Opportunities and Challenges for Plant-Based Vaccines

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Vaccination has become an important and effective public-health measure for safeguarding against devastating outcomes of infectious diseases. Current vaccines rely on the use of either attenuated (weakened) or killed strains of pathogens, *e.g.* against diphtheria, tetanus, measles and mumps. For some vaccines, such as the one against human smallpox, a strain from a different species (cowpox) is used instead. Some of these vaccines (especially parenteral vaccines) contain toxic preservatives such as formaldehyde, thiomersal (a mercury-based compound), and aluminum phosphate (Buetow and Korban, 2000; Streatfield and Howard, 2003). In recent years there has been a move towards developing subunit vaccines, linear immunogenic epitopes of the pathogen that elicit production of antibodies. This alleviates concerns over risk of reversion of attenuated strains to aggressive forms in pathogen-based vaccines (Buetow and Korban, 2000). Scale-up production of current vaccines takes place either in specific pathogen-free (SPF) eggs or in mammalian cells grown in large fermentors or bioreactors. Therefore, these vaccines require purification before they are available for use. Moreover, most are delivered via intramuscular injection, and, therefore, require the use of sterile hypodermic needles.

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In the last several years, a novel approach for developing subunit vaccines has emerged as a result of the genetic engineering technology: the use of plants as hosts—biological bioreactors. At this time, the most economical and technically feasible class of products using this approach involves the engineering of genes to express novel proteins. This has resulted in a $40-billion industry of new therapeutics and industrial enzymes (Howard, 2005).

Opportunities

The important features of any effective vaccine include safety, protective immunity that is sustained for long periods of time (preferably a lifetime), stability, ease of administration, low cost and few side-effects. In recent years, plants have emerged as alternative production systems for subunit vaccines as they are likely to contribute to all of these critical features of effective vaccines. Plants that have been engineered with genes encoding antigenic proteins of various pathogenic viral and bacterial organisms have been shown to correctly express the proteins that elicit production of antibodies in mammalian hosts. Plants can readily and properly handle the downstream processing of foreign proteins, including expression, folding, assembly, and glycosylation, all contributing to the fidelity of antigenic proteins (Wycoff, 2005). As a result, these proteins maintain their activity and efficacy, thus contributing to their viability as subunit-vaccine candidates (Figure 1). Plants can produce not only single, simple foreign proteins, but also complex multimeres, such as secretory proteins and antibodies (Wycoff, 2005). All these capabilities render

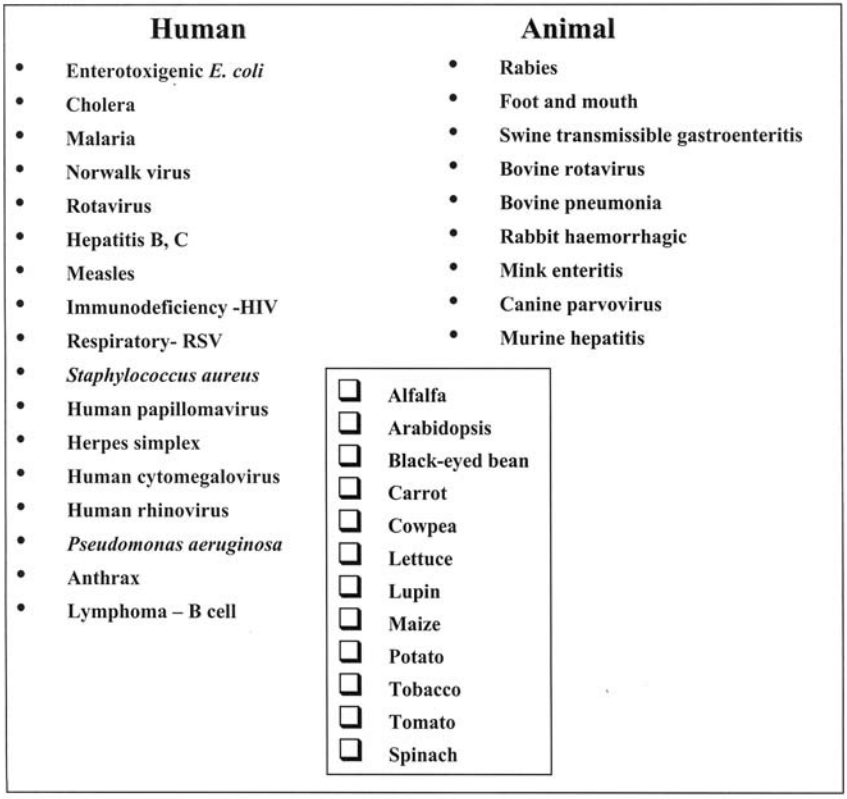

Figure 1. Subunit-vaccine candidates against human and animal diseases for possible production in various crops.

plants as targets of opportunity for marketing of high-value protein products. However, that's not all that plants have to offer.

