Clinical Trials Fuel the Promise of Plant-Derived Vaccines

Kathleen Hefferon, PhD

Abstract

Heat-stable plant-made (edible) vaccines are inexpensive to produce, can be administered orally, and could be utilized to enhance vaccine coverage in children, particularly in developing countries. Plant-made vaccines can deliver undegraded antigens to the enteric mucosal immune system. A number of clinical trials have produced encouraging results. This review summarizes plant-derived vaccines, the mucosal immune response, and the evidence regarding their use and efficacy.

Introduction

Infection from vaccinable pathogens is a leading cause of mortality in underdeveloped countries. In 1992, an assembly of philanthropic groups, in conjunction with the World Health Organization, established the Children's Vaccine Initiative to develop novel oral vaccines and to improve global accessibility.[1,2] Ideal vaccines would be cheap, safe, portable, and durable. Of note, transgenic plants offer a novel delivery system for vaccine proteins.[3,4] Plants are capable of producing recombinant antigens that retain the same structural integrity and activity as their mammalian-derived counterparts. These transgenic plants safely and effectively deliver non-replicative subunit vaccines through the consumption of edible plants.[5]

The first genetically modified crops were disease-resistant soybean and corn and appeared on the US market in 1996. Since then, transgenic plants have been commercialized in many other countries. Transgenic plants, which exhibit increased pest- and disease-resistance, prevent substantial global production losses. Transgenic plants may become a cost-effective and safe system for large-scale production of proteins for industrial, pharmaceutical, veterinary, and agricultural uses.

The induction of an immune response usually precedes control of mucosally acquired infections. Specifically, the nature of the antigen, the route of administration, and the delivery system utilized determine the systemic and secretory immune responses. Traditional parenteral vaccines, for example, primarily induce IgM and IgG responses, whereas mucosal vaccinations induce both IgG and secretory IgA responses.

Infantile diarrhea and other enteral pathologies are leading causes of morbidity and mortality in developing countries. Heat-stable plant-made vaccines that are administered orally, therefore, have the potential to enhance vaccine coverage in children and infants, particularly in resource-poor regions. Plant-based vaccines delivered orally are well suited for combating gastrointestinal diseases, and this has been the focus of a number of Phase 1 clinical trials.

Plant-derived vaccines deliver protein immunogens to the gut – an active part of the immune system. A significant hurdle impacting protein delivery to the intestinal immune system stems from the fact that many antigens are rapidly degraded within the harsh environment of the digestive tract. Plant-made vaccines offer an advantage as plant cells provide protection and prevent degradation of the antigen as it passes through the gut. Another problem is that many antigens do not become recognized by the gut as foreign and, therefore, do not serve adequately as immunogens. One way to overcome this problem is to use adjuvants, which largely affect the immunogenic context in which an antigen is encountered.

Plants Can Express Vaccine Epitopes and Proteins

Plant transformation, meaning the stable integration of the gene of interest into a plant genome, was originally conducted using

a modified strain of *Agrobacterium tumefaciens,* the bacterial strain responsible for crown-gall disease. Stable plant transformation has several disadvantages, such as long production times and contamination via the escape of transgenes into the environment.[7] These concerns have prompted the development of alternative methods of protein expression, such as the use of plant cell culture bioreactors rather than plants grown in outdoor fields.

Another option is the utilization of plant virus expression systems, which produce large quantities in short intervals. Two types of expression systems based on plant viruses have been developed for the production of immunogenic peptides and proteins in plants: epitope presentation systems (short antigenic peptides fused to the coat protein [CP] that are displayed on the surface of assembled viral particles) and polypeptide expression systems (these systems express the whole unfused recombinant protein that accumulates within the plant). However, insert size limitations and host range restrictions preclude the widespread use of such virus expression vectors for every plant species.[6-8] The choice of expression of the vaccine protein, therefore, becomes a matter of choosing the optimal plant species, whether it be whole plant or cell culture and whether stable transformation or transient expression best fits the nature of the therapeutic protein under investigation and its proposed applications.

