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Commercialization of plant-based vaccines from research and development to manufacturing

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Abstract

The benefits of using plant-based oral vaccines are discussed. Transgenic maize expressing an antigen of transmissible gastroenteritis virus (TGEV) is reported as a model to demonstrate efficacy. Young pigs that were fed the TGEV corn orally were protected against challenge with virulent TGEV. Additional parameters important in providing a reliable and consistent supply of plant-based vaccines are discussed. Finally, vaccines developed in maize are evaluated for their potential to contaminate either the food supply or the environment.

Keywords: plant-based vaccines; commercialization

Introduction

There are many methods used for the production of vaccines. Some of the preferred methods involve recombinant systems using microbial host cells. While these systems have proven to be extremely useful, bacteria do not glycosylate proteins, and yeast can hyperglycosylate proteins. This feature can make microbes ineffective for producing some vaccines. Animal cells have been very useful, in many cases requiring downstream processing. However, the cost for animal cell culture systems is much higher than that for microbial systems. These limitations provide an opportunity for plants to play a role in the production of biologicals. Plant systems have the potential for increased safety from potential pathogens because no animal source is used for production. Other factors, such as rapid scale-up, lower cost and ease of delivery, provide a compelling case for plants (Kusnadi *et al.*, 1997; Ma *et al.*, 2003; Jilka *et al.*, 1999).

The commercial production of recombinant proteins from plants has been demonstrated (Hood *et al.*, 1997; Witcher *et al.*, 1998; Woodard *et al.*, 2003), and in these cases the proteins were shown to be functionally equivalent to native proteins. This opens up the potential for vaccines to be produced in plants. In addition to being a source of raw materials for production, plants offer the ability to provide a direct delivery mechanism for many applications. 'Direct delivery' refers to a product that is produced in plants and the plant tissue is used without purification. This could also apply to producing a protein in plants and using the plant tissue as a food source, feed source or a feedstock in industrial products.

This approach is applicable to plant-based oral vaccines. Oral vaccines have the potential to increase the convenience and compliance of vaccines. If the vaccines are expressed in edible plant tissue, the need to purify the vaccine is eliminated, making this a much more economical proposition. This system also has the potential to reduce dependence on needle delivery with its associated problems, the necessity to maintain the cold chain for storage, concerns over inadvertent incorporation of animal pathogens, the need for assistance in administration and the overall cost of vaccines (Streatfield and Howard, 2003).

One example for illustration is that of vaccination against transmissible gastroenteritis virus (TGEV). TGEV causes a disease that affects young pigs and can lead to mortality (Laude *et al.*, 1990). Transgenic corn was produced containing the S protein from this virus, thought to be useful as a vaccine. This corn was then fed to the pigs and the animals were observed to see if an immune

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response was induced. Pigs that were fed the TGEV corn were shown to have dramatically increased antibody titers to the virus compared with control pigs (Streatfield *et al.*, 2001). Experiments were performed to investigate if this would also give protection from the disease when the animals were challenged. The incidence of morbidity in pigs that were fed control corn was 50%. Pigs that were fed TGEV corn for 4 days showed no disease signs, compared with a morbidity incidence of 10% for pigs that were vaccinated with a commercial modified live TGEV vaccine (Lamphear *et al.*, 2002).

To commercialize an animal vaccine, not only must the product be efficacious but it must also be produced at a reasonable cost. This is a challenge for many animal vaccines because they cannot absorb the same cost of production as human vaccines. In addition, for oral vaccines to be efficacious, typical immunogenic doses may be 100 or even 1000 times the dose of the injected product. The oral approach is not at all economically practical if the vaccine is made from a purified product. However, plant systems have the potential to provide, in an edible portion of the plant, a dose 1000 times that used for injectable products and at a competitive cost. The raw material cost, not including formulations, quality control etc., can be below US$0.01 per dose. This will vary depending on the dose needed and expression level of the antigen, but it clearly demonstrates the potential.

In addition to cost, there are needs for consistent and reliable production, safety and regulatory compliance. The primary concerns for regulatory approval are product safety and efficacy. Products must be produced in a reliable manner and in stable form for administration. These requirements have been established for other systems but have not yet been determined for plant-based products. They will involve setting up a master seed bank for both parent seed and production seed. These lines will need to be well characterized using chemical, biochemical, genetic and agronomic criteria.

