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ABSTRACT

Plant derived veterinary vaccines

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Abstract Infectious diseases remain one of the main causes of death and economic losses in animals despite the fact that prophylactic vaccination has been extremely successful in disease prevention. New effective viral, bacterial and parasitic vaccines are needed, but unfortunately production costs still remain prohibitive. In this respect plants can offer a valid alternative. Production of antigenic proteins in plants relies on a well developed and proven technology, several expression platforms are available and many different plant species can be utilized. Plant based veterinary vaccine studies have addressed protection issues in model animals and, more interestingly, some of them have examined the relevant challenge model in the specific species of interest. A general overview of the topic will be outlined together with a few selected promising examples.

Keywords Animal health · Molecular pharming · Plant biotechnology · Vaccines

Introduction

Vaccines have significantly reduced the impact of a number of important pathologies and are of paramount importance in disease control and eradication programs. However, infectious diseases remain one of the main causes of death and economic losses in animals. For this reason there is an increasing demand to develop new vaccines, not only against bacterial and viral disease, but also against different kinds of parasites. Although vaccination programs are one of the most cost efficient health interventions, the development of a new vaccine is an enormous economic effort, mainly due to the research and manufactory costs and to the expense of complying with the legal and regulatory requirements needed for market authorization (Heldens et al. 2008). Most of the currently marketed vaccines are still based on the conventional strategies used in the last 200 years.

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In some cases these vaccines are less than optimal in many respects including virulence, safety and efficacy. Traditional vaccines currently on the market are mainly: i) killed vaccines which contain the pathogen usually inactivated by chemicals or heat, ii) toxoids which are the inactivated toxic components of the pathogen, iii) live vaccines which consist of attenuated variants of the pathogen that is still able to replicate, contains the desired antigenic features but does not retain its original virulent properties. These vaccines still bear a minimal theoretical risk; in fact, partial inactivation of killed vaccines and reversion of attenuated strains could lead to infection instead of protection. In recent times vaccine development has improved thanks to the progress made in advanced biotechnology, molecular immunology and to a deeper understanding of many pathogenic processes. Different tools in antigen discovery such as phage display expression libraries have been developed (Wang and Yu 2004). In addition complete pathogen genome sequences coupled with bioinformatics have opened the era of "reverse vaccinology", an approach no longer based on growing the micro-organism but on selecting candidate protective antigens by analyzing the genetic information of the pathogen (Rappuoli and Covacci 2003). These strategies have provided powerful methods for the identification of novel putative immunogenic proteins that can be used isolated from the context of the infectious agent and directly administered as subunit vaccines. Genetic engineering has allowed the isolation of these immunogenic proteins and their expression in heterologous systems, with the benefit of avoiding the purification of the proteins of interest from the virulent agent, maximizing expression levels and engineering antigenic proteins with new desired features. Recombinant subunit vaccines are predominantly expressed in suspension cultures of *Escherichia coli* or mammalian cells with a few exceptions of yeast or insect cells (Yin et al. 2007). Their production relies on fermentation technology of suspension cells in bio-reactors which requires an enormous upfront capital investment, it has intrinsic severe constraints in scalability and, thereby a considerable impact on manufacturing costs and ultimately on market price. Plant expression platforms are inexpensive and safe alternatives to the conventional systems in the production of novel recombinant subunit vaccines.

Plant expression platforms

A growing number of laboratories are investing in plant-derived vaccines, expanding on the seminal work that first proposed this idea (Mason et al. 1992). Plants are attractive as protein factories because they can produce large volumes of products efficiently and sustainably and, under certain conditions, can have significant advantages in decreasing manufacturing costs (Hood et al. 1999; Giddings 2001) . Plant systems are far less likely than mammalian cells or whole transgenic animal systems to harbor microbes pathogenic to humans. In addition, they are able to perform post-translational modifications typical of eukaryotic organisms (Vitale and Pedrazzini 2005). These cost, scale, and safety advantages make plant-made pharmaceuticals very promising for the development of new improved animal vaccines.

There are three main methods for production of recombinant vaccines in plants: stable transformation of the nuclear genome, stable transformation of the chloroplast genome and viral transient infection. These strategies have been used on a great variety of plant species which include: maize (Streatfield et al. 2001), rice (Wu et al. 2007), potato (Li et al. 2006), tomato (Pan et al. 2008), tobacco (Santi et al. 2006) and others specific for animal consumption such as pigeon pea (Satyavathi et al. 2003), alfalfa (Dus Santos et al. 2005)

and clover (Huang et al. 2006). Stable transformation allows the integration of the transgene into the plant genetic material, consequently the acquired character is transferred to the next generations while with viral transient infection the acquired trait is not genetically transmissible and a new infection will have to be performed on every new plant.

Stable nuclear transformants can be obtained by particle bombardment, also known as the biolistic method. Metal particles are coated with naked DNA and shot inside the plant tissue using a gene gun (Christou 1995), alternatively for the delivery of exogenous DNA constructs into plant cell nucleus it is possible to use the gram negative bacterium *Agrobacterium tumefaciens* (Herrera-Estrella et al. 1992). Efficient transformation protocols have been developed for several plants, including crop species. In this case the transferred DNA integrates randomly in the genome so that its expression is subject to positional effects. Transgenic plants have the advantage of permitting large scale, low-cost biomass production of selected high-expressing genes using agricultural practice and the potential for crossing transgenic lines to obtain multiple proteins expressed in the same plant. Moreover, they are able to confer heat stability to the heterologous protein, especially when expressed in grains and, if edible plants are used, there is the intriguing possibility of direct delivery through oral administration avoiding antigen purification and needle administration.