There are significant differences between plant-derived and traditional vaccines. Although plants present a promising system for the production of human therapeutic proteins, the majority are glycoproteins. These proteins may have modification pathways that produce a mammalian immune response; humanized plants expressing glycoproteins, which are correctly sialylated and O-glycosylated, may facilitate the production of plant-derived proteins in medicine.[9]

Plant-Derived Vaccines and the Mucosal Immune System

The mucosa of the digestive, respiratory, and urogenital tracts are the sites for most infections. The epithelial interface is protected by innate and adaptive immune pathways which can recognize and eradicate pathogens. This mucosal epithelium overlies organized lymphoid follicles and consists of mucin-producing glandular cells, lymphocytes, plasma cells, dendritic cells, macrophages, cytokines, and chemokines. Antigen uptake, processing, and presentation for induction of mucosal responses take place within this tissue.[11,12]

In the intestine, gut-associated lymphoid tissue (GALT) represents approximately 70% of the body's entire immune system. Peyer's Patches, which form large clusters of lymphoid follicles and are distributed along the length of the small intestine, are involved in the immune surveillance of the intestinal lumen. Peyer's Patches contain various, highly specialized cells known as M (minifold) cells, which deliver antigen from the lumen to antigen-presenting cells, followed by the activation of T cells, B cells, and dendritic cells, which are involved in initiating the primary immune response.[13,14,15]

In the respiratory tract, antigen is taken up into alveolar spaces by antigen-presenting cells, most likely via lymphatosis, to regional lymph nodes, the site of the primary immune response. Antigen-specific B cells are produced and return to the lung, where they differentiate into either antibody-secreting plasma cells or memory cells. The cells migrate via the lymphatic system to regional lymph nodes, where the primary immune response occurs.[16]

Strong mucosal immune responses take place upon introduction of an antigen directly into the respiratory tract. Antibody responses in the respiratory tract can occur either quickly through activation of resident memory B cells, if there has been prior exposure to the pathogen, or, if the host is naive to the pathogen, more slowly through the induction of both systemic and local mucosal immunity. Both IgG and IgA assist in the clearance of invading pathogens with the site of exposure determining the nature of the antibody that is produced. In the case of respiratory pathogens, systemic vaccination, which stimulates systemic IgG and elicits a modest mucosal IgA response, is less effective than mucosal vaccination, which stimulates rapid local and systemic IgA and IgG responses.[16,17]

IgA, the major antibody isotype in mucosal secretions, performs several functions in mucosal immunity. For example, sIgA antibodies can block the entry of antigens into the epithelium. IgA antibodies present in the *lamina propria* adhere to and excrete antigen into the lumen, IgA antibodies transported through the epithelium can neutralize virus production and proinflammatory antigens as well as trigger the release of inflammatory mediators.

Phase 1 – Clinical Trials and Plant-Derived Vaccines

In 1990, *Streptococcus mutans* surface protein A was expressed in transgenic tobacco and given to mice. This transgenic plant material successfully induces an antibody response through a demonstration that serum from immunized mice could react with intact *S. mutans*.[18] Plants were then developed which expressed *E. coli* enterotoxin B subunit (LT-B) and which exhibited successful induction of both mucosal and sera antibody responses.[19,20] Multiple animal and human antigenicity and challenge trials have proven the efficacy of such plant-made vaccines (Table 1).

Plant-Made Vaccines to Treat Diarrheal Diseases

Enterotoxigenic *E. coli* (ETEC) and Norwalk Virus or Norovirus (NV) are devastating diarrheal diseases prevalent in Third World countries with *E. coli*, causing three million infant deaths a year. Administering plant vaccine to nursing or gravid women

Table 1: Examples of Mucosal Immune Response Generated to Plant-Derived Vaccines

DISEASE	PLANT USED	ANTISERA RAISED AGAINST	REFERENCE
Enterotoxigenic E. coli			
ETEC	potato, maize	LT-B	19, 20, 22
Norwalk Virus	potato, maize	NV	21
Hepatitis B Virus	potato	HBsAg	23
Rabies Virus	spinach	Spike antigen	31
Human Papillomavirus	potato, tobacco	L1 capsid protein	25, 26, 27
Anthrax	tobacco	Protective antigen (PA)	28, 29
SARS	tomato, tobacco	S protein	30
Measles Virus	lettuce	MV-H protein	32, 33
Swine transmissible gastroenteritis virus	maize	Spike protein	46
Staphylococcus aureus	cowpea	D2 peptide of fibronectin-binding protein (FnBP)	47
E. coli 0157:H7	tobacco	Intimin protein	48
Strain K88 of enterotoxigenic E. coli	tobacco	FaeG of K88 fimbrial antigen	49
Japanese Cedar pollen allergens	rice	Cry jI, Cry jII	42
Foot and Mouth Disease Virus	alfalfa	VP1	50
Respiratory Syncytial Virus	tomato	F protein	51
Sunflower seed albumin	narrow leaf lupin	SSA	44
Norwalk Virus	tobacco, potato	VLP	21
Influenza Virus	tobacco	B5	34
Plague	tomato	F1-V fusion protein	52
Canine Parvovirus	tobacco, chloroplast	2L2I peptide	53
Tuberculosis	arabidopsis	ESAT-6 antigen	54
Rotavirus	alfalfa	VP6	55

