

Production of recombinant antigens in plants for animal and human immunization - a review

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ABSTRACT

Plants present a cost effective production system for high value proteins. There is an increasing world demand for cheap vaccines that can be readily administered to the population, especially in economically less developed regions. A promising concept is the production of vaccines in plants that could be grown locally. Expression of antigenic peptides in the palatable parts of plants can lead to the production of edible active vaccines. Two major strategies are: i) to express antigens in transgenic plants, and ii) to produce antigenic peptides on the surface of plant viruses that could be used to infect host plants. This review considers the experimental data and early results for both strategies, and discusses the potential and problems of this new technology.

INTRODUCTION

Bacterial and viral infections are the leading cause of infant mortality worldwide, accounting for the loss of millions of children per year. Broad-spectrum antibiotics are often vital for treatment, and are administered where available. On the other hand, they are also often inappropriately applied, which results in the increasing occurrence of bacterial resistance to these agents. New, specific immunization strategies are therefore needed to prevent rather than cure infectious diseases. Many bacteria and viruses, transmitted by sexual contact, contaminated food or water, colonize or invade epithelial membranes. In general, mucosal immune response is more effectively achieved by oral rather than parenteral antigen delivery. Parenteral vaccination with whole cell and whole-virus preparations can lead to protective serum IgG and delayed type hypersensitivity reactions against

organisms that have a significant serum phase in their pathogenesis; however, it has limitations. For example, it does not elicit mucosal secretory IgA (sIgA) responses, and hence is ineffective against bacteria invading mucosal surfaces (Ruedl and Wolf, 1995). In contrast, oral immunization can successfully induce specific sIgA responses and systemic immune reactions if antigens are presented to T and B lymphocytes and accessory cells. New, oral immunization strategies should use antigens that lead to specific mucosal immunity, and are cheap, easy to produce, store and apply, in order to meet the needs of the developing world.

State-of-the-art industrial antigen manufacture is production of recombinant immunogenic proteins in culture of genetically transformed cells, e.g., yeast. After identification and isolation, a gene coding for an antigen can be introduced into any cell type and produced in large scales in recombinant cell-culture systems. Fermentation technology and stringent purification are required to yield large amounts of very pure recombinant protein, which is extremely expensive. This certainly does not result in vaccines below the US\$1

mark per dose, which is required to counter the problems of world health care costs (Fox, 1996).

Plants are the most important renewable resource commodity that we know. Over the last decade progress has been made in genetical transformation of plants, control and stabilization of recombinant protein expression. As a result, it may be possible to use genetically modified plants as novel manufacturing units for a variety of high-value therapeutic proteins. Since it has been possible to produce antibodies in transgenic plants, composed of the correctly assembled heavy and light chains (Hiatt *et al.*, 1989; Düring *et al.*, 1990), several research groups have focused on manufacturing antibodies binding to specific antigens of human pathogens (Ma *et al.*, 1995; Richardson and Marasco, 1995; Ma and Hiatt, 1996). The yield of synthesized whole antibody may be as much as 1% of the total soluble protein content in stably transformed plants (Hiatt, 1990). These antibodies can be isolated from plant tissue and used as vaccines. A somewhat different path is based on oral delivery of antigens to humans in order to stimulate sIgA production (Bergmann and Waldman, 1988). In 1992, Charles Arntzen's team from Texas A & M University in Houston (now at Boyce Thompson Institute for Plant Research, Ithaca) introduced the concept of antigen production in transgenic plants for vaccine use (Mason *et al.*, 1992). Large amounts of antigens can be produced at relatively low cost in an agriculturally based system. Furthermore, plant viruses can be genetically modified in order to display vaccine epitopes on their surfaces. Infection of plants with these recombinant viruses can lead to production of high antigen levels within the plant tissue. In either case, manufacture of these vaccines in the edible tissue of plants would combine production and delivery systems. Immunization might be possible simply through food consumption.

This review focuses on production of antigenic proteins as vaccines in transgenic plants and recombinant plant viruses. The progress that has been made by several research groups will be presented, discussed, and problems that arise from this new methodology will be evaluated.

