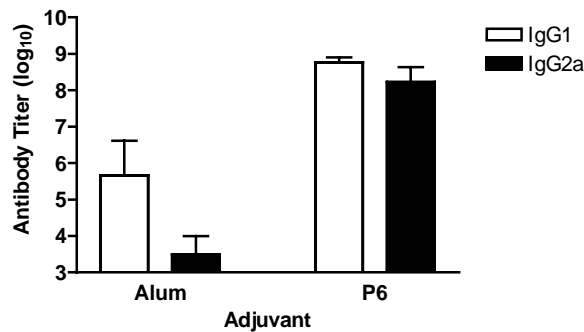


Figure 2: Comparison of immune responses against a recombinant protein following vaccination with an alum-based formulation or a novel polymer-based formulation.