may protect the child through maternal antibodies transferred transplacentally or through breast milk. Norwalk Virus, on the other hand, is composed of a single capsid protein that can self-assemble into virus-like particles (VLPs), which act further to stimulate the immune response.

The first clinical trial to examine whether similar immune responses could be generated in humans using these two antigens involved the feeding of transgenic potato or corn expressing either LT-B or NV to adult volunteers.[20,22] Fourteen healthy adults ingested either 50 or 100 g of raw transgenic potato expressing the vaccine protein or nontransformed potato used as a control; these were randomized in a double-blind fashion. Second or third doses were administered on days seven and twenty-one. Antibody-secreting cells were detected seven days after ingestion of transgenic potato expressing LT-B. Volunteers who ingested potato or corn-based LT-B vaccines developed high increases in LT-B-specific IgG; many of these developed four-fold rises in IgA anti-LT. LT neutralization assays were also performed using Y-1 adrenal cells. Out of eleven volunteers, eight developed neutralization titres which were greater than one. For individuals who ingested two or three doses of transgenic potatoes expressing the NV CP as antigen, 95% developed significant rises in IgA titre. Based on these preliminary studies, both humoral and systemic immune responses can appear to be successfully induced through antigen delivered in consumed plant material.

Hepatitis B Virus (HBV)

Hepatitis B, which causes chronic liver disease, affects over 300 million people worldwide. Hepatitis B Virus surface antigen (HBsAg), the principal antigen used for vaccine production, is a potential transgenic plant product. Like NV capsid protein, HBsAg has been demonstrated to form intact immunogenic virus-like particles. The efficacy of HBsAg produced in transgenic plants and delivered orally has been compared with the oral delivery of the yeast-derived rHBsAg, which is currently being used as an injectable vaccine in mice.[23] Peeled potato tubers were fed to mice at a dose of 42 µg HBsAg per feeding once a week for three weeks. A week after the first two doses were administered, anti-HBsAg antibodies were observed in mice fed transgenic tubers but not in mice fed yeast-derived HBsAg. Antibody levels peaked four weeks after the third dose and returned to baseline levels eleven weeks later. Control mice fed nontransgenic potato did not exhibit an elevated anti-HBsAg antibody response.[23] The strong primary response exhibited by mice fed HBsAg derived from plants may result from the protective encapsulation of the antigen within the potato cell. Digestion of plant tissue within the gut would increase the like-

liness of antigen release near the Peyer's patches and result in a more robust immune response. That intact VLPs comprised of HBsAg were visualized in these potatoes suggests a more immunogenic presentation than the yeast-derived vaccine. Mice primed initially with potato-derived HbsAg, then boosted parenterally with yeast-derived rHBsAg, were also examined in a separate study to determine whether memory B cells had also been established. These mice exhibited a strong secondary response lasting for over five months.

More recently, a double-blind and placebo-controlled Phase 1 human clinical trial was performed using plant-derived HBV vaccine.[24] Transgenic potato tubers that had not been cooked and which expressed approximately 8.5 µg/g HBsAg were fed to previously vaccinated individual volunteers. More than half of those volunteers who ingested one hundred grams of the transgenic potato tubers in the form of three doses exhibited a substantial increase in anti-HBsAg serum titres. No volunteer who ate the nontransformed potatoes provided as controls displayed an increase in antibody titre (Thanavala *et al.* 2005). Results of this study and similar studies conducted by other groups highlight the potential of plant-derived vaccines for those countries which have limited access to therapeutic proteins and modern medical infrastructure.

