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Plant-Derived Human Vaccines; An Overview

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Abstract: Biotechnology has offered important and efficient means for improving human life and health. However in spite of incredible development of biotechnological procedures, there are problems in point of economical view, especially in the case of products which are needed in huge amounts and relate to human health, such as vaccines. Application of biotechnology in such way that eliminates or reduces time-consuming and expensive processes, regarding production and subsequent quality control steps, can help better vaccination programs for large population, especially in the developing countries. The aim of this study was to summarize all data about human plant-based vaccine development including candidate antigens, transgenic plants and corresponding immunological responses in animal models or human using complete literature bibliography. The conclusion is that viral vaccines have been studied more than bacterial ones. Crude extracts of transformed plant materials as well as purified recombinant antigens expressed in plants have been found to induce immunological response in some investigations. Most of animal studies have been done with great success. Although few studies have been performed in humans but most of them have lead to hopeful results. Presently none of the commercially available products are produced in plants while most of biotechnology products which are comprised of proteins and possibly DNA-based vaccines are good potential candidates for plant-based production. Continuing investigations on plant-based vaccines is very crucial.

Key words: Vaccine, plant, human pathogens, bacteria, viruses

INTRODUCTION

Vaccines are administered to humans and animals for induction of their immune response against viruses, bacteria and other types of pathogenic organisms as well as some autoimmune diseases (Carter and Langridge, 2002; Ma and Jevnikar, 1999).

However, manufacturing of vaccines is time-consuming and expensive process yet regardless of being provided from whole microorgamism (live, killed or attenuated) or structural subunits like toxins (Streatfield *et al*., 2002). Parenteral route is the most common vaccine administration.

Many healthy hazardous infective agents (enteric, respiratory and sexually transmitted pathogens) use mucosal epithelium for attachment and penetrating inside the body. Mucosal immunization against infectious disease and for treatment of some autoimmune diseases (e.g., rheumatoid arthritis, inflammatory bowel diseases, Bechet's disease and lupus erythematosus) has recently attracted much interest (Rezaie *et al*., 2005; Hadjbabaie *et al*., 2005; Rigano and Walmsley, 2005). Orally administrated vaccines are effective means for induction of mucosal immunization using IgA response and subsequent mucosal immunologic memory. There are also evidences for arising of humoral (Marquet-Blouin *et al*., 2003) and cellular immunological reactions via B and T lymphocytes ($CD8^+$ cytotoxic cells and $CD4^+$ helper cells), as well as natural killer (NK) cells (Rigano and Walmsley, 2005; Walmsley and Arntzen, 2003; Holmgren *et al*., 2003). There are some reports about mucosal adjutants and these necessary components of such vaccines have been developed especially based on native or detoxified bacterial toxins (Holmgren *et al*., 2003; Lycke, 1997; Pizzia *et al*., 2001), derivatives or CpG motif-containing DNA (McCluskie *et al*., 2000).

Oral vaccines have some advantages such as better uptake and higher efficacy (Streatfield *et al*., 2002). However, of all the vaccines being produced today, only a few are being produced for oral administration (polio, cholera, typhoid and tuberculosis).

Anyway, there are many candidate pathogens which could be subjected for mucosal vaccine development; these include vaccines for intestinal pathogens (e.g.,

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Helicobacter pylori, hepatitis virus and entero-toxigenic *E. coli*), respiratory pathogens (rhinovirus, influenza and tuberculosis) and genito-urinary sexually transmitted diseases (e.g., HSV, HIV). This list of microbial and viral pathogens is become increasingly larger considering pathogens that are common between animals and humans.

Subunit vaccines and role of biotechnology: Subunit vaccines are purified antigens that have been made especially based on specific proteins of infectious agents by biotechnological tools and administrated parenterally or orally and can induce systemic as well as mucosal immunization (Nemchinov *et al*., 2000; Lauterslager *et al*., 2001; Rigano *et al*., 2006).

Application of recombinant DNA technology has increased both safety and efficacy of biopharmaceuticals but not the cost of industrial production of some products such as subunit vaccines, considering expensive materials and procedures (e.g., purification steps).

According to the report published by the Pharmaceutical Research and Manufactures of America (PhRMA; Washington, DC), vaccines are the largest category of products amongst different biopharmaceutical products which reached clinical trial phase annually, followed by monoclonal antibody-based products (PhRMA, 2002).