STRATEGY I: EXPRESSION OF FOREIGN ANTIGENS IN PLANTS VIA STABLE TRANSFORMATION

Production of transgenic plants with foreign protein expression is a multistep procedure. Depending on the plant species, it can be rather quick (a few weeks) or slow (more than a year). Antigenic protein produc-

tion that will be used in active vaccination has to target a plant tissue that is palatable without further processing. Fruit like banana or tomato that are eaten raw might be suitable candidates for vaccine production.

The steps involved in the production of antigen-expressing transgenic plants are simplified in Figure 1. First of all, a gene coding for the antigen that would stimulate serum or mucosal antibody production against the selected pathogen has to be identified and isolated. Next, this gene has to be cloned into a plant expression vector. This is usually a plasmid suitable for plant transformation. It should contain a strong plant tissue specific or constitutive promoter that controls antigen sequence expression within the plant, as well as marker genes such as an antibiotic resistance marker for selection of transformed plant tissue. In order to find the ideal promoter, it might be necessary to isolate one that is specifically expressed in the target organ of the plant, e.g., the fruit, using one of the available methods (Hansen and Harper, 1997). It should be remembered, however, that not only the promoter but also enhancers and intronic or exonic 3' sequences may play a role in the quantitative expression of plant genes (Dean *et al.*, 1989; Chinn *et al.*, 1996) or in specific activation events (Elliot *et al.*, 1989; Douglas *et al.*, 1991; Dietrich *et al.*, 1992).

Recent reviews demonstrate the important role that post-transcriptional regulation of gene expression plays in plants (Abler and Green, 1996), and discuss ways to optimize transgene expression with an emphasis on post-transcriptional events (Kozziel *et al.*, 1996). Furthermore, chromosomal position effects of the transgene integration site can lead to variability in transgene expression (Matzke *et al.*, 1989). Methylation events can completely silence a transgene (Matzke and Matzke, 1995; Meyer, 1995). Following production of transgenic plants, a larger number of individuals should be analyzed in order to obtain a clear picture of transgene expression patterns. Transformation can be carried out using one of the following established systems (Lindsey, 1996): i) *Agrobacterium tumefaciens*- or *A. rhizogenes*-mediated transformation of plant tissue cultured *in vitro*; ii) particle bombardment to transfer DNA into plant cells or tissues, or iii) direct gene transfer into plant cells or protoplasts by electroporation or chemical methods. The choice of method depends on the plant used (not all plants, especially monocotyledonae, are susceptible to transformation with *Agrobacterium*) and the equipment available.

Following transformation, a callus-like tissue develops from transformed cells. Using selective pressure (e.g., an antibiotic) in the culture medium, plant tissue that expresses the foreign DNA is selected.

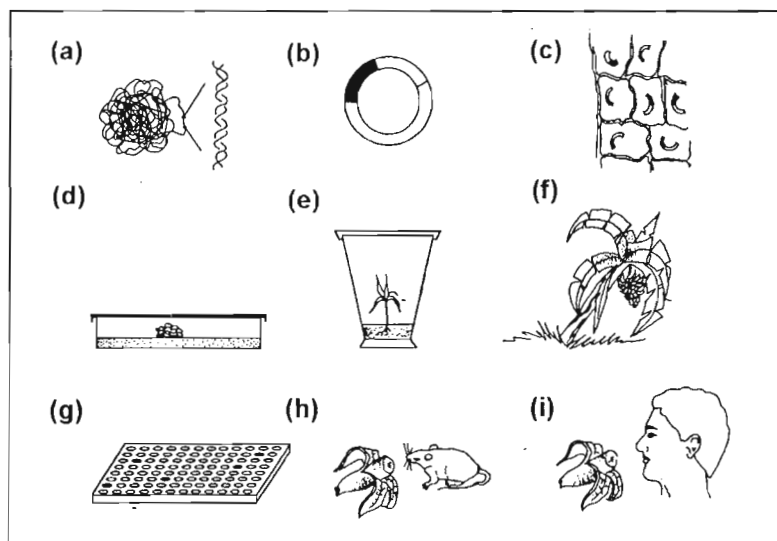


Figure 1 - Steps involved in the generation of a recombinant antigen for oral vaccination in transgenic plants. (a) Identification and isolation of antigen-coding DNA. (b) Cloning the gene into a plant expression vector. (c) Stable transformation of plant tissue. (d) Selection of transformed plant tissue. (e), (f) Regeneration of transgenic plants, expressing the antigen in edible tissue. (g) Antigenicity tests of protein extracts from transgenic plants. (h) Feeding experiments and immunogenicity tests in animals. (i) Immunization of humans with antigen-expressing, edible plant tissue.

