Transgenic plants as edible vaccines — reality and future prospects

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This review describes the recent progress in the construction of transgenic plants for vaccine production. Transgenic plants are an attractive and cost-effective alternative to microbial systems for the production of proteins with pharmaceutical value. Advances in biotechnology are enabling plants to be exploited for expression of candidate vaccine antigens with the goal of using the edible plant organs for economical delivery of oral vaccines. It has recently been shown that genes encoding antigens of bacterial and viral pathogens can be expressed in plants in a form in which they retain native immunogenic properties. Transgenic potato tubers expressing bacterial antigens stimulated humoral and mucosal immune response, when they were provided as a food. Although the utility of «edible vaccines» to prevent disease remains to be established, the successful implementation of this strategy can be the first step on the way to modern vaccines of new generation.

Introduction. Research on new vaccines has used molecular biology to identify the antigenic determinants of infectious disease agents and to develop genetic engineering approaches to produce and deliver these antigens as subunit vaccines. In recent studies, tools of plant biotechnology have been added to these efforts. It has been found that transgenic plants provide a novel system for production of recombinant proteins that act as oral immunogens when the plant products are consumed as food.

Many infectious agents colonize or invade epithelial membranes; these include bacteria and viruses that are transmitted via contaminated food or water or by sexual contact.

Vaccines that are effective against these infections must stimulate the mucosal immune system to produce secretory IgA (S-IgA) at mucosal surfaces such as the gut and respiratory epithelia. In general, a mucosal immune response is more effectively achieved by oral, rather then parenteral, antigen delivery. Several particulate antigens have proven to be effective oral immunogens, including live and killed microorganisms. By comparison with parenteral immunization, oral immunization using subunit or soluble antigens is often inefficient at stimulating an immune response, and requires larger amounts (mg versus μg) of antigen.

Subunit vaccines based upon recombinant cell-culture expression systems are feasible but, for commercial-scale production, these systems require fermentation technology and stringent purification protocols so that sufficient amounts of recombinant protein can be obtained for oral delivery. Even with technological improvements, fermentation-based subunit vaccine production may be prohibitively expensive technology for developing countries where novel oral vaccines are urgently needed. Transgenic plants that express antigens in their edible tissue might be possible simply through consumption of an «edible vaccine».

The concept of vaccine production in transgenic plants was introduced about 6 years ago by Charles Arntzen and Hugh Mason at Texas A&M University [1] greatly stimulating research in this direction [2—8].

In general, research in this field falls into three general categories. First, experiments have been conducted to determine the capacity of plants to produce foreign proteins that retain antigenic determinants necessary for effective immunization. Second, the oral immunogenicity of plant-derived proteins has been evaluated with special emphasis on the

immunogenicity of food samples. Third, research has been conducted to find an appropriate food crop that could be used for both production and distribution of vaccines, with special emphasis on the developing world.

Hepatitis B surface proteins. The first studies of candidate vaccine expression in transgenic plants have been carried out using the gene encoding hepatitis B surface antigen (HBsAg) [1, 3]. This protein was chosen because the commercially available vaccine and the associated human immune response have been very well characterized, because the structure of the immunogenic form of that protein was known, and because the availability of a cost-effective recombinant HBV vaccine is a high priority especially for the developing countries. Moreover, the existence of commercially available test systems for HBV detection substantially simplified the procedure of HBsAg detection in plant tissues.

The envelope of hepatitis B virus (HBV) consists of three polypeptides which comprise the large (L), middle (M) and major or small (S or HBsAg) protein components. These three proteins are encoded in a large open reading frame, which is divided into preS1, preS2 and the S gene. The S protein or HBsAg is a major component of the hepatitis B virions and contains 226 amino acids. The HBV M protein contains additional 55 amino acid residues at the N-terminal of the S protein, usually called the preS2 antigen. Similarly, the L protein contains additional 108—119 amino acids, depending on the serotype of the virion, at the N-terminal of the M protein [9].

