Pharmaceutical foodstuffs—Oral immunization with transgenic plants

When plants genetically engineered to produce bacterial or viral proteins are consumed as food, they trigger a protective immune response in animals and humans.

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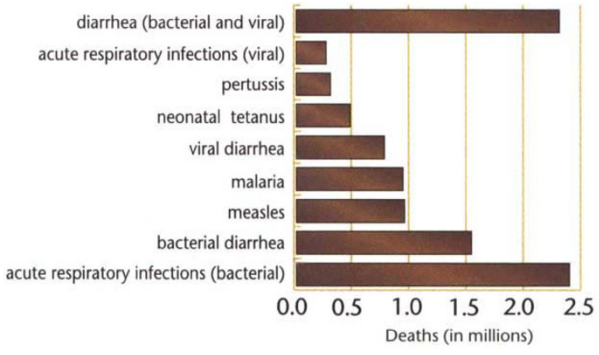
SUBUNIT VACCINES OFFER enormous promise for global reduction or eradication of infectious diseases. Molecular biology now enables scientists to isolate genes from human pathogens (viruses and bacteria) and to transfer the DNA into another organism (such as yeast or *Escherichia coli*), which serves as a transgenic (containing the transferred gene) host. The transgenic organism then produces a protein (subunit of the pathogen) that contains the antigenic fingerprint of the disease-causing agent but does not itself cause disease. These subunit vaccines are purified from the transgenic hosts and injected into vaccinees to induce immunity against a specific disease. Transgenic plants comprise a new production system that does not require vaccine purification or injection.

The incredible achievements of vaccination to date are attributable, in part, to the simplicity of the vaccines used. In the case of smallpox, attenuated poxvirus (a weakened form of the organism that does not cause disease) was produced inexpensively and transported even to remote villages in a robust formulation for injection. For polio, the simple incorporation of attenuated polio viruses on sugar cubes created an oral vaccine that was universally accepted. Unfortunately, these approaches cannot be extended to many other vaccines, and definitely not to the new generation of subunit vaccines, which hold the greatest promise for disease prevention in the next century. A new breakthrough is needed to introduce simplicity into the technically sophisticated field of subunit vaccines to make them available on a global scale, and especially in the poorer countries of the world where infectious diseases are still the primary cause of death (see figure).

The last seven years have seen the evaluation of transgenic plants as production systems for subunit vaccines. Studies with prototype vaccines have verified that immunogenic proteins can be produced in plants[1–6] and that the subunits can trigger oral immunity if the plant tissues are consumed as food[3–5].

The creation of transgenic crops containing foreign genes (such as those providing resistance to insects) is now routine. About 30 million acres of transgenic canola, maize (corn), cotton and soybeans were grown in the United States and Canada last year and the new traits are genetically stable over many generations. After North America, the region with the next highest acreage of transgenic crops is China, indicating that transgenic plant technology can be, and is, practiced in the developing world.

Early research on vaccine production in plants focused on designing vaccines to protect against pathogens that cause diarrhea[7]. The reasons are twofold. First, diarrhea caused by bacteria and viruses is responsible for approximately three million infant deaths per year, mostly in the developing world. Secondly, oral immunization is likely to induce localized protection against enteric diseases. Oral immunization, either with attenuated whole microbes or with subunit vaccines, stimulates an immune response at effector sites (lymphoid tissue) that line the gut. Responses include the appearance of specific antibodies in blood serum (which is also achieved by vaccine injection), and of specific secretory antibodies. The latter are found in the mucosal secretions of the intestines (and also in saliva, respiratory and reproductive tract secretions). They are a major indicator of mucosal immunity and are important for the prevention of infection by the myriad microbes that attack mucosal surfaces, causing respiratory, enteric, or sexually transmitted diseases. Subunit vaccines (against viruses and bacteria that cause diarrhea) produced in plants such as potato induced oral immunization in mice that ate the tissues[3–5]. Both serum and secretory antibodies, specific for subunits of the infectious agents, were detected in these preclinical studies.



Infectious diseases are a major cause of death in children under five years of age, especially in developing countries (Source: World Health Organization).

To create vaccine-producing transgenic plants, the gene encoding the antigenic subunit is placed under the control of plant-specific DNA regulatory sequences, and is then induced to integrate into the nuclear chromosome of the plant cell (where it will follow Mendelian inheritance in subsequent generations)[1–7]. For some antigens this has been relatively straightforward; the capsid protein of Norwalk virus (causal agent of acute gastroenteritis), for example, accumulates to significant levels in potatoes[4]. However, in the case of two other bacterial genes—those encoding LT-B, the binding subunit of the heat-labile toxin of enterotoxigenic *E. coli*, and CT-B, the binding subunit of cholera toxin—introduction into plant cells has yielded unsatisfactory levels of protein. Two strategies have been used to enhance production in these cases. A plant cell localization signal fused to the antigenic protein has been shown to promote increased cellular accumulation of the subunit[3,5]. Alternatively, a completely synthetic gene that is 'plant-optimized' can be created. A synthetic LT-B gene has been designed that lacks problematic sequences such as cryptic plant-specific polyadenylation signals and that produces a protein with the authentic amino acid sequence specified by codons that are preferred by plant cells[8]. It seems likely that there is no theoretical limitation to the use of plant cells for expression of antigen-encoded DNA sequences from human pathogens. It is likely, however, that sophisticated gene manipulations will usually be required to drive the high levels of protein accumula-

tion necessary to make plants efficient vaccine production systems.