Integration of the chimeric gene into the plant genome can be verified using DNA-DNA hybridization techniques. Plant shoot and root development can be induced through variation of plant hormone levels in the growth medium. Complete and fertile plants can be regenerated, cloned, selfed and crossed. Evaluation of antigen expression on mRNA and protein levels using the appropriate tests (Northern hybridization for mRNA levels, polyacrylamide gel electrophoresis of proteins, Western blot and enzyme-linked immunosorbent assay) can be carried out for different plant organs and tissues. It is especially important to evaluate the stability of expressional antigen levels in the edible target plant tissues. Biological activity of the antigens has to be tested in animal immunization studies: development and titers of serum and mucosal antibodies have to be analyzed following either injection of crude or purified protein extracts, or after feeding antigen-expressing plant tissue.

TRANSGENIC PLANT PRODUCTION OF ANTIGENS AGAINST BACTERIAL DISEASES

Two research groups have focused on vaccines against enteric diseases that are a major problem in developing countries. Enterotoxigenic *Escherichia coli* (ETEC) and *Vibrio cholerae* cause watery diarrhea by

colonizing the small intestine and producing enterotoxins. Untreated, the diseases lead to death, and are the leading cause of infant mortality worldwide.

Enterotoxigenic *Escherichia coli*

Arntzen's group (Haq *et al.*, 1995) produced vaccines against ETEC in plants. The structure of the heat labile enterotoxin (LT) of ETEC is well known, with its 27-kD A subunit and a pentamer of 11.6-kD B binding subunits (LT-B). The latter bind to G_{M1} ganglioside on the epithelial surface. The rationale for using the B subunits as vaccine components is that the production of antibodies would interfere with this binding process, and thus block the toxin activity. The gene encoding LT-B was cloned into a plant expression vector behind the cauliflower mosaic virus (CaMV) 35S promoter and the 5' untranslated region of the tobacco etch virus (TEV). The 3' sequence of the soybean *vspB* gene functioned as a termination

sequence. A second plasmid was constructed, where the short coding sequence for a signal peptide that leads to endoplasmatic (ER) retention of the protein was ligated to the 3' end of the LT-B coding sequence. The two chimeric constructs were independently transferred into tobacco and potato plants via the *Agrobacterium tumefaciens* transformation system. Following selection and regeneration of transformed plants, the antigen amounts in tobacco leaves and potato microtubers were quantified by ELISA. The amount of antigen produced varied among plants, even when the same plasmid was used. The maximum was 0.0014% of total soluble protein in leaves and 0.011% of total soluble microtuber protein. Plants transformed with the construct that included the ER retention signal sequence showed significantly higher expression of the antigen than the transgenic plants lacking this signal. This suggests that cellular compartmentalization of the fusion protein had taken place, facilitating oligomerization. Oligomerization of plant-derived recombinant LT-B was verified by gel filtration methods. Immunogenicity was determined by administering four doses of soluble extracts of tobacco leaves containing 12.5 µg of the antigen to BALB/c mice. The same amount of recombinant *E.coli*-expressed rLT-B was given to control mice. Both groups of mice developed equal amounts of antibodies in the serum and mucosa, and the biological activity of LT was neutralized by these antibodies to the same extent. Oral doses of 5 g of transgenic, rLT-B-expressing

potato microtubers (containing 15 to 20 μg of antigen) were fed to mice. The animals developed LT-specific serum IgG and mucosal IgA upon consumption of the tubers; however, the immune response was weaker than in control mice that received an oral gavage of 20 μg of purified bacterial rLT-B. Haq *et al.* (1995) suggest that this might indicate some interference of plant factors with antigen reactivity. They propose that a higher antigen concentration in plant tissue might overcome these limitations.