The S gene was introduced into cells of tobacco [1] and potato [3] plants and individual transformants were regenerated. When extracts from transgenic plant tissues were examined the presence of HBsAg were revealed by ELISA using monoclonal antibodies directed against human-serum-derived HBsAg. Further examination of plant-derived HBsAg purified by immunoaffinity chromatography revealed the presence of spherical virus-like particles with an average size of 22 nm. These particles exhibited properties that were very similar to the subviral particles obtained from human serum and to the recombinant HBsAg which is formulated in the commercial vaccine produced in yeast cells [1]. Importantly, HBsAg in the particle form was found to be much more immunogenic than that in the form of the peptide alone [10].

To evaluate the immunogenicity of plant-derived HBsAg it was used for parenteral immunization of mice. Anti-HBsAg antibodies were recovered which reacted with authentic HBsAg from human serum. This was the first indication, that antigenic properties

of the protein were maintained in transgenic plants. Subsequently, T cells were isolated from mice immunized with plant-derived HBsAg. When grown in culture, these T cells could be activated using commercial vaccine as well as a synthetic peptide which mimics the «a» epitope determinant of HBsAg. In total, the immunology studies conducted to date show that the recombinant HBsAg recovered from plant cells retain both B and T cell epitopes [5].

Although recombinant HBV vaccines have shown that HBsAg alone is sufficient to induce a highly protective immunity, experiments in animals have highlighted the potential benefits which might result from the inclusion of the preS2 domain in anti-HBV vaccines [11]. The preS2 domain is also immunogenic in humans and elicit anti-preS2 responses during natural HBV infection, which often occur prior to an other anti-HBV response [12]. For this reason, the HBV M protein gene (preS2 containing HBsAg) has been recently expressed in plants [8] and physical and immunological properties of this protein were evaluated [13]. These studies have demonstrated that plant cells have the capacity to not only synthesize M protein but also to allow it to be assembled in an immunologically active form.

To evaluate the immune response to plant-derived M protein and to compare it to the response to HBsAg from serum (preS2 containing HBsAg), HBV vaccine and plant-derived HBsAg, Balb/c mice were immunized intraperitoneally with corresponding antigens. Kinetics of antibody responses were studies up to 14 weeks after primary immunization. The results presented in Fig. 1 indicate that both plant-derived HBV proteins can elicit the anti-HBsAg antibodies in mice and that the plant-derived M protein is nearly as immunogenic as the control preS2 containing HBsAg isolated from serum. Moreover, the presence of anti-preS2 antibodies in the sera of immunized Balb/c mice was detected in mice immunized with the preS2 containing proteins (Fig. 2). These results suggest that plant system can provide an alternative method of producing the HBV M protein suitable for vaccination.

Recently, it was also shown that the plant-derived HBV M protein given to mice by oral intubation (gavage) stimulated serum antibody response and corresponding specific antibodies were detected [14].