As an example, the *E. coli* labile toxin (LtB) has been examined for reproducibility of the product from a variety of different plant lines. The results showed a consistent pattern of DNA incorporation across a variety of different lines for the same transformation event. Furthermore, when the protein LtB was extracted from individual seeds and examined by western immunoblot analysis, remarkable uniformity of expression was also observed (Streatfield *et al.*, 2003).

In the end it is critical that the final product be uniform year after year. However, it is more realistic to expect that there will be variations of expression from year to year and at different locations. Therefore, it would be prudent to blend grain lots together in the final formulations, such that the overall concentration will be consistent. Although the experiments referred to above do not demonstrate this, they do support the belief that there should be no major discrepancies, and thus blending for consistent quality should be readily achievable.

The whole corn seed may be ground up into meal and blended. Alternatively, corn may be processed and divided into fractions of bran, grits and germ. This allows the use of only the germ, bran or grits. One option is to use only the germ fraction, which has a high content of protein and specific recombinant DNA proteins. Using this technique, fractions with a 5- to 10-fold enrichment of a recombinant protein based on dry weight have been observed (Lamphear *et al.*, 2002). This technique may offer additional advantages if delivery of a high concentration of the antigen is needed or if expression is relatively low.

Final processing and formulation of the product should be compatible with existing feed operations. However, some proteins may not be able to survive the high temperatures usual in processing. In the case of LtB, processing temperatures would be predicted to convert the active tetramer into its monomeric form. Experiments have shown that processing temperatures can be reduced to 178°C, which is sufficient to make many types of formulated products and still retain full activity of the LtB (Streatfield *et al.*, 2002)

Another concern is the storage of plant-based products. At what temperature must storage occur? In another experiment, the LtB was stored at either 4°C or 25°C over a period of 1 year and no diminished activity of LtB was seen. This gives great flexibility to both the manufacturer and the end user (Lamphear *et al.*, 2002).

In addition to safety and efficacy requirements for the product *per se*, the USDA also regulates the growing of transgenic plants to ensure a safe environment and particularly to prevent inadvertent entry of these products into the human food supply. The current situation with plant-based animal vaccines calls for a safety and risk assessment that would be accepted by the industry, regulatory agencies, special interest groups and the public. The risk assessment needs to be science-based and could be similar to that used for other systems. A proposed system for evaluating the risk for unintentional exposure has been suggested. Formulas existing for other regulated substances can be modified for non-food products produced in plants, to permit a quantitative assessment of exposure risk. (J. A. Howard and K. C. Donnelly, submitted for publication).

The compliance requirements for producing non-food products in transgenic plants are considerably different from those used for producing food products (Table 1). These include physical isolation, delayed planting times compared with food crops, agronomic support, dedicated equipment and frequent monitoring. When these practices are taken into account, the amount of transgenic corn that may inadvertently end up in the food supply and the associated risk can be calculated. In one case, aprotinin, it has been shown that even in the absence of the required confinement practices, the

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Table 1 Comparison of compliance requirements

Feature	Standard agriculture	Confinement program
Training	None	Extensive
Quality control	None	All step
Quality assurance	None	All steps
Audits	None	Internal and external
Documentation	Some	Electronic and paper
Corrective action	None	Documented
Compliance officer	None	Dedicated
Communication	Some	All levels
Accidential release procedures	Not applicable	Yes

amount of aprotinin that could inadvertently end up in the food supply would be well below the level needed to show an effect. This means that there is no hazard to human health even if the plants are grown and harvested as a typical commodity crop. When the required containment practices are taken into account, the calculated risk could be a million times below this level.

In conclusion, the technology to produce plant-based protein products has been proven to work. The first plant-produced protein products (avidin and β-glucuronidase) are now on the market. Experimentally, TGEV has been used to demonstrate the efficacy of plant-based oral vaccines in a veterinary species. This new technology may allow many new products to market that are cost-effective, convenient and free of extraneous animal pathogens. Regulatory guidelines for transgenic plant production address confinement practices that reduce the risk of unintentional exposure, and show that this risk is orders of magnitude below the minimal concern for food safety. However, safety assessment models need to be standardized and accepted by the public, regulatory agencies and special interest groups. Ultimately, we need to consider the production of plant-made vaccines as we do other pharmaceutical production systems, such as eggs or yeast, rather than as value-added agriculture.

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