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COMMENTARY

# The Mucosal Immune Response to Plant-Derived Vaccines

Kathleen Laura Hefferon

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**ABSTRACT** Transgenic plants present enormous potential as one of the most cost-effective and safe systems for large-scale production of proteins for industrial, pharmaceutical, veterinary and agricultural uses. Heat-stable plant-derived vaccines that are administered orally could in effect enhance vaccine coverage in children and infants, particularly in developing countries. Here we discuss the current status of plant-derived vaccines and their potential to champion the battle against infectious diseases in the least developed countries.

**KEYWORDS** plant-derived biopharmaceuticals · mucosal immunity · plant biotechnology

## INTRODUCTION

The concept of using transgenic plants as delivery systems for vaccine proteins first appeared on the horizon as a direct response to a dire need in developing countries for vaccines that were cost effective, safe, and easy to store or transport. Plant-derived vaccines offer an alternative to non-conventional vaccines which combines both safety and effectiveness by facilitating oral delivery through consumption of edible plant tissue (1). Many plant-derived vaccines have been demonstrated to be just as efficacious as conventional vaccines and do not impose additional adverse reactions or side effects. Plants are capable of producing recombinant antigens that undergo similar post-translational modifications as their mammalian-derived counterparts. Depending on the specific production platform, yields of plant-derived bio-pharmaceuticals can reach as high as 45% of a plant cell's total soluble protein. Indeed, quantities approaching 250 mg of protein per litre have been determined for foreign protein production in some plant cell culture systems (2). After purification steps, plant-derived vaccines tally up to only a fraction of the cost compared to that of vaccines produced from analogous mammalian cell culture systems.

The mode of entry for many infectious diseases is through the host's mucosal surfaces, such as the gut. The fact that many antigens cannot survive the harsh environment of the digestive tract poses a significant obstacle for the delivery of proteins to the intestinal immune system. Plant-made vaccines offer an advantage as plant tissue provides protection and prevents degradation of the antigen as it passes through the gut. A sufficient amount of vaccine antigen is able to reach the Peyer's patches, where it can elicit an adequate immune response. Another issue to be addressed is the fact that since many antigens do not become recognized by the gut as foreign, they do not serve adequately as immunogens. One solution is the use of adjuvants to alter the immunogenic context by which an antigen may be encountered by the body. Cholera toxin subunit B (or CT-B toxin), a prototype adjuvant, can modify the cellular environment for highly efficient antigen presentation. CT-B can act either as an adjuvant for coadministered antigens or as an efficient transmucosal carrier molecule and delivery system for plant-derived subunit vaccines (3). In the latter case, proteins which are weakly immunogenic can be coupled to CT-B as part of a fusion protein and be expressed in plant tissue, thus increasing their antigenicity within the gut. There is no evidence that delivery of vaccines via edible plant tissues also induces immune reactions against any bystander food antigens.

A number of significant differences exist between plant-derived and traditional vaccines. For example, the majority of

K. L. Hefferon (✉)
Cornell Research Foundation, Cornell University
395 Pine Tree Rd Suite 310
Ithaca, NY 14850, USA
e-mail: klh22@cornell.edu

human therapeutic proteins are in fact glycoproteins. While transgenic plants possess the intrinsic ability to produce glycoproteins in general, some of the N-glycoproteins synthesized in plants differ from those derived from their mammalian counterparts. Some of these differences may lead to an increase in allergenicity and an undesirable immune response. Efforts have been made to further 'humanize' plant-derived therapeutic proteins and immunoglobulins by altering a number of glycosylation pathways found in plants (4). Additional steps include the retention of proteins within the ER, the design of mutant plants which lack enzymes necessary to produce plant-specific glycan structures or which acquire mammalian–specific glycosylation machinery. Future work, such as the engineering of plants to express glycoproteins which are correctly sialylated and O-glycosylated, will further enhance the development and applications of plant-derived proteins in medicine.

## CLINICAL TRIALS OF PLANT-DERIVED VACCINES

Initial plant-derived vaccines were designed to prioritize diseases which affect the intestinal tract and are major causes of mortality in developing countries. Two of the most devastating diarrheal diseases for children residing in developing countries are enterotoxigenic *E. coli* (ETEC) and Norwalk Virus or Norovirus (NV). Administration of a plant-derived vaccine against ETEC or NV in mothers may prove useful in immunizing the fetus *in utero* by transplacental transfer of maternal antibodies to the infant or through expression in breast milk. The first clinical trial involved feeding transgenic potato or corn expressing either LT-B or NV to healthy adult volunteers in a randomized, double-blind fashion. This preliminary study led to the conclusion that both humoral and systemic immune responses can be induced through antigen delivered in consumed plant material. Since then, further studies have been conducted using LT-B expressed in soybean and cornseed, respectively (5). Upon administration, mice developed both IgG and IgA responses and were partially protected against LT challenge. In addition to this, LT-B was able to successfully act as an adjuvant for co-administered proteins. Similarly, mice fed transgenic potato expressing NV CP developed IgM, IgG and IgA responses.