Escherichia coli heat-labile enterotoxin B subunit. The choice of which antigens to use in the initial studies has been strongly influenced by the desire to determine if transgenic plant materials containing foreign antigens will result in oral immunization and stimulate a mucosal immune response
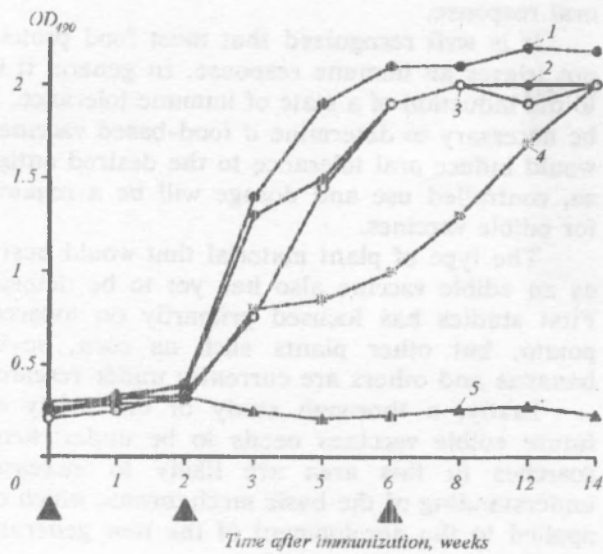
Fig. 1. Kinetics of anti-HBsAg antibody responses in Balb/c mice: *1* — serum HBsAg; *2* — M protein; *3* — vaccine; *4* — S protein; *5* — negative control. Mice were immunized intraperitoneally with the same amounts of plant-derived M and S proteins, HBsAg from serum and Engerix vaccine. Solid arrows indicate the time of vaccination (0, 2nd and 6th week). The presence of anti-HBsAg antibody were monitored by ELISA
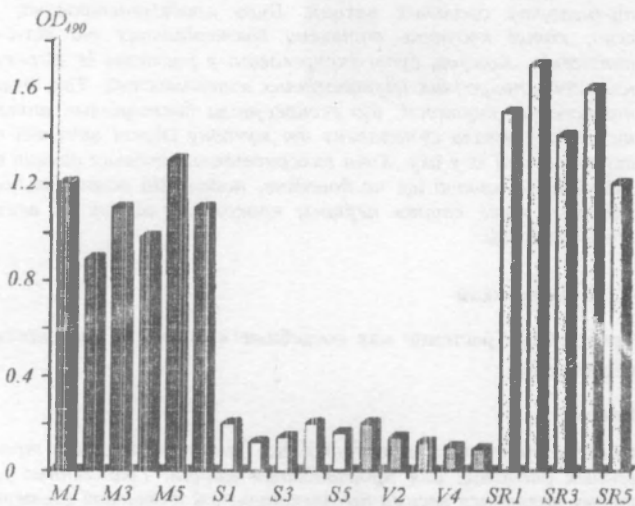

Fig. 2. Comparison of anti-preS2 antibody responses in mice. 12 weeks post-immunization sera from individual mice immunized with plant-derived M protein (M1—M6), plant-derived S protein (S1—S5), Engerix vaccine (V1—V5) and HBsAg from serum (SR1—SR5) were tested for the presence of anti-preS2 antibody by ELISA

Thus, antigens with the high mucosal immune response have been the early targets for plant-based expression.

The binding subunit of the heat-labile enterotoxin of *E. coli* (LT-B) was an obvious candidate for evaluation in plant expression system since it has been extensively characterized in structural and immunological studies.

The heat-labile enterotoxin (LT) from *E. coli* is a multimeric protein that is structurally, functionally and antigenically very similar to cholera toxin (CT). It was found that LT has one A subunit (LT-A) and a pentamer of B subunits (LT-B). Specific binding of the nontoxic LT-B pentamer to the G_{M1} gangliosides present on epithelial cell surfaces allows entry of the toxic LT-A subunit into cells [15]. Antibody interference with binding of the B subunit to cells, thus blocking toxin activity, is the basis of attempts to use the B subunit as a vaccine component. Because LT-t is very similar in structure and immunological properties to the CT-B, immunization with CT-B leads to cross-protection against enterotoxigenic *E. coli*. LT-B and CT-B are both potent strong oral immunogens.

LT-B also has recently been expressed in plants although the levels of expression were low [2]. The oral immunogenicity of recombinant LT-B was tested in mice and compared with bacterial LT-B. When given orally to mice by gastric intubation, the plant-derived antigen stimulated humoral and mucosal immune responses with titers comparable to the bacteria-derived LT-B. In addition, the antibodies produced against the tobacco-derived LT-B were able to neutralize LT activity, indicating the potential protective value of the immune response.

The oral immunogenicity of unpurified recombinant LT-B was also assessed by feeding raw transgenic potato tubers to mice. After only four feedings of 5 g tuber samples to mice, mucosal and serum antibodies were recovered. No immune response was observed in animals that were fed non-transformed tubers.

It should be also noted that CT and LT are excellent oral adjuvants, which stimulate immune responses against co-fed antigens at concentrations well below those that cause diarrhea.