Vibrio cholerae

Cholera toxin (ctx) is a very well-characterized protein, and it can function as an antigen to stimulate oral immunity, which leads to high titer, specific antibody responses in both serum and mucosa after feeding the toxin without inducing oral tolerance (Elson and Ealding, 1984b). Pentameric B subunits of ctx (ctB) are responsible for ctx recognition by the G_{M1} ganglioside on the surface of various mammalian cell types (Spangler, 1992). Recombinant ctB on its own has been shown to be a potent oral vaccine against the cholera toxin (Jertborn *et al.*, 1992). The A chain (ctA) is an NAD-dependent ADP ribosyl transferase that is responsible for the diarrheogenic effect of the enterotoxin of *Vibrio cholerae* (Spangler, 1992). It enhances ctx antigen characteristics when orally administered (Sun *et al.*, 1994). Using primers designed for the previously published DNA sequences of the ctx genes (Mekalanos *et al.*, 1983), Hein *et al.* (1996) amplified the genes encoding ctA and ctB by polymerase chain reaction, and cloned them into plant expression vectors. Expression of the genes was controlled by the strong, constitutive CaMV 35S promoter and a reiterated 35S enhancer. Variants of the ctA gene were prepared and consisted of the mature ctA chain-coding sequence preceded by: i) the native ctA signal peptide sequence, ii) the coding sequence for a signal peptide of murine origin. A third construct based on (ii) included a single amino acid exchange in order to eliminate enzymatic activity of ctA. The chimeric plasmid constructs were separately introduced into tobacco via *Agrobacterium tumefaciens*. Transgenic tobacco plants containing the genes for ctA preceded by either the native or the murine peptide leader sequences expressed ctA protein in leaves. Under non-reducing conditions, most of this protein migrated identically to the 27 kD native ctA extracted from *V. cholerae*, demonstrating that it had been correctly processed in the plant, cleaving the leader sequences. The protein encoded by the third mutated ctA construct was expressed at lower levels in transgenic tobacco and migrated to 29 kD, which most likely represents the

uncleaved protein that still contains the leader sequence. Transgenic plants containing the ctB genes expressed the ctB protein in leaves. In SDS-PAGE, the protein migrated identically to the monomeric ctB isolated from *V. cholerae*, but pentameric ctB was not found. It is worth noting that Hein *et al.* (1996) did not find any alteration in the morphology of the transgenic plants.

CTX HOLOENZYME AS AN EFFECTIVE ANTIGEN CARRIER

Apart from functioning as a specific oral immunogen, the ctx holoenzyme has the characteristics of an adjuvant to generally stimulate systemic and mucosal immunity (Elson and Ealding, 1984a). Furthermore, it has been shown to be a very effective antigen carrier and adjuvant for coadministered antigens (Jobling and Holmes, 1992; Hajishengallis *et al.*, 1995). Expression of both the ctA and ctB subunits in the same tissue of one transgenic plant could lead to the assembly of the ctx holoenzyme. Sexual crosses of the plants transgenic for ctA and ctB produced by Hein *et al.* (1996) could result in a progeny expressing the ctx holoenzyme. This progeny could provide the opportunity to produce complex vaccines in plants that would induce immune responses against linked or coadministered antigen proteins.

TRANSGENIC PLANT PRODUCTION OF ANTIGENS AGAINST VIRAL DISEASES

Rabies virus

McGarvey *et al.* (1995) studied the expression of a rabies virus antigen in transgenic plants. Orally administered glycoprotein (G-protein), which coats the outer surface of the rabies virus, had been shown earlier to elicit protective immunity in animals (Hooper *et al.*, 1994). The gene for the G-protein, including the signal peptide, was linked to the CaMV 35S promoter, and the recombinant DNA was introduced into tomato plants by *Agrobacterium tumefaciens*-mediated transformation. Leaves and fruits from transgenic plants expressed the recombinant G-protein, which was detected in Western blots using different antisera. The protein was localized within leaf cells in Golgi bodies, vesicles, plasmalemma and parenchyma cell walls. Tomato derived G-protein apparently has a lower molecular mass (60 and 62 kDa) than the virus (66 kDa), and immunology data have not been obtained yet. If recombinant protein expression levels in tomato fruit are high, the system could be a

very promising one for development of an edible vaccine.