Monoclonal antibodies (Mab), or "plantibodies," have also been produced in plants. The development of Guy's 13 secretory IgA plantibody technology originated with the crossing of four transgenic plant lines, each expressing both heavy and light immunoglobulin domains, the J chain and the secretory component, so that the resulting progeny could express all four proteins simultaneously. Assembly of these proteins into a single macromolecule was shown to be highly efficient in the transgenic tobacco plant format. Furthermore, functional studies showed that this plant-produced sIgA bound specifically to its native antigen, and in a clinical trial was able to effectively prevent oral colonization by *S. mutans* via passive immunization of the mucosal surfaces. This study represented the first demonstration of a therapeutic protein produced in plants which had a clinical application in humans. Since then, other Mabs have been produced in plants. For example, anti-rabies human Mab developed in tobacco exhibited an anti-rabies virus-neutralizing activity and affinity comparable to its mammalian-derived counterpart HRIG (6). Recently, full-sized monoclonal IgG antibodies against the tumor-associated antigen tenascin-C (TNC) have been produced in tobacco lines and determined to be functional, further increasing the potential of plant-derived therapeutic products for use in modern medicine (7).

## ALLERGIES, ORAL TOLERANCE AND DOSE RESPONSE RELATIONSHIPS

The examples of LT-B and NV described in the previous section detail the use of plant-based oral vaccines to induce an immune response. In these cases, the amount of vaccine antigen ingested was adjusted to minimize the possible emergence of oral tolerance. Plant-derived vaccines have also been examined for their ability to induce oral tolerance to common allergies. For example, transgenic rice plants which accumulate mouse T-cell epitope peptides corresponding to pollen allergens of Japanese Cedar have been developed as a proof-of-concept study of the ability of oral tolerance to be induced by plant-derived antigens (8). Mice who consumed transgenic rice prior to systemic challenge resulted in allergen-induced oral tolerance. Although the systemic unresponsiveness corresponded with a reduction of pollen allergen-specific Th2-mediated IgE responses and histamine release, the CD4+ T-cell proliferative response remained unaffected. Plant-derived vaccine strategies can also be used to suppress asthma-based allergies. For example, transgenic narrow leaf lupin plants have been developed which express the allergen sunflower seed albumin (SSA). Oral consumption of SSA induced an antigen-specific IgG2a antibody response and prevented a delayed-type hypersensitivity response from taking place (9).

On the other hand, some apprehension remains that plant-derived vaccines which are orally administered may in fact promote the development of tolerance to vaccines or allergies to co-administered food proteins. Accidental consumption of a plant-derived vaccine may alter the way in which a person would later respond to a similar vaccine antigen, leading to vaccine inefficiency as well as reduced ability of the immune system to eliminate infection. In an effort to address these concerns, corn-derived LT-B has been used as a model system

to determine the maximum nonstimulatory dose in mice (10). These studies identified a threshold level of orally administered plant-derived LT-B which did not stimulate detectable levels of antibody but could nonetheless induce immune priming. Further research will be required to fully investigate the relationship between oral administration of plant-derived antigens and the immune response.

## MAKING PLANT-DERIVED PHARMACEUTICALS A REALITY

The ease of administration and low cost have made the concept of plant-derived vaccines, antibodies and other therapeutic agents a feasible solution for providing relief to developing countries. The fact remains that −20% of the world's infants are not immunized, and infectious diseases are responsible for over 2 million preventable deaths a year as a result of constraints on vaccine production, distribution and delivery. Plant-derived vaccines would also be useful against those diseases that are less prominent and whose treatments are poorly financed, such as dengue fever, hookworm and rabies.

Plant-derived vaccines have been produced in the greenhouse and outdoor fields and through cell culture. Unlike field-grown plants, where variations in soil and weather prohibit good manufacturing practice conditions which are indispensible for pharmaceutical production, cell suspensions can be grown in precisely controlled environments. In particular, plant cells which secrete the specific protein product can be grown continuously, resulting in less expensive downstream processing. Expression levels are constantly increasing as technologies associated with plant-derived vaccines continue to advance. Purification from plant cell culture is in general easier and significantly less expensive than purification from their mammalian and bacterial counterparts. Indeed, some plant-derived biopharmaceuticals, such as topically applied monoclonal antibodies, may require only partial purification and thus be even less intensive in terms of labour and cost. Recently, the first plant-derived vaccine, a poultry vaccine for Newcastle disease which was produced in plant cell culture, has received approval for market release (www.dowagro.com/newsroom/corporatenews/2006/20060131b.htm).

## CONCLUDING REMARKS

When plant-made pharmaceuticals were first introduced to the general media and in scientific literature, the technology was referred to as "edible vaccine" production. Researchers speculated that plant-made pharmaceuticals could be produced in the field and consumed as a routine/local food source. In the world's developing countries, vaccines could potentially be derived from fresh produce, even from an individual's own garden. The problem with this approach became apparent as the requirement for maintaining plant tissue containing the vaccine protein at consistent levels for oral consumption became paramount. Indeed, the final product will most likely be packaged as a capsule, juice, paste or even perhaps a suspension for oral delivery, rather than in the form of an entire fruit or vegetable. The production of plant-derived vaccines for the international marketplace is under extensive regulatory control. The regulatory framework and approval process for a given plant-derived vaccine is far more extensive than any followed by traditional vaccines used commercially and includes authorization by not only the FDA as a new therapeutic product that is safe for medical use, but also by the USDA and EPA as a food product grown under environmentally safe conditions.

Plant-derived vaccines continue to provide hope for more immunogenic, more effective and less expensive vaccination strategies against mucosal pathogens. The results of the studies presented here hold great promise for the use of plant-derived vaccines in the future.

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