Plant systems do not harbor human or animal pathogens (such as virions or prions) and, therefore, they do not transmit such pathogens along with the target subunit vaccine. Moreover, they cost less to produce than via fermentation or bioreactors; plants can be grown in the field or in a greenhouse relatively inexpensively (Howard, 2005). When produced in edible parts of the plant, such as grain, fruit or even leaves, subunit vaccines may not require purification. Maintaining the antigenic protein within plant cells that are edible may also contribute to stability and reduce degradation. Another advantage of producing subunit vaccines in edible parts of a plant is the potential to deliver them orally rather than intramuscularly (Streatfield and Howard, 2003), providing a simple and easy means of administration to humans and animals. Moreover, oral delivery stimulates mucosal immunity (the first line of defense) in the tissues lining the mouth, nose and esophagus (among others) that provide the first target of opportunity for pathogens to enter and infect the human body. In addition, production in plants reduces the overall cost of vaccinations, which is often prohibitive in developing countries; for example, sterile hypodermic syringes are not required.

The advantages and opportunities from producing subunit vaccines in plants may be summarized as follows:

- Elimination of risk of contamination with infectious agents
- With oral delivery, they activate the mucosal immune system—the first line of defense
- Avoidance of injections
  - Improved patient compliance
  - Reduced risk of transmission of other infectious agents through contaminated needles
- Longer shelf-life
- Cost-effective in large quantities

However, myriad challenges are yet to be overcome before the promise of this technology will be fully realized.

Challenges

The challenges facing plant-based-vaccine development include technical, regulatory and economic aspects and public perception. Among the technical challenges it is critical to select a plant system that can be grown under conditions that minimize environmental risks, such as transfer of pollen from transgenic to conventional varieties or to related

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species. Expression of antigens in plants is a major regulatory concern. Whether or not the protein is confined to specific tissues will enable or nullify exposure to the environment. Targeting expression via a tissue-specific promoter driving the transgene may reduce regulatory concerns (Korban, 2002). For example, elimination of expression of the transgene in pollen will reduce dissemination of the antigenic protein to other plants and alleviate environmental contamination, although not completely.

Among other technical challenges to be considered, the crop should provide ample biomass for accumulation of a sufficient quantity of the antigenic protein. Whether it is a grain, vegetable or fruit crop, protocols will be needed to ensure transcription, translation, intracellular localization, tissue specificity, adequate gene-copy number, and metabolism and accumulation of the protein of interest (Buetow and Korban, 2000; Streatfield and Howard, 2003). Determining the level of expression of the transgene and stability of expression over generations of the transgenic line will be essential for determining the economic feasibility of a proposed plant-based vaccine. Depending on the target protein product, levels of 10 mg/kg of plant dry weight of a crop may be sufficient, although levels of 100 mg/kg or higher are more likely to be necessary (Howard, 2005). Approximately 50 kg per year of a particular antigenic protein would certainly meet economic feasibility. Other issues related to technical challenges include formulation: will the vaccine be marketed as an encapsulated powder, a concentrated liquid or a nasal aerosol? By what route will it be delivered? What will be the proper dosage? Would a single dosage suffice, or will (a) booster(s) be necessary? All of these technical questions are yet to be answered for plant-based vaccines.

Among regulatory challenges, issues relevant to any genetically modified (GM) crop that have to gain regulatory approval from the USDA, FDA and/or EPA apply to plant-based vaccines. In addition, issues related to separation of a pharmaceutical product from the original crop targeted for the food chain have become increasingly important as concerns over adventitious presence of medicinal products in the food supply have surfaced in recent years. Physical separation of dual-purpose crops is needed—whether achieved by geographical isolation or by greenhouse containment—as is dedicated equipment for harvesting and handling, as well as standardized monitoring procedures. Concerns over the use of food crops for production of plant-based vaccines have been accompanied with calls for targeting non-food crops for pharmaceutical purposes, whether for the production of therapeutic proteins or plant-based vaccines. However, as indicated above, food crops remain highly desirable as targets for production of plant-based vaccines because of their amenability for oral delivery, avoiding the necessity for isolation and purification of the subunit vaccine prior to delivery. In addition, regulatory issues related to clinical trials, going through phase I–IV trials—similar to any other pharmaceutical product—must be pursued to assess efficacy, safety and reliability, followed by FDA approvals. For more than 30 years, live attenuated vaccines have been produced in SPF eggs, and successfully used to immunize infants and adults against common diseases such as measles and mumps. So, how can we take advantage of the regulatory history already established by the vaccine industry to push for plant-based vaccines?