Chloroplast transformation can only be achieved by the biolistic method. Stable transformation of the chloroplast offers several distinct advantages in areas of transgene targeting, product yield, and regulatory compliance. Since the chloroplast genome allows for homologous recombination, the gene of interest can be precisely targeted to a specific locus of the genome. The chloroplast is inherited maternally, hence this technology reduces the risk of potential transgene escape by pollen dissemination. Unfortunately, chloroplasts are unable to perform typical eukaryotic posttranslational modifications, such as glycosylation and only a few plant species have been transformed with this technology, primarily tobacco (Bock 2007).

Different molecular tools are available to insert genes of interest into a viral genome in such a way that the heterologous protein is produced in the plant as a “byproduct” of virus replication. The use of plant viral vectors offers several advantages. Recombinant protein expression can reach very high levels in a relatively short time ranging from 3 to 14 days post-infection, depending on the system used. The small genome size of most plant viruses facilitates molecular engineering, allowing the facile generation of large numbers of different constructs that can be quickly tested. Fully functional and systemic infectious vectors are easily transmissible by mechanical inoculation, making large-scale infections feasible. Several expression vectors have been developed using different types of plant viruses; the most common are based on single stranded positive RNA viruses like the tobacco mosaic virus (Lico et al. 2008).

Plant derived veterinary vaccines

The production of subunit vaccines for animal health have been widely validated using all the different plant heterologous gene expression approaches previously described; numerous candidate vaccines have been proven to elicit humoral and mucosal immune responses against toxins, viruses, bacteria and parasitic pathogens. Regrettably there are still no examples on the market although recently a tobacco plant cell derived vaccine for Newcastle disease (Cardinau et al. 2004), developed by Dow AgroSciences, has gained regulatory approval by the United States Department of Agriculture (USDA) Center for Veterinary Biologics. Different studies have addressed protection issues upon pathogen

challenge in model animal species, but just a few of them have investigated the relevant challenge model in the specific species of interest. Three of those will be briefly described.

Swine transmissible gastroenteritis

One of the most promising studies showing protection with a mucosally delivered plant-based vaccine involved the swine transmissible gastroenteritis virus (TGEV) S protein expressed in corn (Streatfield et al. 2001; Lamphear et al. 2002). Ten-day-old, specific-pathogen-free, TGEV-seronegative piglets were fed daily with non-transgenic corn, mixed with 50 g of transgenic corn, containing 2 mg of the S protein, in a medicated milk replacer over a 10-day period, and control groups were orally immunized with either a commercial modified live vaccine or non-transgenic corn. All piglets were then orally challenged with a virulent form of the virus, and clinical symptoms were evaluated. While 50 % of the pigs vaccinated with transgenic corn developed diarrhea, 78 % in the group immunized by the commercially available vaccine and 100 % of those that were fed with non-transgenic corn became ill. These observations suggest that the corn-derived S protein generated an immune response adequate to confer partial protection from the virus.

Foot-and-mouth-disease

Recently Yang and colleagues reported the induction of protecting immunity against foot-and-mouth disease virus (FMDV) in swine using a plant chimeric virus particles (CVPs) approach (Yang et al. 2007). Plant viral display vectors can be used to provide molecular scaffolds to different kinds of peptides fused with the plant viral coat protein (CP) and, therefore, exposed and displayed on the surface of CVPs. In this specific case the CP gene of the bamboo mosaic virus (BaMV) was genetically modified to support fusion with the coding sequences of an antigenic peptide of the VPI protein of the FMDV. The BaMV and *C. quinoa*, the plant host, provided the expression system to produce large quantities of the chimeric fusion particles. Several groups of two month old specific pathogen free swine were immunized with different doses of CVPs by intramuscular injection and, six weeks later, boosted by the same route with the same amount. Four weeks after the boost all swine were challenged with the FMDV and monitored for symptoms. All of the negative control group animals showed serious symptoms of FMD, while all swine immunized with the CVPs showed no symptoms after challenge.

Infectious bursal disease

Protection studies on chickens have been conducted for the infectious bursal disease (IBD) using leaf extracts of transgenic *Arabidopsis thaliana* stably expressing the major host-protective immunogen VP2 protein of the IBD virus. Vaccine efficacy orally administered by gavage was evaluated and compared to a commercial live attenuated vaccine. Moreover, plant derived transgenic material has been used as an oral booster of chickens primed with the conventional vaccine. Upon challenge infection chickens orally immunized with the plant extract showed 80 % protection while chickens primed with the commercial vaccine followed by an oral boost of plant expressed VP2 proved 90% protection, the commercial vaccine showed 78 % of survival and all the control sham immunized animals died from the exposure (Wu et al. 2004). Recently, another group used the same VP2 antigen but this time stably transformed and specifically expressed in rice seeds. Specific pathogen-free chickens, orally vaccinated with transgenic seeds, produced neutralizing antibodies against

IBDV and had a protection rate of more than 80 % when challenged with a highly virulent IBDV strain (Wu et al. 2007). All together these data demonstrate that IBD can be efficiently controlled using a plant derived vaccine strategy.

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