The choice of an expression system for the production of recombinant proteins depends on many parameters, regarding technical and economical aspects. As mentioned above, addition of any extraordinary process for purification, refolding, posttranslational modification, long-term storage, scaling up, maintenance of biological activity of the protein, as well as quality control procedures in the production of therapeutic proteins increase the cost of successful and commercial production of a recombinant protein (Macrides, 1996; Datar *et al*., 1993).

PLANT-BASED PRODUCTS

Amongst living organisms employed for production of recombinant proteins; either with clinical use or not, plant systems have recently attracted much interest as a means for production of purified recombinant proteins especially in exactly or partially folded structure and also as edible products in which express and deliver subunit vaccines.

Molecular farming has been applied for two decades for production of wide range of recombinant proteins. The most important future of plant-based expression systems have been reviewed elsewhere (Streatfield *et al*., 2002; Faye *et al*., 2005; Goldstein and Thomas, 2004) and recent advances in genetic engineering have provided the efficient tools for transformation of plants by foreign genes and expression of variety of biofarmaceutics in such a level appropriate for commercial purpose (Rigano and Walmsley, 2005; Schillberg *et al*., 2005). The first generation of recombinant proteins produced in transgenic plants is now reaching commercial status (Fischer *et al*., 2004). Research now underway for production of other therapeutic agents including monoclonal antibodies (Verch *et al*., 1998), antimicrobial agents (Chong *et al*., 2000), hormones (Barta *et al*., 1996), blood components (Sijmons *et al*., 1996) and various interferons (De Zoeten *et al*., 1989). It should not be forgotten that presently none of the commercially available products are produced in plants while most of biotechnology products which are comprised of proteins and possibly DNA-based vaccines are good potential candidates for plant-based production (Goldstein and Thomas, 2004).

The first clinical trial of an edible plant vaccine which expressed in potatoes was done in 1997 with permission of US Food and Drug Administration (Tacket *et al*., 1998). In spite of numerous studies that currently underway in field of edible plant vaccines, the majority remain in the phase I/II of clinical trials and a few have been tested on human volunteers (Thanavala *et al*., 2005; Tacket *et al*., 2000, 2004; Yusibov *et al*., 2002; Kapusta *et al*., 2001). The most important future of clinical trial phases and commercialization of plant vaccines have been reviewed by Kirk and Webb (2005).

The main objective of the present paper is to summarize all investigations about plant vaccines production against human pathogens including bacteria and viruses.

Plants: The variety of plant species have been used for the production of recombinant proteins like alfalfa (Due Santos *et al*., 2005), potato (Arakawa *et al*., 1997), tobacco (Ghosh *et al*., 2002), maize (Chikwamba *et al*., 2002), arabidopsis (Rigano *et al*., 2006), corn (Streatfield *et al*., 2002), tomato (Walmsley *et al*., 2003), carrot (Bouche *et al*., 2003), lettuce (Kapusta *et al*., 2001), cowpea (Durrani *et al*., 1998), spinach (Karasev *et al*., 2005) and even unicellular algae such as *Chlamydomonas* SPP (Sun *et al*., 2003; Goldschmidt-Clermont, 1991). Fruits (apple, banana, grape, melon, kiwi, peanut), barley, canola, cauliflower, cranberry, cucumber, pea, pepper, raspberry, rice, service berry, soybean, squash, strawberry, sugar beet, sugarcane, sunflower and sweet potato have also been used (Richter and Kipp, 1999). Some of mentioned plants have advantages in regard of easy cultivation and

high volume yield, especially in the case of production of proposed plant vaccines for domestic animals. However many human vaccines that should be administered to infants, have to be consumed uncooked for prevention of protein denaturation and must have no toxic materials if applied as edible and unprocessed vaccines (direct ingestion of plant materials). In the case of transformation of tobacco, the expressed protein must be extracted and purified (Koya *et al*., 2005; Watson *et al*., 2004; Aziz *et al*., 2002). Low nicotinic tobacco has been used in some studies (Pogrebnyak *et al*., 2005). Tobacco has been used in many studies according to ease of transformation and extensive genomic sequence knowledge (Sala *et al*., 2003). Various parts of plants (leaves, seeds, fruits, root hairs, chloroplasts) can also be used as vehicles for the biomedical products.

