USE OF PLANTS AS VECTORS FOR PRODUCTION OF BIOMEDICAL PRODUCTS.

INTRODUCTION

Biotechnology applied to plants is a rapidly evolving field, offering worldwide new perspectives for agriculture. Besides the food applications, transgenic or modified plants may be used for the production of high-value molecules such as lipids, carbohydrates, bioplastics and therapeutic proteins like antibodies, enzymes and viral and bacterial vaccines (Peeters et al. 2001, Goddijn & Pen 1995, Mor et al. 1998, Tacket & Mason 1999, Koprowski & Yusibov 2001). The main advantages of the use of the so called molecular farming in plants are the generally low costs of the production, the possibility of scaling up production to industrial level, absence of animal or human pathogens in the products and the possibility of long storage of the molecules in seeds, tubers or leaves. This short review present basic data on vaccine and antibodies preparation in plants.

Vaccine production.

It is well known that sub-unit vaccines for human and animal immunization may be prepared in genetically modified yeast, bacteria or mammalian cells. With the advent of the biotechnology applied to higher plants, transgenic plants were also included in the list of substrates used for vaccine production. The main goal is to obtain plants organs like leaves or fruits and crude extracts or purified proteins, which by oral or parenteral administration, may induce an immune response to the expressed antigens (Sala et al. 2003).

The first demonstration of expression of a vaccine antigen in plants was presented in 1990, when Streptococcus mutans surface protein antigen A was expressed in tobacco leaves (Curtiss & Cardineau 1990). Soon others data have been published like the expression in plants of rabies virus glycoprotein (McCarvey et al. 1995), hepatitis B antigen (HbsAg) (Kapusta et al. 1999), E.coli enterotoxin (Streatfield et al. 2000).
2003) and Norwalk virus capsid protein (Mason et al. 1996; Walmsley & Arntzen 2000). Proteins produced in plants have been shown to induce specific mucosal IgA and serum IgG when applied orally to mice and humans. The production of autoantigens for oral therapy of autoimmune diseases has been also described (Carter & Langridge 2002). Production of epitopes in plants that may be tailored to cytotoxic activity against tumors, has been also demonstrated as well as the use of plants cells as bioreactors, for the production of antibodies for use in immunotherapy (Ma 2001).

Theoretically the production of recombinant vaccines in plants present clear advantages over the traditional subunits vaccines prepared in other substracts. A high stability of the proteins can be achieved, even in absence of refrigeration. The use of the oral route and the induction of mucosal and serum response are important points characteristic of most products prepared in plants. An optimised expression system of the proteins in plants can be developed, being the plant cells engineered to accumulate the desired antigen(s) in specific cell compartments like the endoplasmic reticulum (ER) and vacuoles. The safety of the product due to the absence of contaminants of human or animal origin in the plant is also an important issue to be raised. Vaccines prepared in plants may be easily used for animal immunization, administrated as a food supply.

The expression of the vaccine proteins in plants may be achieved by a stable or by a transient transformation. In the stable transformation the foreign gene may be introduced in the nucleus or into the chloroplast genome. For transient transformation the desired gene is integrated in a plant virus and which is used to infect the susceptible plant, where the vaccine antigen will accumulate along the virus replication.

In the stable integration an infection with a bacteria, like the Agrobacterium tumefaciens containing the choosen gene is generally used and allow the integration in the plant genome at random chromosomal sites (Ishida et al. 1996). Another approach is the integration of the gene or epitope on the circular DNA of the chloroplast by the use of a virus containing the desired antigen(s). For the plant infection with the virus, mechanical devices have been developed and the integration is mostly site-specific.

The use of the chloroplast present some advantages (Sala et al. 2003) since its sequence is well known in many plants and they are able to process correctly eucaryotic proteins (Daniell et al. 2001). By the use of flanking sequences, precise insertions may be obtained in the chloroplast DNA. Another advantage is the large amount of the vaccine protein which can be obtained, up to 45 % of the total soluble protein content, compared with 0.001 to 0.4% in nuclear inserts (Sala et al. 2003). The photosynthetic capacity of the DNA is not modified by the insertion of foreign genes and the accumulation of new proteins. The procedure however, is well stablised in tobacco plants but not fully developed for edible species (Kuroda & Maliga 2001).