Human Papillomavirus (HPV)

A major cause of cervical cancer in women, particularly in developing countries, is human papillomavirus. Current vaccines are too expensive and are difficult to distribute widely in these countries. A number of immunization studies involving a plant-derived vaccine against human papillomavirus have been performed using a mouse model. Initial studies by Biemelt et al. (2003) demonstrated that either plant- or insect-derived VLPs, consisting of the L1 capsid protein of HPV, were both immunogenic to an equal degree.[25] Half of mice fed transgenic potatoes expressing HPV VLPs developed L1-specific antibodies. A few years later, Warzecha et al. introduced a plant-optimized version of the L1 capsid protein of HPV into tobacco potato plants, which accumulated higher levels of VLPs.[26] Mice who consumed potato tubers expressing this altered version of L1 elicited a significant enhanced serum antibody response.

The potential of producing a plant-made vaccine against a papillomavirus using a plant virus-based expression vector system has also been investigated. In this instance, the L1 capsid protein of control rabbit papillomavirus (CRPV), often used as a model system for papillomavirus-host interaction studies, was incorporated into a tobacco mosaic virus (TMV)-based vector. Extracts from plants infected with TMV-L1 were shown to protect rabbits from infectious virus upon inoculation.[27]

Anthrax

Anthrax is an acute and fatal disease acquired by inhalation or ingestion of spores and caused by *Bacillus anthracis*, a gram-positive spore-forming bacteria. As a result, anthrax has been classified as a category A biological warfare agent. Protective Antigen (PA), one of the proteins expressed by *B. anthracis*, is named for its ability to elicit a protective immune response. Transgenic tobacco chloroplasts have been shown to accumulate PA to levels as great as 14.2% of total soluble protein.[28] An in vitro macrophage lysis assay demonstrated that PA derived from chloroplasts was fully functional at levels comparable to *B. anthracis*-derived PA used as a positive control. Neutralization of PA was successfully accomplished with sera taken from mice 15 days after the third immunization with extracts of tobacco chloroplast expressing PA. Survival of immunized mice challenged with a lethal dose of anthrax LT (lethal toxin) further demonstrated the immunoprotective properties of chloroplast-derived PA.[29]

SARS

Due to recent outbreaks, there has been an increased incentive for an effective vaccine against the coronavirus which causes SARS (severe acute respiratory syndrome). Pogrebnyak et al. (2005) expressed the N-terminal fragment of the coronavirus spike protein (S1) at high levels in both tomato and tobacco plants.[30] Tomato fruit was lyophilized and fed to mice who exhibited increased IgA titres toward S1 in their feces. When mice were immunized parenterally and later boosted with S1 protein expressed in tobacco roots, IgG titres corresponding to S1 were detected in their sera. More significantly, high IgG1 immune responses and significant IgG2a and IgG2b responses were observed, suggesting that these animals elicited a Th2-type response, as opposed to the Th1-type response found for mice.

Rabies Virus

Rabies causes approximately 55,000 deaths a year in Southeast Asia and Africa but does not receive significant financial attention because it is not a major killer in the industrialized world. The vaccine currently available is too expensive for developing countries. A recombinant plant virus expression vector has been engineered to express the rabies virus spike antigen.[31] Mice fed spinach leaves infected with the recombinant virus particles were able to display an immune response. Further studies indicated that mice, which were immunized orally with this engineered virus and then infected with an attenuated strain of rabies virus, were able to recover rapidly.

Measles Virus

Measles is contracted through the respiratory tract and is highly contagious. The case-fatality rate of measles can be several hundred times greater in the Third World than in developing nations. Over 30 million cases of measles were reported in 2004. Eradication of the virus has been confounded by its highly contagious nature, combined with the difficulty of maintaining and administering the vaccine in countries in which there is a scarcity of refrigeration, medical infrastructure, and syringes required for subcutaneous administration.

Preliminary studies have illustrated that a DNA measles vaccine, when used in conjunction with a plant-derived antigen booster, can evoke a substantial immune response. High-titre MV-neutralizing antibodies were shown to be generated in mice when a plant-derived MV-H protein vaccine was combined with a MV-H DNA vaccine in a prime-boost vaccination strategy.[32] Almost all mice administered first with an intramuscular dose of MV-H and later with orally administered plant-derived MV-H exhibited an IgG response. The results of this study suggest that this heterologous prime-boost approach will be successful for other plant-derived vaccines as well.