Systems: Plant transformation is achieved by two main tools including stable plant transformation (stable integration of desired genes into the plant genome, either nuclear DNA or chloroplast DNA) and transient transformation of plants through infection of plants by modified plant viruses which have a desired gene.

Stable plant transformation: *Agrobacterium tumefacience* is most frequently studied plant parasite which is used for integration of the gene of interest to nuclear genome. However integration occurs at random chromosomal sites by this mean. Chloroplast can be transformed in stable system. Chloroplast genome is a circular DNA which is present in multiple copies (up to 10000 copies) in plant cells and can accept large and multiple coding sequences as well as nuclear DNA. Furthermore, site-specific integration of genes to chloroplast DNA, the presence of great knowledge about its nucleotide sequence (which ensures proper integration of foreign genes using well known flanking sequences) and the ability of chloroplast for production of correct folded eukaryotic proteins are important futures of stable transformation of plants via integration of genes to chloroplast (Daniell *et al*., 2002). Beside the higher production of recombinant protein than nuclear system, production and accumulation of the foreign protein in the chloroplast does not significantly affect photosynthetic efficiency (Sala *et al*., 2003). However, the main limitation for application of chloroplast-based transformation system is that chloroplast DNA transformation still requires optimization in many plant species (Kuroda and Maliga, 2001) expect of tobacco (Koya *et al*., 2005).

Transient plant transformation: Transient expression systems are used for expression of foreign genes which are not integrated to genomic DNA and cannot pass over the generations. Virus-based systems are frequently used approach and the viral genome is designed in such a way to express the interested gene within coat glycoprotein without any interfering with self assembly properties of virions. Plant viruses having plus-sense, single-stranded RNA as genome have been applied for this purpose (Zhang *et al*., 2000; Yusibov *et al*., 1997) with shorter time for cloning of the foreign gene in the viral genome as compared with time required to transform the plant cells, the ease at which antigen production can be scaled up and the wide host range of plant viruses that allow the use of multiple plant species as biofactories (Koprowki and Yusibov, 2001).

BENEFITS OF PLANT-BASED VACCINES

The most important futures of plant-based system for production of vaccine can be listed as follow:

- Inexpensive large scale production; cost will be reduced 100-1000 times as compared with that of traditional vaccines (Sala *et al*., 2003).
- Easy storage (heat stability and prevention of contamination by microorganisms except those are produced in tomato and tobacco which should be kept at 4°C and frozen, respectively (Stoger *et al*., 2002).
- Easy processing (use as raw food or dry powder, or partially or completely purified materials) (Sala *et al*., 2003).
- Convenient, easy and safe administration (in oral route) and applicable as parentral (Bouche *et al*., 2003) or nasal (Tregoning *et al*., 2005) products when be purified.
- Good result in the case of systemic and mucosal immunity induction (Lauterslager *et al*., 2001; Rigano, 2006).
- Localization of expressed protein in desired cellular compartment (e.g., chloroplast) (Koya *et al*., 2005; Daniell *et al*., 2001; Tregoning *et al*., 2003).
- A proposed application against bio-terrorism or biological weapons (Sala *et al*., 2003).
- A proposed application for large scale vaccination of domestic animals

Besides important future of plant-based system for vaccine production listed above two other opportunities can be achieved using such systems:

Formulation of multicomponent vaccines: Another important future of plant-derived vaccine technology is development of vaccines combining numerous antigens. For example, it could be possible to make a plant producing antigens to stimulate effective immune

response to cholera, enterotoxigenic *E. coli* (ETEC) and rotavirus. In the mentioned study, a cDNA which contains cholera toxin (CT) B and A2 subunit coding sequence and rotavirus enterotoxin and enterotoxigenic *E. coli* fimbrial antigen genes was expressed in potato. Orally immunized mice showed detectable levels of serum and intestinal antibodies against 3 pathogens as well as significant increase in CD^{4+} lymphocyte numbers in their spleens (YU and Langridge, 2001). In another study, an edible vaccine for hepatitis B and HIV have been designed (Schelkunov *et al*., 2004).