Norwalk virus capsid protein. Further evidence to support the concept of edible vaccines have recently been obtained in experiments with plant-derived Norwalk virus capsid protein (NVCP). Norwalk virus is a member of the *Caliciviridae* family and causes epidemic acute gastroenteritis in humans. As in the case of HBV, expression of NVCP in plant cells yields a protein that self-assembles in plant cells into virus

like particles. The plant-derived NVCP was orally immunogenic in mice. Extracts of tobacco leaf expressing NVCP were given to CD1 mice by gavage and the treated mice developed both serum IgG and secretory IgA specific for Norwalk virus-like particles. Furthermore, when potato tubers expressing NVCP were fed directly to mice, they developed serum IgA specific for Norwalk virus [7].

Vaccines for animal diseases. Edible vaccines can also provide efficient and humane strategies for disease prevention in production of companion animals, as well as feral populations. It is practically possible to generate vaccines against viral and bacterial infections by expressing corresponding antigens in plant tissues edible for animals. The already mentioned LT-B subunit is the most likely candidate for the first commercial vaccine, as enterotoxigenic *E. coli* strains readily infect animals as well. Admittedly, vaccines for animals are a more likely target for edible-vaccine technology in the near future than vaccines for human as the latter need more detailed inspections for safety.

Recently, transgenic plants have been generated that expressed the gene encoding the glycoprotein (G-protein) that coats the outer surface of the rabies virus [6, 14].

Although the immunogenicity of these material has yet to be evaluated, it is encouraging to note that bait containing some G-protein produced in a more traditional *in vitro* system was effective in immunizing raccoons orally, providing protection against «street virus» challenge.

Future prospects. The research conducted to date has demonstrated that transgenic plants have the capacity to synthesize and accumulate subunit antigenic proteins that retain immunological properties of their native counterparts. In the case of HBV proteins and NVCP, virus-like particles accumulated in plant cells. It is very significant as the particulate form is very important in determining immunogenic properties and has greater oral immunogenicity than soluble proteins.

Studies remaining to be conducted will involve the evaluation of dosage requirements for plant-delivered vaccines. Successful experiments conducted thus far have used proteins (LT-B and NVCP) with very high oral immunogenicity. It will be necessary to determine if other proteins, which may not be normally transmitted orally, will be as effective in inducing an oral response. From this point of view the results of the oral immunization with the plant-derived HBV M protein are rather encouraging. Multi-subunit vaccines, including oral adjuvants such as LT or CT (or derivatives thereof), and various

fused proteins could be also used for enhancing the oral response.

It is well recognized that most food proteins do not trigger an immune response. In general it is due to the induction of a state of immune tolerance. It will be necessary to determine if food-based vaccines also would induce oral tolerance to the desired antigen. If so, controlled use and dosage will be a requirement for edible vaccines.

The type of plant material that would best serve as an edible vaccine also has yet to be determined. First studies has focused primarily on tobacco and potato, but other plants such as corn, soybean, bananas and others are currently under research.

Lastly, a thorough study of the safety of the future edible vaccines needs to be undertaken. Researches in this area are likely to increase our understanding of the basic mechanisms, which can be applied to the development of the new generation of vaccines.

М. М. Доманський

Трансгенні рослини як істівні вакцини — реальність та перспективи

Резюме

Огляд сучасних літературних даних про створення трансген них рослин для виробництва вакцин. Трансгенні рослини є дуж привабливою та дешевою альтернативою існуючим мікро біологічним системам виробництва білків для фармацевтики. Успіхи сучасної біотехнології відкрили можливість експресува ти у рослинах різні антигени, що використовуються для вакцинації, з метою використання істівних частин рослин для транспорту оральних вакцин. Було продемонстровано, що гени, котрі кодують антигени бактеріальних та вірусних патогенів, можуть бути експресовані у рослинах із збереженням їхніх природних імунологічних властивостей. Так, бульби трансгенної картоплі, що експресували бактеріальні антиге ни, стимулювали гуморальну та мукозну імунні відповіді при використанні їх у їжу. Хоча використання істівних вакцин для запобігання хвороб ще не доведене, подальший розвиток цього напряму може стати першим кроком на шляху до вакцин нової генерації.