In the case of transient expression, vectors positive-sense single-stranded plant RNA plant virus are used as carriers of desired epitopes, inserted in the coat protein gene. Since no genome integrations occur, only the infected plant generation express the vaccine protein. Transformed alffalfa mosaic virus has been able to express three different epitopes of rabies and HIV (Koprowski & Yusibov 2001). Another important advantage of the transient viral
expression in plants is the shorter time for cloning the desired gene in the viral genome, when compared with the time necessary to transform the plants by nuclear expression.

Gene constructs, expression signals and peptide design for optimal vaccine production of vaccines in plants have been recently summarized (Sala et al. 2003).

The plant species used include many examples. Initially tobacco and potato have been used but now banana, tomato, corn, lettuce, lupine, carrots and others are being introduced for vaccine production.

Antibody response.

The infection with infectious agents occur mostly through mucosal membranes and immunity is best obtained when the vaccine is applied directly to the mucosa, and a stimulation of IgA occurs. It is well known that parenteral vaccines induce in general low levels of IgA. The vaccines prepared in plant have the capacity of induce both systemic and mucosal responses. When the antigens are still inside of the plant cell, protected by the cell wall, occurs a slow release of the antigen at the lower intestine, simulating the results obtained by the use of liposomes and capsules. The addition of peptides like HbsAg, has been shown effective to increase the immunogenicity of some oral delivered vaccines (Walmsley & Arntzen 2000). Antigens obtained from plant cells may be eventually purified for parenteral use like cancer therapy.

Safety issues.

The possible presence of agents like endogenous viruses or agents like prions in the vaccines prepared in mammalian cells, is a difficult problem. Vaccines prepared in plants may be considered free of these agents and is not known any plant DNA which might be able to interact with animal DNA.

Concerns about the use of genetic modified plants may pose difficulties for the general acceptance of vaccine plant-derived. The use of the transient expression technology may be the solution, considering that no genetic transformation of the plant is induced in this method.

The chemical constitution of the subunit vaccines developed in plants, as well the production process used to generate the proteins in plant tissue culture, can be more easily defined by the use of cultivation media without sera or other animal fluids.

Examples of candidate vaccines / products obtained in plants.

The first human trial with a transgenic plant-derived vaccine was performed in 1977, when 14 volunteers received by oral route, transgenic potatoes containing 3.7 to 15.7 mg/g of heat labile toxin of enterotoxigenic E. coli and placebo. A fourfold increase in levels of serum IgG anti-toxin was recorded in 10 out of 11 volunteers and 5 persons of the group, present IgA antibodies for the toxin in fecal specimens (Tacket et al. 1998).

Transgenic lettuce expressing HbsAg antigen, when taken as an unprocessed food, stimulate the production of significant levels of antibody in human volunteers (Kapusta et al. 1999).

Oral feeding of mice at laboratory conditions, with potato tubers expressing either the Norwalk virus capsid protein or the heat-labile enterotoxin of E. coli, induced both mucosal (IgA) and serum (IgG) antibodies for the two agents. The response could be enhanced by the association of colera toxin to the product (Koprowski & Yusibov 2001).
The Lt-B protein of enterotoxigenic E. coli has been expressed in defatted corn germ of transgenic plants. At cell surface, the Lt-B accumulated at approximately 1.8% of the total soluble protein (TSP), in the first generation of the maize seed. In the cell vacuoles, up to 12% of TSP were obtained. The corn grains when given to mice, induced protective immunogenic responses (Stratfield et al. 2003).

The same antigen has been expressed in potato tubers of transgenic plants transformed by Agrobacterium tumefaciens mediation. Fresh potato tubers containing about 13 mg of Lt-B per gram of fresh weight were used for immunization of mice. The subcutaneous inoculation with an extract of Lt-B, show an antibody response similar to the inoculation of extracts of Lt-B of bacterial origin. The mice primed by a previous inoculation of the extract by subcutaneous route, show a good response of IgA in serum and feces, when the extract was given by oral route. The oral route alone did not induce detectable antibodies (Lauterslager et al. 2001).

The B subunit of E.coli heat-labile enterotoxin (Lt-B) and the spike protein of swine transmissible gastroenteritis virus, have been expressed at high levels in corn and both antigens induce protective antibodies when given by oral route to mice and piglets, respectively (Stratfield et al. 2001).

MV surface glycoproteins measles antigens have been obtained in carrots, as well as the hemagglutinin protein. Both products induced antibodies in mice and humans after oral administration. However in previously primed mice, the oral vaccination stimulated a considerably higher response. The authors suggest that a plant-based edible measles vaccine may be useful in adults that have been primed by an early natural infection or vaccination (Muller et al. 2003).

Production of antibodies and antibody fragments in plants.