Many antigenic proteins from infectious viruses are chemically modified in host cells, for example, by the addition of sugars to produce glycoproteins. Although plants will glycosylate proteins, the carbohydrate additions are different from those of mammalian cells. This could be a hindrance in expression of certain immunogens if the sugar component of glycoproteins determines protective epitopes. Ongoing studies with a rabies virus glycoprotein produced in plants may answer this question[6]. Some vaccines require the presence of structural epitopes determined by protein folding and association. Notably, plants produce virus-like particles that mimic the structure of the authentic viral proteins[1,2,4]. In the case of hepatitis B surface antigen, the virus-like particles from plants preserve both the B- and T-cell epitopes that are present in the currently available commercial vaccine[2].

Preclinical studies of plant-expressed bacterial antigens, LT-B and CT-B, have provided indirect evidence for protective immunity in mice. The animals produce antibodies that neutralize the native bacterial toxins in mammalian cell assays and in the fluid accumulated in the intestines of animals challenged with the bacterial toxin[3,5,8]. Based upon animal trials, the U.S. Food and Drug Administration approved human clinical testing of raw potatoes containing LT-B in 1997. The results of this trial are described in the current issue of *Nature Medicine*[9]. The study concludes that edible vaccines are feasible for humans. Volunteers who consumed raw potatoes developed LT-B-specific IgG and IgA; the amplitude of the responses was comparable to a challenge with 10^6 virulent enterotoxigenic *E. coli* (an amount sufficient to induce severe diarrhea). A companion paper in the same issue by Ma *et al.*[10] reports data from another clinical trial that used secretory antibodies produced in transgenic plants to passively immunize human volunteers. The investigators found that colonization of teeth and gums by *Streptomyces mutans*, the bacterium which is the major cause of dental caries, could be prevented by the plant-derived antibodies. This is especially noteworthy because plants offer the only experimental system that can produce these antibodies in a quantity that is pharmaceutically useful.

Research on plant-produced vaccines has moved from theory to proof-of-principle. But challenging questions remain to be answered. Will 'non-traditional' oral subunit vaccines function at effector sites in the gut? Is it possible that oral tolerance[11] could develop with food-borne antigens? Lastly, what is the most appropriate plant tissue to deliver subunit vaccines? Bananas are a particularly attractive choice for developing countries because the fruit is eaten uncooked even by infants[7]. Although many obstacles have yet to be overcome, it is my opinion that they are implementation issues—not roadblocks. Vaccines produced in plants offer a new strategy for safe and cost-effective global immunization against infectious diseases.

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The global vaccine enterprise: A developing world perspective

The industrialization of the vaccine enterprise has implications for the supply of vaccines to the developing world.

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THE MOST IMPORTANT factor driving the transformation of the vaccine enterprise (which encompasses the development, clinical testing, production, licensure and distribution of vaccines) is the increasingly complex scientific and technological base that is required to develop and manufacture the newest generation of vaccines. The traditional (and highly successful) vaccines, such as those against small pox, diphtheria, tetanus, pertussis and tuberculosis, were based on the pioneering work of Jenner and Pasteur. The pathogen was grown in quantity in a simple facility, purified in a few steps, killed with an inactivating agent (where appropriate), and blended into the final product. Within a few decades of Pasteur's death, his disciples established Pasteur Institutes or similar public institutions in many parts of the world that produced vaccines as a public service[1].

In contrast, today's new vaccines are 'high tech' products that require expertise in multiple scientific disciplines, large numbers of skilled staff, and costly advance investment in research and manufacturing facilities. New generation vaccines, such as genetically engineered subunit vaccines against hepatitis B virus, cell-free vaccines against whooping cough (pertussis), and the protein-polysaccharide conjugate vaccines against invasive bacterial diseases, for example those caused by *Haemophilus influenzae* type b (a major cause of bacterial meningitis in small children) and *Streptococcus pneumoniae*, bear little resemblance to the traditional vaccines in the way that they are produced.

The second factor driving the transformation of the vaccine enterprise is the changing nature of technology ownership. Even though the basic research supporting development of vaccines is conducted at public and academic research centers supported by public funds, vaccine development has become primarily the purview of large industrial laboratories, often augmented in key segments by specialized biotechnology companies funded by venture capital. Thus, most key technologies for future vaccines will be developed and owned by companies that will diligently protect their new inven-