Easier multiple boosting: Immunization against some infectious agents such as malaria causative agents, hepatitis viruses, HIV and measles virus need a broad immune response that is achieved via multiple boosting of available vaccines (e.g., hepatitis B). However simultaneous administration of DNA vaccines and plant materials expressed measles proteins could arise immune response in mice (Webster *et al*., 2002). Similar strategy could be achieved to overcome the mentioned problem which could also decrease the risk of blood born agent transmission. The immunization strategy for a plant-derived measles virus (MV) vaccine was optimized and resulted in a significant increase in MV-neutralizing antibodies. An enhanced immune response to a prime-boost vaccination strategy combining a DNA vaccine with orally delivered plant-derived vaccines was demonstrated (Webster *et al*., 2002).

PLANT-BASED VACCINES INVESTIGATIONS

Production of bacterial and viral plant vaccines have been studied in some investigations. Here almost all of such experiments have been shown in Table 1 and 2. Only vaccines with future application in human have been considered in the present study.

Table 1: Plant-based vaccine investigations for human bacterial pathogens and corresponding immunological responses

Pathogen	Antigen	Plant species	Immunological response	References
Bacillus anthracis		Tobacco (Chloroplast)	Subcutaneous immunization of mice yielded IgG	Koya *et al.* (2005)
	83 kDa protective antigen	Tobacco	None tested	Waston *et al.* (2004)
	83 kDa protective antigen	Tobacco (*Agrobacterium* - *mediated*)	Cytotoxicity of expressed lethal toxin was confirmed in macrophage cell line (RAW 264.7)	Aziz *et al.* (2002)
Vibrio cholerae	Cholera toxin B subunit) (ctxB)	*Nicotiana tabacum*	Immunization of mice has showed the effects of recombinant Cholera toxin B subunit on T-cell proliferation and cytokine levels	Jani *et al.* (2004)
	LT-B of *E. coli*	Maize	Induction of semm and mucosal immunity in maize-fed mice	Chikwamba *et al.* (2002)
	Cholera toxin B subunit (ctxB)	Tobacco (Chloroplast)*	None tested	Daniell *et al.* (2001)
	Cholera toxin B subunit oligomers	Potato	None tested	Arakawa *et al.* (1997)
	Cholera toxin B subunit	Potato	Cholera toxin B subunit -specific antibodies were induced in orally immunized mice	Arakawa *et al.* (1980)
	Cholera toxin B subunit	Tomato	None tested	Jani *et al.* (2002)
M. tuberculosis **	Antigen ESAT-6 fused to the B subunit of *E. coli* heat-labile enterotoxin (LTB)	*Arabidopsis thaliana*	Induction of Th1 response (antigen-specific responses from CD4+ cells and increased IFN-gamma production) and Th2 response was induced in the Peyer's patch.	Rigano *et al.* (2006)
Helicobacter pylori	Urease subunit B	*Nicotiana tabacum*	None tested	Gu *et al.* (2005)
Clostridium tetani	TetC	Tobacco Chloroplast	Both intranasal and oral administration could induce CD4+ T cell driven B cell antibody production.	Tregoning *et al.* (2003)
	TetC		This is the first study documenting protective immunity by a single intranasal dose of plant vaccine	Tregoning *et al.* (2005)
E. coli (enterotoxigenic)		Potato	Oral administration could arise low level systemic and local antibody production	Haq *et al.* (1995)
	Heat-labile enterotoxin B subunit (LT-B)	Potato	Mice immunized with potato LT-B had higher levels of serum and mucosal anti-LT-B than those gavaged with bacterial LT-B	Mason *et al.* (1998)

Table 1: Countinued

Pathogen	Antigen	Plant species	Immunological response	References
	Heat-labile enterotoxin B subunit (LT-B)	Potato (*Agrobacterium* mediated)	Recombinant protein was immunogenic and oral administration of tubers elicited both systemic and local Ig A responses in parentally primed, but not naive, animals.	Lauterslager *et al.* (2001)
	Heat-labile enterotoxin B subunit (LT-B)	Tobacco (Chloroplast)	Heat-labile enterotoxin (LTB) protein with biochemical properties (binding to GM1-ganglioside receptors) identical to native LTB protein was expressed	Kang *et al.* (2003)
	Major F4ac fimbrial subunit protein (fae G)	Tobacco	The plant-produced FaeG could bind to the receptors on the villi and subsequently inhibit F4 ETEC binding in a dose-dependent manner	Joensuu *et al.* (2004)
	Heat-labile enterotoxin B subunit (LT-B)	*Nicotiana benthamiana* (virus based)	Purified rLTB from plant extracts was capable of binding G(M)1 ganglioside and intranasal application of rLTB (15 microg per mouse) induced LTB-specific IgG1 antibodies	Wagner *et al.* (2004)
	Heat-labile enterotoxin B subunit (LT-B)	Maize kernel	None tested.	Chikwamba *et al.* (2003)
	GM1 receptor binding (B) subunit of the heat-labile toxin (Lt)	Coru	Binding to the receptors on the villi inhibit F4 ETEC binding in a dose-dependent manner	Streatfield *et al.* (2002)
	Heat-labile enterotoxin B subunit (LT-B)	Tomato	None tested	Walmsley *et al.* (2003)
Pseudomonas aeruginosa	A synthetic peptide (peptide 10) of outer -membrane protein F fused to protein F	Cowpea	Subcutaneous administration in mice could induce *P. aeruginosa*-specific opsonic IgG(2a)	Brennen *et al.* (1999)
Porphyromonas gingivalis	C-terminal binding portion of *P. gingivalis* fimbrial protein (FimA)	Potato	None tested	Shin *et al.* (2005)

