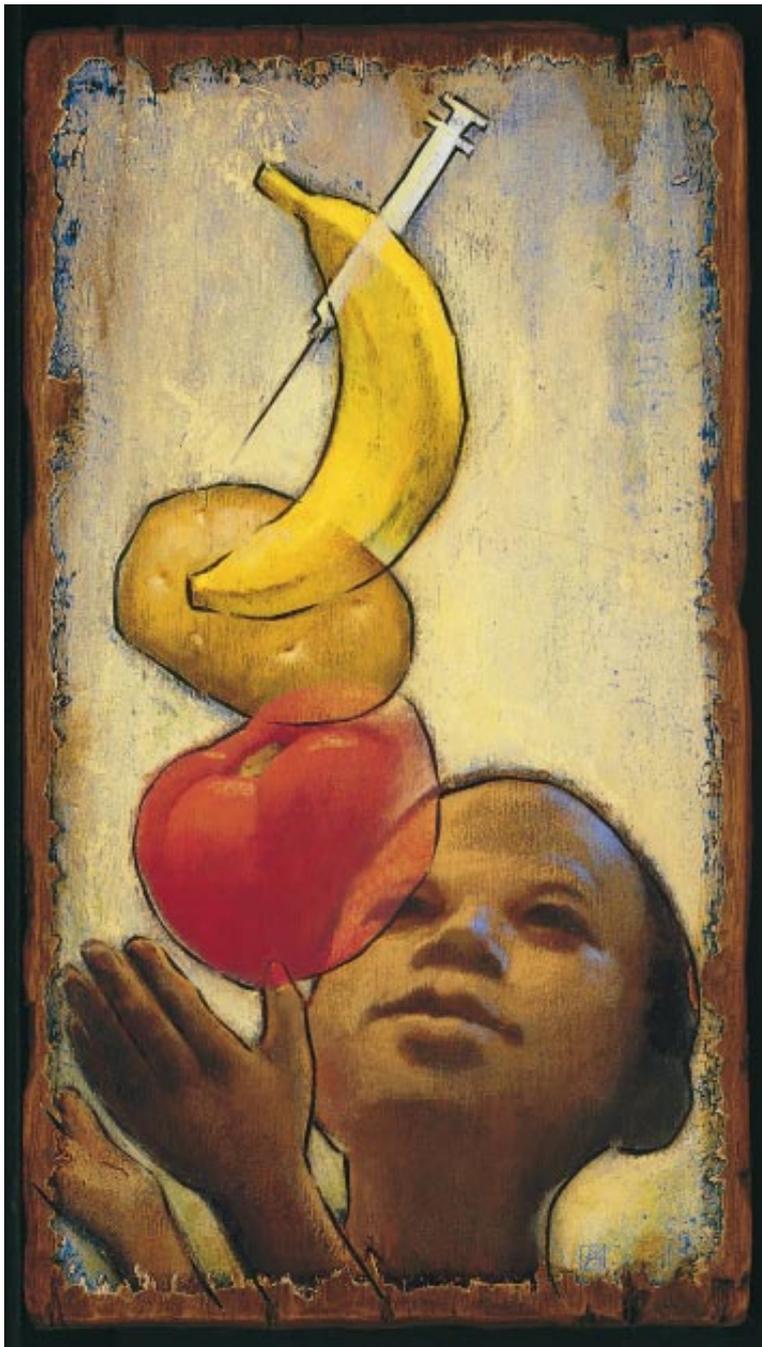


One day children may get immunized by munching on foods instead of enduring shots. More important, food vaccines might save millions who now die for lack of access to traditional inoculants

Edible Vaccines

by William H. R. Langridge



Vaccines have accomplished near miracles in the fight against infectious disease. They have consigned smallpox to history and should soon do the same for polio. By the late 1990s an international campaign to immunize all the world's children against six devastating diseases was reportedly reaching 80 percent of infants (up from about 5 percent in the mid-1970s) and was reducing the annual death toll from those infections by roughly three million.