Hepatitis B virus

Novel approaches to vaccine development against hepatitis B virus (HBV) were based on Mason *et al.*'s (1992) experiments. They demonstrated that tobacco leaves can express recombinant hepatitis B surface antigen (HBsAg). Thanavala's group at the Roswell Park Cancer Institute in collaboration with Arntzen's group investigated the immunogenicity of this recombinant HBsAg derived from transgenic plants (Thanavala *et al.*, 1995). The tobacco-derived rHBsAg self-assembled into subviral particles that were virtually identical in size, density, sedimentation and antibody binding to the HBsAg derived from serum or a recombinant yeast expression system (Mason *et al.*, 1992). This is important as the particle form is required for immunogenicity. BALB/c mice were immunized with a crude protein preparation from transgenic tobacco leaves in which the rHBsAg was <3% of the total protein; a specific antibody response was detected a week after the last antigen injection (Thanavala *et al.*, 1995). This indicates that elaborate purification procedures may not be necessary to ensure the immunogenic activity of plant-derived antigen. The results obtained compared favorably with immunizations from commercially available yeast-derived rHBsAg (Recombivax[®], Merck, Sharp and Dohme). They also demonstrated that HBsAg-specific antibodies of all IgG subclasses as well as IgM antibodies were produced after immunization with tobacco rHBsAg. IgG3 was the most abundant idiotype in the tobacco antigen-treated mice, whereas yeast antigen-treated mice showed very high values of IgG1 and very low levels of IgG3. This is very important as in humans the IgG3 subclass of antibodies is the most efficient in activating the complement pathway. T-cell-mediated immunity is the basis for hepatitis B prevention. Thanavala *et al.* (1995) investigated the fidelity of the T-cell epitope by examining *in vitro* proliferative response of mouse lymph node T cells. The results demonstrated that tobacco-derived HBsAg had primed T cells *in vivo* that could be recalled *in vitro* to proliferate upon stimulation not only by the original immunogen (derived from tobacco) but also by Recombivax[®] and a small HBsAg peptide. This study shows that the immunogenic capacity of HBsAg is preserved when the antigen is expressed in plants, and that its features are comparable to a commercially available vaccine. The authors are now extending their efforts to produce rHBsAg in edible plant tissue.

Norwalk virus

Arntzen's group also studied the production of antigens against Norwalk virus, which causes acute epidemic gastroenteritis in humans (Mason *et al.*, 1996). The gene coding for Norwalk virus capsid protein (NVCP) was fused to CaMV 35S promoter or potato tuber specific patatin promoter, using also TEV translational enhancer. The constructs were transferred into tobacco and potato using *Agrobacterium tumefaciens*. Expressional levels of recombinant NVCP were very high, up to 0.23% of total soluble protein in tobacco leaves and up to 0.37% of soluble protein in potato tubers (34 µg per 1 g of tuber tissue). This striking difference between expression levels of recombinant NVCP and recombinant LT-B (Haq *et al.*, 1995) might be due to the fact that NVCP accumulates in the cytoplasm and does not require subcellular targeting as is the case for LT-B. Immunogenicity of plant-derived NVCP was demonstrated. When delivered orally to mice, it stimulated the production of humoral and mucosal antibody responses. However, immune response to antigen delivered in potato tuber by normal feeding was lower than that obtained by gavage of partially purified rNVCP. This finding might be due to lower purity levels of the tuber material or because the rNVCP extracted from tuber was to a lesser extent in the virus-like particle form than the partially purified antigen (Mason *et al.*, 1996) and hence less stable in the stomach.

STRATEGY II: DELIVERY OF VACCINE EPITOPES USING PLANT VIRUSES

An alternative strategy to the production of transgenic antigen-expressing plants is the use of modified plant viruses to express large amounts of antigenic proteins in plants following infection. If epitopes within a known antigen have already been identified, smaller DNA fragments encoding these epitopes can be fused to the gene coding for the coat protein of plant virus. The recombinant virus carrying the chimeric gene is then used to infect plants, which after replication will spread throughout the plant. Finally, viral particles expressing the antigen epitope on their surface are isolated and used in immunization studies. The ideal candidate virus needs to have high replication levels in infected plant tissue and should already be sufficiently characterized at molecular level to allow genetic manipulations. Furthermore, it should be stable and easily purified from plant tissue. One important aspect when designing a modified virus coat

protein is to ensure that the chimeric protein molecules retain the capacity to assemble and also to systemically infect the host plant. In some cases, fused proteins limited infection or caused local necrosis (Beachy *et al.*, 1996). Strategies to overcome this problem have to consider inserting the foreign peptide in a virus capsid site that is not involved in intramolecular structure or intersubunit assembly.