*This is the first report of transgenic chloroplasts manufacturing a plant-derived vaccine. ** This is the first report of an orally delivered, subunit, tuberculosis vaccine priming an antigen-specific, Th1 response

Table 2: Plant-based vaccine investigations for human viral pathogens and corresponding immunological responses

Pathogen	Antigen	Plant species	Immunological response	References
Measles virus	Tandem repeats of a protective loop-forming B cell epitope (H386-400) of the measles virus hemagglutinin protein with a human promiscuous, measles-unrelated T cell epitope (tt830-844).	Carrot	I.P. administration in mice resulted in high titers of antibodies and the sera could neutralize field isolates of different geographic origins and genotypes.	Bouche *et al.* (2003)
	Multiple copies of the loop-forming hemagglutinin noose epitope (designated as "L"; aa386-400)	Carrot	Chimeric molecules expressing multiple copies of a protective B cell epitope of the measles vims could induce a repertoire of B cells diverse enough to overcome the genetic diversity of field viruses.	Bouche *et al.* (2005)
	Coding region of the measles virus hemagglutinin (H)	Tobacco	The plant-derived measles H protein was immunogenic when administered orally	Huang *et al.* (2001)
	Hemagglutinin (H) protein	Tobacco	A single-dose DNA immunization followed by multiple boosters (orally delivered plant materials) could induce measles virus-neutralizing antibodies	Webster *et al.* (2002)
Hepatitis B	Hepatitis B surface antigen (HbsAg)	Tobacco	None tested	Mason *et al.* (1992)
	Hepatitis B surface antigen (HbsAg)	*Lupinus luteus* and *Lactuca sativa*	Hepatitis B virus surface antigen, could develop specific semm-IgG response in human volunteers	Kapusta *et al.* (1999, 2001)
	Hepatitis B surface antigen (HBsAg)	Potato	HBsAg-specific serum antibodies induced in mice fed HbsAg-transgenic potatoes and a parenteral boosting, generated a strong longlasting secondary antibody response	Kong *et al.* (2001)
	Hepatitis B surface antigen (HBsAg)	Potato	None tested	Smith *et al.* (2003)