Expanding Markets

Until recently, the vaccine market was considered low-margin, but that is changing as technology advances and new diseases are being addressed with vaccines. Worldwide, the market is $6 billion according to Peter Young, CEO of AlphaVax (Research Triangle Park, NC), which is developing viral-vector vaccines for HIV, malaria, Marburg virus and cancer, among others. At least one plant-based vaccine must prove to be an economic success story in order to pave the way for others to make it through to commercialization. This new technology may also serve as a platform for delivery of multiple antigens against several economically important diseases. This would certainly alleviate economic concerns over the plant-based vaccine approach, and boost its impact on the market.

Three years ago, the *Partnering for Global Health Forum* [co-sponsored by the Biotechnology Industry Organization (Washington, DC) and the Bill and Melinda Gates Foundation (Seattle)] brought together individuals from biotechnology companies, government agencies, foundations, and NGOs interested in pursuing biotechnology-based solutions for overcoming malaria, tuberculosis, typhoid, cholera, dengue fever, river blindness, AIDS and other diseases that plague developing nations. This was the beginning of an ongoing process to match funding sources and biotech companies, and to influence legislation and the regulatory process to encourage drug development for impoverished markets. Many biotechnology laboratories currently have proven technology and compounds ready for late-stage development, but lack funding to bring them to fruition to assist the individuals who need them. The message of the meeting was that funding from foundations, the government, and not-for-profit groups is available to further these efforts. Unfortunately, plant-based vaccines were not specifically spelled out in the announcement for request for proposals, although it was clear that this technology has great potential to help meet the goals of this major worldwide initiative.

As for the issue of the public's acceptance of plant-based vaccine technology, it is important to point out that the pharmaceutical industry has become a target for critics, and negative opinion is reflected in public polls. In a Kasier Family Foundation poll (spring 2005), pharmaceutical companies were ranked seventh in a list of nine industries, deemed less trustworthy than HMOs, but more trustworthy than oil and tobacco companies.

The pharmaceutical industry estimates that the cost of bringing a new chemical entity to market is around $800 million (including time-value of money; *i.e.*, factoring in the interest a company has to pay to borrow capital). Therefore, it is deemed justifiable that the public pays more in order for these drug companies to see returns on their investments. This, in turn, has contributed to the public's anger over drug prices. The shortage of influenza vaccine supply in the winter of 2004–2005 revived an issue that predates the biotech industry: what is the best way to make vaccines? For the influenza vaccine, a confluence of cost, pricing legal liability, and inertia provides an odd, but now familiar answer. Influenza vaccine is produced in chicken eggs, a manufacturing process blessed by regulatory bodies worldwide despite the fact that it has not been substantially upgraded in 60 years. The plant-based technology may circumvent long-standing production problems inherent in the egg-based system.

Summary

Producing vaccines in plants offers numerous advantages over current vaccine methodologies. Among them, safety, ease of production and low cost of production provide strong justification for developing the technology. However, many challenges remain within the pharmaceutical industry; requirements for generating non-food products in transgenic plants are different from those for food products. These challenges include technical, regulatory, economic, and public-perception issues. Physical isolation, delayed planting, agronomic support, dedicated equipment and frequent monitoring all contribute to the technical challenges involved.

As the technology to produce vaccines in plants goes through the regulatory pathway and demonstrates its economic feasibility, it may also overcome public-perception concerns that seem to have been dodged by the pharmaceutical industry. The likelihood that plant-based vaccines can be administered via oral or intranasal delivery systems will also add to their desirability as well as their economic benefits. There is potential for major impacts on global health, particularly in developing countries. However, standardized safety-assessment models must meet with approval from the general public along with the regulatory agencies and other interested parties. Risk assessment must be science-based in order for the results to be believable and trustworthy. Funding of research will accelerate the advances made thus far, and bring this technology closer to commercialization and worldwide use.

Producing vaccines in plants offers numerous advantages over current vaccine methodologies. However, many challenges remain.

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His research has been featured in the mass media, including NBC's *Today* show, TVE (TV-Spain), BBC, *St. Louis Post-Dispatch*, and *US News and World Report*. He has been an invited speaker at numerous institutions and conferences in the US and abroad, and has organized several symposia and workshops for various national and international conferences. He is (co)author of a dozen patent applications and has published over a hundred refereed scientific articles and thirteen chapters/reviews.

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