EPITOPE DELIVERY IN TOBACCO MOSAIC VIRUS

Malaria

Turpen *et al.* (1995) expressed selected B-cell epitopes derived from malarial sporozoites in tobacco mosaic virus (TMV) capsid protein. The epitope sequence was fused to the COOH terminus using the leaky stop codon derived from the replicase protein reading frame. The virus particles produced in infected plants included both wild type and engineered coat protein at the predicted ratio of 20:1. Furthermore, an internal fusion construct inserting the foreign DNA into the surface loop region of the coat protein was made. Both constructs yielded high titer of genetically stable virus in tobacco. The recombinant coat protein was recognized by specific anti-malaria antibodies.

Contraception

The ZP3 protein of the zona pellucida of mammalian oocytes has been a target for contraception. A small epitope was shown to induce antibody-mediated contraception in mice (Millar *et al.*, 1989). Based on earlier published X-ray crystallography data of the TMV coat protein, Fitchen *et al.* (1995) inserted the DNA coding for a 13-amino acid epitope between codons 154 and 155 of the TMV capsid protein gene. Amino acids 154 and 155 are orientated to the outside of the virus coat and are not involved in intramolecular structure. Plants infected with this recombinant virus accumulated high levels of virus particles, demonstrating that antigen insertion did not interfere with virus particle assembly. Preparations of these particles parenterally administered to female mice generated antibodies against ZP3. Histological and immunological analyses of ovaries showed that antibodies were bound to the zona pellucida, although an impact on fertility of immunized mice could not be demonstrated in these experiments. It is possible that other or extended epitopes could lead to positive results.

EPITOPE DELIVERY IN COWPEA MOSAIC VIRUS

Foot and mouth disease

The surface of cowpea mosaic virus (CPMV) is composed of large and small coat protein subunits. The DNA coding for an epitope of 25 amino acids from the VP1 protein of foot and mouth disease virus (FMDV) was cloned into the infectious cDNA of CPMV small capsid subunit (Usha *et al.*, 1993). Whole plants and protoplasts were inoculated, and virus particles recovered from plant material reacted with antibodies raised against FMDV. However, systemic spread of the virus in the plant was restricted, and the FMDV sequences in the virus were lost after serial passaging.

Human immunodeficiency virus

Subsequently, the CPMV system was improved. Foreign sequences were introduced between the amino acids 22 and 23 of the small capsid subunit (Porta *et al.*, 1994), which lie within a loop on the surface of the virus and is not involved in subunit interactions. Stable fusions that lead to immunogenicity in animal experiments included epitopes of human rhinovirus 14 (HRV-14), and a 22-amino acid peptide of the gp41 glycoprotein of human immunodeficiency virus type 1 (HIV-1 IIIb) (Porta *et al.*, 1994). Three different HIV-1 strains were neutralized *in vitro* and *in vivo* by antibodies recovered from mice previously injected with the gp41 modified CPMV (McLain and Dimmock, 1994; McLain *et al.*, 1995). Two injections of 1 µg of CPMV-HIV-1 chimera (containing 17 ng of the HIV peptide) in three different mouse strains resulted in mouse sera that neutralized up to 97% of the HIV-1 IIIb virus (McLain *et al.*, 1996). These results demonstrate that stable, functional vaccine epitopes can be produced by recombinant CPMV.

Mink enteritis virus

Dalsgaard *et al.* (1997) used the CPMV system to express antigens that produce a short linear epitope from the VP2 capsid protein of mink enteritis virus (MEV). The chimeric CPMV was propagated in *Vigna unguiculata*, the black-eyed bean. The course of plant infection (primary viral lesions, systemic spread of the virus) resembled infection by the wild-type virus. Formation of viral particles was confirmed by electron microscopy. One single subcutaneous dose of chimeric viral particle (0.1 to 1.0 mg) protected mink against MEV in a dose-dependent manner. The authors con-

cluded that the vaccine meets the potency requirements for inactivated MEV vaccines as defined by the United States' FDA. This work is so far the most complete one using CPMV as a vaccine production system. Commercial development of this work is being considered.