Hiatt et al. (1989) have been the first to report the use of transgenic plants for antibody production, using a murine signal sequence. In mammals cells at least two chaperone proteins direct the correct folding and assembly of heavy and light chains (Gething & Sambrook 1992). Similar proteins have been described in plants which suggest that the protein folding and assembly do not differ substantially in plants and animals (Peeters et al. 2001, Fischer et al. 2003).

One of the most critical aspects of antibody production is the glycosylation step, which is correctly performed in plant cells, but not in E. coli for instance; yeast cells have the tendency to hyperglycosylate proteins (Streatfield et al. 2003).

Once the appropriate cDNA has been isolated, starting from a hybridoma cell line, it has to be inserted in a chosen vector, as recently reviewed (Schillberg et al. 2002).

An important point to be considered in antibody production in plants is that recombinant antibodies are more stable in some intracellular structures than in others.

It has been shown that the folding and assembly of the immunoglobulin chains, occur in the ER and the glycosylation in both, ER and Golgi apparatus (Fischer et al. 2003).

The majority of plant-derived antibodies have been obtained in transgenic plants and this require a stable transformation, as described for vaccine production in plants. The transformation may be rapidly obtained but it take about 2 years to establish a routine production, including the scale up of the product.

As alternative to reduce the time is to establish a transient expression which can be obtained by the infection of the plants with modified A. tumefaciens or plant viruses like TMV
and potato virus X, both used for production of scFv fragments and full size immunoglobulins (McCormick et al. 1999, Verch et al. 1998).

In plant cell cultures the secretion of small molecules of the antigen like scFv in the media, has been observed but larger molecules remain in the apoplast of the cell in culture; both tobacco and rice cells in suspension, have been used for production of recombinant antibodies (Fischer et al. 2003).

The plant assembed antibodies like IgGs and Fab fragments can be translocated through the cell wall and accumulate in the intercellular spaces of the leaf. Antibodies may also accumulate in chorooplasts and in the endoplasmic reticulum (Düring et al. 1990).

Most of the work in antibody expression in plants have been developed in tobacco plants and Arabidopsis thaliana. An output around 1% of the TSP of antibody protein has been obtained in both plants, under optimal conditions.

Not only full-length antibodies and fragments like scFv, which contain the variable regions of the heavy and light chains, have been obtained in plants. Multivalent antibodies like IgA (S1gA) may be also induced in plants, through sexual crosses of transgenic plants, each synthesizing one of the proteins chains, reaching the expression of the different genes in one plant and the final assembly of a functional S1gA functional molecule (Ma et al. 1995). Due to the complexity of the S1gA molecule, plants are at present the only economic viable expression system for large-scale production of this important antibody (Peeters et al. 2001).

Another important point in the production of antibody in plants is the downstream processes of isolation and purification by targeting the products to specific cellular structures or tissues, that can be more easily separated from other plant materials (Peeters et al. 2001). As stated for vaccine preparation, the antibodies prepared in plants are free of potentially pathogenic microorganisms and other harmful substances associated to production in animal systems. Long storage of the products at room temperature without losses in activity has been also documented (Fiedler & Conrad 1995).

Examples of antibodies produced in plants for therapeutic purposes.

A plant derived monoclonal S1gA and a IgG for topical application in the the oral cavity, could reduce he recolonisation of Streptococcus mutans, the agent of dental caries, for at least 4 months (Ma et al. 1995).

Another study showed that antibodies produced in soybean, protected mice against genital herpes simplex type 2. The product have similar physical properties of the one produced in animal cells in culture (Zeitlin et al. 1998).

Other products developed in plants are an anti-sperm antibodies in corn, which prevents pregnancy in rabbits, anti-HIV antibodies and antibodies for treatment of non-Hodkins lymphoma by using vector technology (Peeters et al. 2001).

CONCLUSIONS

Research in plant biotechnology provided new insights in biology and allowed the production in plants of vaccines and antibodies for prevention, treatment, diagnosis and analytical purposes. Further research in aspects like transformation technology, downstream processes and clinical trials, will open new possibilities for economical production of both products. These research lines will bring a more close integration in the fields of plant and animal virology, since virus diseases for both animals and humans, are important targets for the application of vaccines and antibodies.

As described, virus are also essential tools for many steps in the processes of induction of the protein synthesis in plants.
Not at least it seems essential that projects and research lines in this field should be implemented and supported in Brazil, considering the importance of our agriculture and the impact of virus diseases in man, animals and plants in our country.

REFERENCES