In a later study, the MV-H protein was expressed in lettuce and proven to be immunogenic in mice following intraperitoneal injection without an adjuvant or intranasal inoculation with adjuvant.[33] Mice primed with MV-H DNA and boosted with an oral formulation of freeze-dried lettuce expressing MV-H in the presence of an adjuvant elicited the greatest response. Furthermore, the nature of the immune response depended upon the manner in which the MV-H antigen is presented to the immune system. For example, both soluble as well as secreted forms of MV-H were demonstrated to induce a Th2 type response, whereas membrane-bound MV-H protein elicited a Th1 response.

Influenza Virus

Influenza virus is responsible for 300,000-500,000 deaths and three to five million hospitalizations annually. Every flu season, new epidemic strains of influenza A arise due to point mutations within the surface glycoproteins hemmaglutinin (HA) and neuraminidase (NA). These changes enable any new emerging virus strains to evade the host's immune system. Currently, vaccines against influenza virus are produced in chicken eggs, an expensive process with a long production time.

More recently, tobacco plants, which express the full-length HA from the Awyoming/03/03 strain of influenza virus, were developed.[34] This plant-derived HA has been demonstrated to be antigenic both by ELISA and by single radial immunodiffusion assay (SRID). Moreover, plant-derived HA was found to be immunogenic in mice. A high serum IgG titre was observed following the first antigen boost and was enhanced following the second boost to levels comparable to the commercially available egg-produced, formalin-inactivated virus. IgG subtypes were analyzed, with IgG1, IgG2a and IgG2b antibody responses identified, suggesting that both Th1 and Th2 responses were stimulated using the plant-derived vaccine. Additionally, an ELISPOT analysis of spleen cells was used to show that the increase in production of both gamma-IFN and IL-5 in response to challenge resembled that of the commercially purchased inactivated virus. Plant-derived influenza vaccine also induced significant serum hemagglutinin inhibition (HI) and virus neutralizing (VN) antibody titres. The serum HI and VN titres found in mice immunized with plant-derived HA correlated well with levels observed in serum from mice immunized with the commercial virus. The high quality of immune response determined from these experiments demonstrates well the potential for developing an effective influenza vaccine using a plant-based approach.

Monoclonal Antibodies Generated in Plants

Plants have also been engineered to produce a variety of functional Mab. The development of Guy's 13 secretory IgA plantibody technology commenced with the work of Ma et al. (2005) and involved the sexual crossing of four transgenic plants, each expressing both heavy and light immunoglobulin domains, the J chain, and the secretory component.[36] Plants, which could express and correctly assemble all four proteins simultaneously, were screened. Preliminary clinical trials indicated that plant-derived IgA prevented oral colonization by *S. mutans* via passive immunization of the mucosal surfaces by topical application. Since this first study, many Mabs have been produced in plants. A well-studied plant-derived Mab is the anti-rabies human monoclonal antibody, which was developed in tobacco and has been demonstrated to exhibit an anti-rabies virus neutralizing activity and affinity comparable to mammalian-derived counterpart HRIG.[37]

Plant-Made Vaccines, Allergies, and Oral Tolerance

Most substances in the gut are not immunogenic due to the cellular environment at the site of antigen presentation. This lack of response prevents the onset of unnecessary and damaging inflammatory responses to benign substances, which may lead to conditions such as inflammatory bowel syndrome and food allergies.[38,39,40] Oral tolerance, the phenomenon of feeding with a specific protein resulting in the abolishment of subsequent responses to systemic challenge with the same protein, is a reflection of how antigen is processed and presented to T lymphocytes which reside in the mucosa.[41] To examine the ability of plant-derived antigens to induce oral tolerance, Takagi et al. (2005) developed transgenic rice plants expressing mouse T cell epitope peptides specific for pollen allergens of *Cryptomoeria japonica* (Japanese Cedar).[42] The T cell epitope peptides corresponding to Cry jI and Cry jII pollen antigens were expressed together with soybean storage protein glycinin AlaB1b as part of a fusion protein. Mice which were fed transgenic rice were later challenged by feeding with total protein extracts of pollen as the allergen. Oral consumption of transgenic rice to mice prior to systemic challenge resulted in allergen-induced oral tolerance, accompanied by a dramatic inhibition of sneezing. 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.[43]

The plant-derived vaccine strategy for oral tolerance has also been demonstrated to successfully suppress asthma-based allergies. Allergic asthma, a chronic airway inflammatory disorder,

is often associated with the presence of activated CD4(+) Th2-type lymphocytes, eosinophiles, and mast cells. Sunflower Seed Albumin (SSA), a common allergen, has been expressed in transgenic narrow leaf lupin (*Lupinus angustifolius L.*).[44] Oral consumption of plants expressing SSA prevented a delayed-type hypersensitivity response. Experimental asthmatic symptoms, such as mucus hypersecretion, eosinophilic inflammation, and enhanced bronchial reactivity, were significantly reduced, while the production of CD4(+) T cell-derived IFN-gamma and IL-10 was increased.[44] These data demonstrate that plant-based vaccines may have potential applications in the protection against allergic diseases, such as asthma.