THE PROSPECTS

The medical community has shown an intense interest in production of vaccines in plants, which includes immunogenic antigens as well as antibodies for passive immunotherapy. The possibility of producing domestically grown vaccines at a low price is considered a very exciting prospect, especially in countries with low health care budgets where immunization does not take place because of the costs involved. Oral routes of vaccination, especially when administered as part of the diet, will certainly reduce the need for highly trained personnel and the equipment required for parenteral application. However, before the prospects of this novel method can be evaluated to its full extent, more studies with a variety of different antigens are necessary. The potato-derived recombinant LT-B antigen that elicited a strong immune response in mice (Haq *et al.*, 1995) is an extraordinarily potent immunogen. The results obtained might not be readily transferrable to other inducers of immune responses. Expression of antigenic proteins in plants has to be maximized, and protein stability under post-harvest storage conditions also has to be addressed.

The first human clinical trials for an edible, plant-produced active vaccine will begin in 1997. The LT-B antigen expressed in transgenic potatoes will be tested on 12 volunteers at the Baltimore Vaccine Testing Center in the USA (Johnson, 1996). Raw potato is not the most palatable food; therefore, a variety of candidate plants will have to be tested in the future in order to optimize antigen production, storage and delivery. However, the Baltimore trial results will be watched with great interest as a proof of principle. Further human clinical trials are expected shortly.

It has long been appreciated that oral administration of some protein antigens can lead to hyporesponsiveness in experimental animals, a phenomenon called oral tolerance (e.g., Challacombe and Tomasi, 1980; Ernst *et al.*, 1988). It is important to determine whether the amount of orally administered antigen required to induce the specific immune response is less than the amount that induces oral tolerance. The oral response, immunogenicity or tolerance, may be antigen specific, and needs to be tested for each individual case. There is a possibility that the cholera toxin might be

used to help diminish the oral tolerance problem. Not only did ctx not induce oral tolerance, but it also abrogated oral tolerance to an unrelated protein antigen when administered to mice (Elson and Ealding, 1984b). Further studies are needed to completely understand and maybe use the underlying mechanism of this phenomenon.

Surely, as with all transgenic plant experiments, environmental safety has to be evaluated before any genetically modified organism can be released into the environment. The escape of engineered transgenes (genes inserted using recombinant DNA techniques) to either cultivated plants of the same species or wild relatives are environmental and health concerns. (Jørgensen *et al.*, 1996). It is especially important to control the possible escape of vaccine genes via unwanted sexual crosses with other palatable plants, where they might be expressed at non-controlled levels. Consumption of these plants might lead to undesired oral tolerance in animals and humans. A viable possibility is to introduce male sterility that could be reversed upon induction (Mariani *et al.*, 1990, 1992) into the antigen-expressing plants to prevent sexual crosses with other plants in the environment.

Studies with vaccine epitopes delivered on the surface of plant viruses show exciting potential. Whereas the past experiments have focused on fidelity and stability of epitope presentation, the latest results by Dalsgaard *et al.* (1997) have demonstrated a functional and potent vaccine produced in CPMV that would meet US FDA standards for non-living vaccines.

In all cases, regulatory considerations have to be undertaken, and legal standards have to be met. These include genetic stability, microbiological considerations, purity, product comparability as well as environmental impact (Miele, 1997). Safety issues must be an essential part of this research in order to achieve the goal of safe, cheap vaccines for the developing as well as for the developed world.

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RESUMO

Plantas representam um sistema muito rentável para a produção de proteínas de valor elevado. Vacinas de baixo

custo que podem ser administradas com facilidade são muito solicitadas no mercado mundial, especialmente nas regiões menos desenvolvidas. A produção de vacinas em plantas cultivadas em agricultura local é uma área bastante promissora para solucionar esse problema. A expressão de peptídeos antigênicos nas partes comestíveis destas plantas pode resultar na produção das vacinas ativas e edulas. As duas principais estratégias são: i) a expressão de antígenos em plantas transgênicas e ii) a produção de peptídeos antigênicos na superfície de vírus vegetais que serão usados para infectar plantas hospedeiras. Esta revisão considera os dados experimentais e os primeiros resultados utilizando ambas estratégias, e discute as possibilidades e os problemas desta nova tecnologia.

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