Table 2: Continued

Pathogen	Antigen	Plant species	Immunological response	References
	Hepatitis B surface antigen (HbsAg)	Potato	A primary immune response was induced in mice fed transgenic tubers which could be greatly boosted by intraperitoneal delivery of a single subimmunogenic dose of commercial HbsAg vaccine	Richter *et al.* (2000)
	Hepatitis B surface antigen (HbsAg)	Potato	serum anti-HBsAg titers increased in 10 of 16 human volunteers (62.5%) who ate three doses of potatoes	Thanavala *et al.* (2005)
	Hepatitis B surface antigen (HBsAg)	Potato	The virus-like particles produced in plant stimulated serum IgG and IgA responses in mice and humans	Huang *et al.* (2005)
	Hepatitis B surface S and preS2 antigens	*Solanum tuberosum*	Feeding of potatoes to mice could arise antibody response.	Joung *et al.* (2004)
Hepatitis E	Hepatitis E virus (HEV) ORF2 partial gene	Tomato (*Agrobacterium mediated*)	None tested	Ma *et al.* (2003)
	Hepatitis E virus capsid protein (HEV CP)	Potato	Oral immunization of mice with transgenic potatoes failed to elicit detectable anti-CP antibody response in serum.	Maloney *et al.* (2005)
Hepatitis C	Consensus HCV-HVR1 epitope (R9) that antigenically mimics many natural HVR1 variants	Tobacco	Plant-derived HCV antigens could react with specific monoclonal antibodies and immune sera from individuals infected with HCV	El Atter *et al.* (2004)
			Plant-derived antigens (HVR1/CTB) could react with specific monoclonal antibodies	Nemchinov *et al.* (2000)
	Consensus sequence of hypervariable region 1 (HVR1) fused on the C-terminal of the B subunit of cholera toxin (CTB)	Tobacco (Virus- based)	Immune sera from individuals infected with virus from four of the infected with virus from four of the major genotypes of HVC Intranasal immunization of mice with a cmde plant extract arose both anti CTB serum antibody and anti-HVR1 serum antibody	
	HVR1 sequences of the HCV envelope protein E2 (R9 mimotope)	Tobacco (Virus- based)	Specific humeral response in rabbit Down-modulation of the lymphocyte surface density of CD3 and CD8, in patients with chronic HCV infection Induction of a significant release of different cytokines in lymphomonocyte cultures R9 mimotope-specific CD8 T-cell response was achieved in the majority of the patients studied	Piazzolla *et al.* (2005)
Rabies vims	Surface glycoprotein (G-protein) gene	Tomato (*Lycopersicon esculentum* Mill var. UC82b)	None tested	Mc Garvey *et al.* (1995)
	A chimeric peptide containing amino acids 253-275 of GP and nucleoprotein amino acids 404-418 of nucleoprotein	*Spinacia oleracea* (Virus-based)	Clinical trials (orraly) in human volunteer have shown significant antibody responses to plant material extract	Yusibov *et al.* (2002)
	Coding sequence of surface glycoprotein (G protein)	Tobacco leaves (*Nicotiana tabacum*)	Purified G protein from tobacco leaf microsomal fraction could immunized mice (IP) and elicited high level of immune response	Asraf *et al.* (2005)
Transmissible gastroenteritis coronavirus (TGEV)	N-terminal domain (amine acid residues 1-750) and the full-length glycoprotein S of TGEV	Arabidoposis	Leaf extracts from transgenic plants could develop antibodies in immunized mice that reacted specifically with TGEV in ELISA, immunoprecipitated the virus induced protein and neutralized the virus infectivity	Gomez *et al.* (1998)
	N-terminal domain of the glycoprotein S (N-gS)	Potato tuber	Extracts (I.P) and potato tubers expressing N-gS (orally) developed serum IgG specific for TGEV and serum antibodies specific for gS protein, respectively	Gomez *et al.* (2000)
Human respiratory syncytial virus	Two peptides containing amino acids 174-187 of the G-protein of the human RSV A2 strain fused to alfalfa mosaic virus coat protein	*Nicotiana tobacum* (Virus-based)	High levels of serum antibody specific for RSV G-protein was induced in BALB/c mice (IP) and they were protected against infection with RSV long strain	Belanger *et al.* (2000)
	RSV fusion (F) protein gene	Tomato fruit	Induction of both serum and mucosal RSV-F specific antibodies in mice with ripe transgenic tomato fruit	Sandue *et al.* (2000)
Human cytomegalovims	The major glycoprotein (gB)	Tobacco	None tested	Wright *et al.* (2001)
Norwalk virus	Norwalk virus capsid	Potato	Human trial showed an immune response	Tacket *et al.* (2000)