Real-Time Plant-Derived Pharmaceuticals

As mentioned earlier, one original driving force for generating plant-derived vaccines has been to develop new vaccines and therapeutic agents which target the most devastating infectious diseases found in developing countries. Diarrhea, the major cause of global mortality, and other diseases, which prevail in developing countries, are not being prioritized by the private sector, as there is little hope of return on investment. However, the fact remains that 20% of the world's infants have no access to vaccines, and two million deaths take place each year due to preventable infectious diseases. Plant-derived vaccines would also be useful against those diseases which are rare and whose cures are not well financed, such as dengue fever, hookworm, and rabies. Inexpensive and easy-to-administer, plant-derived vaccines could provide relief to the usual constraints involved in vaccine delivery.

Vaccines have been produced in both food crops and in plant species not routinely eaten, in the greenhouse, open field, and through cell suspension culture. Field-grown plants may fall prey to variations in soil and weather, which can negatively impact the good manufacturing practice conditions required for production of pharmaceuticals in general. Cell suspension culture, on the other hand, can be grown in a precisely controlled environment or even grown continuously, resulting in less expensive downstream processing. While purification of vaccine proteins from plants entails some cost, recent advances in this direction have demonstrated that plant-derived protein purification is less costly and requires fewer steps than mammalian and bacterial protein purification. Indeed, some forms of plant-derived therapeutic proteins, such as topically applied monoclonal antibodies, need only be partially purified, and, as a result, would be even less costly and labor-intensive. Approval for release of the first plant-derived pharmaceutical, a veterinary vaccine for Newcastle Disease in poultry, which was generated from plant cell culture, sets the stage for a new range of proteins produced in plants for use in medicine.

Concluding Remarks

When first cited in the literature, plant-derived vaccines were introduced as "edible vaccine." True to form, the first clinical trial performed within the US required volunteers to consume 100-150 g of raw transgenic potato (Richter et al. 2000). Since this initial trial, researchers have 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 or even from an individual's own garden. The advantages to the use of food crops for vaccine production frequently led to public misperceptions as to how these materials would be delivered in a practical sense. Eventually, to control the level of exposure of the antigen or vaccine protein, the production of plant-made vaccines and therapeutic proteins further evolved to meet the standard requirements for the productivity of pharmaceuticals in general by avoidance of the issues of dose variability and assurance of high quality of the product. Edible vaccines are, therefore, more commonly referred to at present as plant-made pharmaceuticals (PMPs), where a plant product is derived from batch-processed plant tissues or a similar processing method, which can then be prescribed by a health-care worker. In the end, the vaccine is more likely to be administered in the form of a capsule, paste, or juice, or even perhaps as a suspension for oral delivery, rather than as a whole tomato or banana.[45]

The results of the pre-clinical and clinical trials of plant-derived vaccines and therapeutic proteins described in this review hallmark the potential of plants to become oral delivery vehicles for vaccines. Those who ingest plant tissue containing vaccine antigen exhibit a greater immune response and recover more rapidly from disease than those who ingest control plants in human volunteer or animal model studies. The provocation of mucosal immunity against a given antigen can be achieved by other means besides oral ingestion. For example, intranasal immunization of vaccine proteins can improve local mucosal immunity and enable large populations to be immunized at less cost. Plant-derived vaccines continue to provide promise and hope for more immunogenic, more effective, and less expensive vaccination strategies against both respiratory as well as intestinal mucosal pathogens of the Third World.

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Kathleen L. Hefferon, Ph.D., Cornell Research Foundation, Cornell University, Ithaca, NY, completed her Ph.D. in Molecular Virology from the Faculty of Medicine, University of Toronto. She is a science writer for the Center for Hepatitis C Research at Rockefeller University.

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