Н. Н. Доманский

Трансгенные растения как съедобные вакцины — реальность и перспективы

Резюме

Обзор современных литературных данных о создании тра генных растений для производства вакцин. Трансгенные р тения являются весьма привлекательной и дешевой альтерна тивой существующим микробиологическим системам произ водства белков для фармацевтики. Успехи современной био технологии открыли возможность экспрессировать в расте ниях различные антигены, используемые при вакцинации, для применения съедобных частей растений при транспорте ора льных вакцин. Было продемонстрировано, что гены, кодирую щие антигены бактериальных и вирусных патогенов, могут

быть экспрессированы в растениях с сохранением их природных иммунологических свойств. Так, клубни трансгенного картофеля, экспрессировавшие бактериальные антигены, стимулировали гуморальный и мукозный иммунные ответы при употреблении их в пищу. Хотя использование съедобных вакцин для предотвращения болезней еще не доказано, дальнейшее развитие этого направления может стать первым шагом на пути к созданию вакцин нового поколения.

REFERENCES

1. *Mason H. S., Lam D. M.-K., Arntzen C. J.* Expression of hepatitis B surface antigen in transgenic plants // Proc. Nat. Acad. Sci. USA.—1992.—89.—P. 11745—11749.

2. *Haq T. A., Mason H. S., Clements J. D., Arntzen C. J.* Oral immunization with a recombinant bacterial antigen produced in transgenic plants // Science.—1995.—268.—P. 714—715.

3. *Domansky N. N., Ehsani P., Salmanian A-H., Medvedeva T.* Organ-specific expression of hepatitis B surface antigen in potato // Biotechnol. Lett.—1995.—17.—P. 863—866.

4. *Mason H. S., Arntzen C. J.* Transgenic plants as vaccine production systems // Trends Biotechnol.—1995.—13.—P. 388—392.

5. *Thanavala Y., Yang Y.-F., Lyons P. et al.* Immunogenicity of transgenic plant-derived hepatitis B surface antigen // Proc. Nat. Acad. Sci. USA.—1995.—92.—P. 3358—2261.

6. *McGarvey P. B., Hammond J., Dienelt et al.* Expression of the rabies virus glycoprotein in transgenic tomatoes // Bio/Technology.—1995.—13.—P. 1484—1487.

7. *Mason H. S., Ball J. M., Shi J-J. et al.* Expression of Norwalk virus capsid protein in transgenic tobacco and potato and its

8. *Ehsani P., Khabiri A., Domansky N. N.* Polypeptides of hepatitis B surface antigen produced in transgenic potato // Gene.—1997.—190.—P. 107—111.

9. *Tiollais P., Pourcel C., Dejean S.* The hepatitis B virus // Nature.—1985.—317.—P. 489—495.

10. *Cabral G. A., Marciano-Cabral F., Funk G. R. et al.* Cellular and humoral immunity in guinea pigs to two major polypeptides derived from hepatitis B surface antigen // J. Gen. Virol.—1978.—38.—P. 339—350.

11. *Millich D. R., Thornton G. B., Neurath et al.* Enhanced immunogenicity of the pre-S region of hepatitis B surface antigen // Science.—1985.—228.—P. 1195—1198.

12. *Petre J., Rutgers T., Hauser P.* Properties of a recombinant yeast-derived hepatitis B surface antigen containing S, preS2 and preS1 antigenic domains // Arch. Virol. (Suppl.).—1992.—24.—P. 137—141.

13. *Khabiri A., Ehsani P., Domansky N.* Characterization of the hepatitis B middle (M) surface protein synthesized in transgenic potato plants // Manuscript submitted.

14. *Domansky N., Khabiri A., Ehsani P. et al.* Enhanced immunogenicity of transgenic plant-derived hepatitis B middle surface antigen // 5th Int. Congr. of Plant Mol. Biol. (Singapore, 21—27th September 1997).—P. 1257.

15. *Sixma T. K., Pronk S. E., Kalk K. H. et al.* Crystal structure of a cholera toxin-related heatlabile enterotoxin from *E. coli* // Nature.—1991.—351.—P. 371—377.