Table 2: Continued

Pathogen	Antigen	Plant species	Immunological response	References
	protein (NVCP), assembled into virus-like particle		(semm IgG in 20% and stool IgA in 30%)	
	Capsid protein (NVCP)	Tobacco and potato	Low titer serum IgG in mice following feeding	Masson (1996)
	Capsid protein (NVCP)	Potato	The virus like particles in fresh potato tuber could stimulate serum IgG and IgA responses in mice and humans when they were delivered by ingestion	Huang *et al.* (2005)
Human papillomavirus	HPV16 L1 coding sequence	Tobacco	None tested	Liu *et al.* (2005)
	HPV major capsid protein L1	Tobacco and potato	Induction of an anti-L1 antibody response in 3 out of 24 mice ate tubers from transgenic potatoes	Biemelt *et al.* (2003)
	Plant codon-optimized version of the HPV type 11 (HPV11) L1 major capsid protein coding sequence	Potato	Plant-expressed L1 self-assembles into VLPs with immunological properties comparable to those of native HPV virions (I.P. and orally)	Warzecha *et al.* (2003)
Rotavirus	Codon-optimized gene (sVP6) encoding the VP6 protein of human group A rotavirus	Alfalfa	Immunized mice developed high titers of anti-VP6 serum IgG and mucosal IgA and passive immunization in their offspring	Dong *et al.* (2005)
	VP7	Potato	Mice immunized with the transformed tubers elicited serum IgG and mucosal IgA specific for VP7	Wu *et al.* (2003)
	Gene six encoding the 41 kDa group specific capsid structural protein VP6	Potato	Detectable humoral and intestinal antibody responses	Yu and Langride (2003)
	VP6	Tobacco	None tested	Birch-Machint *et al.* (2004)
Sars	N-terminal fragment of SARS-CoV S protein (S1)	Tomato and low-nicotine tobacco	Significantly increased levels of SARS-CoV -specific IgA in mice (ingestion of tomato) and detectable level of presence of SARS-CoV-specific IgG	Pogrebnyak *et al.* (2005)
HIV	Env/gp120	*N. benthamiana* (virus-based)	IP administration in Swiss-Webster mice have resulted in production of neutralizing IgG	Yusibov *et al.* (1997)
	Env/gp120(13-amino-acid peptide derived from the V3 loop)	Tomato (virus-based)	SC administrating in NMRI mice caused IgG	Joelson *et al.* (1997)
	Residues 731-752 of the transmembrane gp41 protein of HIV-1	Cowpea (virus- based)	HIV-1-specific IgA in faces and higher levels of specific serum antibody, IgG2a, were produced in mice after intranasal administration.Oral immunization was less effective	Durrrani *et al.* (1998)
	Highly conserved ELDKWA epitope from glycoprotein (gp) 41	Potato (virus-based)	Intraperitoneal or intranasal application of purified recombinant Ag could elicit high levels of HIV-1-specific immunoglobulinG (IgG) and IgA antibodies	Marusic *et al.* (2001)
	(HIV-1) p24 capsid protein	Tobacco (*Agrobacterium*-mediated)	None tested	Zhang *et al.* (2002)
	Env/gp41	Cowpea (virus-based)	The recombinant epitope was immunogenic in rabbits	Porta *et al.* (1994)
	Amino acid residues of 731-752 of the gp41 envelope protein of HIV type 1	Cowpea (virus-based)	Purified self-assembled virions could induce specific antibodies in adult C57/BL6 which gave a strong ELISA and could recognize conformational epitope on the gp41 oligomer	Mc Lain *et al.* (1995, 1996)
	Simian immunodeficiency virus (SIVmac) Gag p27 capsid gene (CTB-Gag) linked to cholera toxin B subunit (CTB)	Potato	None tested	Kim *et al.* (2004)
	Gag capsid protein of Simian immunodeficiency virus type (SIVmac)	*Solanum tuberosum* (*Agrobacterium* mediated)	None tested	Kim *et al.* (2004)
	12-amino acid (aa) HIV-1 Tat transduction peptide fused to a 90-aa murine rotavirus NSP4 enterotoxin protein (Tat-NSP4(90))	Potato	None tested	Kim and Langridge (2004)
	The V3 loop of HIV-1 envelope glycoprotein gp120 fused to the choler toxin B subunit gene (CTB-gp120)	*Solanum tuberosum* (*Agrobacterium* mediated)	GM1-ganglioside enzyme-linked immunosorbent assay (GM1-ELISA) was shown the binding of CTB-gp 120 fusion protein pentamers to intestinal epithelial cell membrane glycolipid receptors	Kim *et al.* (2004)
	Plant codon optimized Tat (1–93 aa),	Spinach (virus-based)	Oral administration could induce detectable specific antibodies when mice boosted with DNA vaccine	Karasev *et al.* (2005)

CONCLUSIONS

Viral vaccines have been studied more than bacterial ones. Crude extracts of transformed plant materials as well as purified recombinant antigens expressed in plants have been found to induce immunological response in some investigations. Most of animal studies have been done with great success and although few studies have been performed in humans, but most of them have lead to hopeful results. The continuing investigations in plant-based vaccines seems very essential.

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