Yet these victories mask tragic gaps in delivery. The 20 percent of infants still missed by the six vaccines—against diphtheria, pertussis (whooping cough), polio, measles, tetanus and tuberculosis—account for about two million unnecessary deaths each year, especially in the most remote and impoverished parts of the globe. Upheavals in many developing nations now threaten to erode the advances of the recent past, and millions still die from infectious diseases for which immunizations are nonexistent, unreliable or too costly.

This situation is worrisome not only for the places that lack health care but for the entire world. Regions harboring infections that have faded from other areas are like bombs ready to explode. When environmental or social disasters undermine sanitation systems or displace communities—bringing people with little immunity into contact with carriers—infections that have been long gone from a population can come roaring back. Further, as international travel and trade make the earth a smaller place, diseases that arise in one locale are increasingly popping up continents away. Until everyone has routine access to vaccines, no one will be entirely safe.

In the early 1990s Charles J. Arntzen, then at Texas A&M University, conceived of a way to solve many of the problems that bar vaccines from reaching all too many children in developing nations. Soon after learning of a World Health Organization call for inexpensive, oral vaccines that needed no refrigeration, Arntzen visited Bangkok, where he saw a mother soothe a crying

FOODS UNDER STUDY as alternatives to injectable vaccines include bananas, potatoes and tomatoes, as well as lettuce, rice, wheat, soybeans and corn.



BANANA TREES AND TOMATO PLANTS growing at the Boyce Thompson Institute for Plant Research at Cornell University have been genetically engineered to produce vaccines in their fruit. Bananas are particularly appealing as vaccines because they grow widely in many parts of the developing world, can be eaten raw and are liked by most children.



baby by offering a piece of banana. Plant biologists had already devised ways of introducing selected genes (the blueprints for proteins) into plants and inducing the altered, or “transgenic,” plants to manufacture the encoded proteins. Perhaps, he mused, food could be genetically engineered to produce vaccines in their edible parts, which could then be eaten when inoculations were needed.

The advantages would be enormous. The plants could be grown locally, and cheaply, using the standard growing methods of a given region. Because many food plants can be regenerated readily, the crops could potentially be produced indefinitely without the growers having to purchase more seeds or plants year after year. Homegrown vaccines would also avoid the logistical and economic problems posed by having to transport traditional preparations over long distances, keeping them cold en route and at their destination. And, being edible, the vaccines would require no syringes—which, aside from costing something, can lead to infections if they become contaminated.

Efforts to make Arntzen’s inspired vision a reality are still quite preliminary. Yet studies carried out in animals over the past 10 years, and small tests in people, encourage hope that edible vaccines can work. The research has also fueled speculation that certain food vaccines might help suppress autoimmunity—in which the body’s defenses mistakenly attack normal, uninfected tissues. Among the autoimmune disorders that might be prevented or eased are type I diabetes (the kind that commonly arises during childhood), multiple sclerosis and rheumatoid arthritis.

By Any Other Name ...

Regardless of how vaccines for infectious diseases are delivered, they all have the same aim: priming the immune system to swiftly destroy specific disease-causing agents, or pathogens, before the agents can multiply enough to cause symptoms. Classically, this priming has been achieved by presenting the immune system with whole viruses or bacteria that have been killed or made too weak to proliferate much.

On detecting the presence of a foreign organism in a vaccine, the immune system behaves as if the body were under attack by a fully potent antagonist. It mobilizes its various forces to root out and destroy the apparent invader—targeting the campaign to specific antigens (proteins recognized as foreign). The acute response soon abates, but it leaves behind sentries, known as “memory” cells, that remain on alert, ready to unleash whole armies of defenders if the real pathogen ever finds its way into the body. Some vaccines

provide lifelong protection; others (such as those for cholera and tetanus) must be readministered periodically.

Classic vaccines pose a small but troubling risk that the vaccine microorganisms will somehow spring back to life, causing the diseases they were meant to forestall. For that reason, vaccine makers today favor so-called subunit preparations, composed primarily of antigenic proteins divorced from a pathogen’s genes. On their own, the proteins have no way of establishing an infection. Subunit vaccines, however, are expensive, in part because they are produced in cultures of bacteria or animal cells and have to be purified out; they also need to be refrigerated.

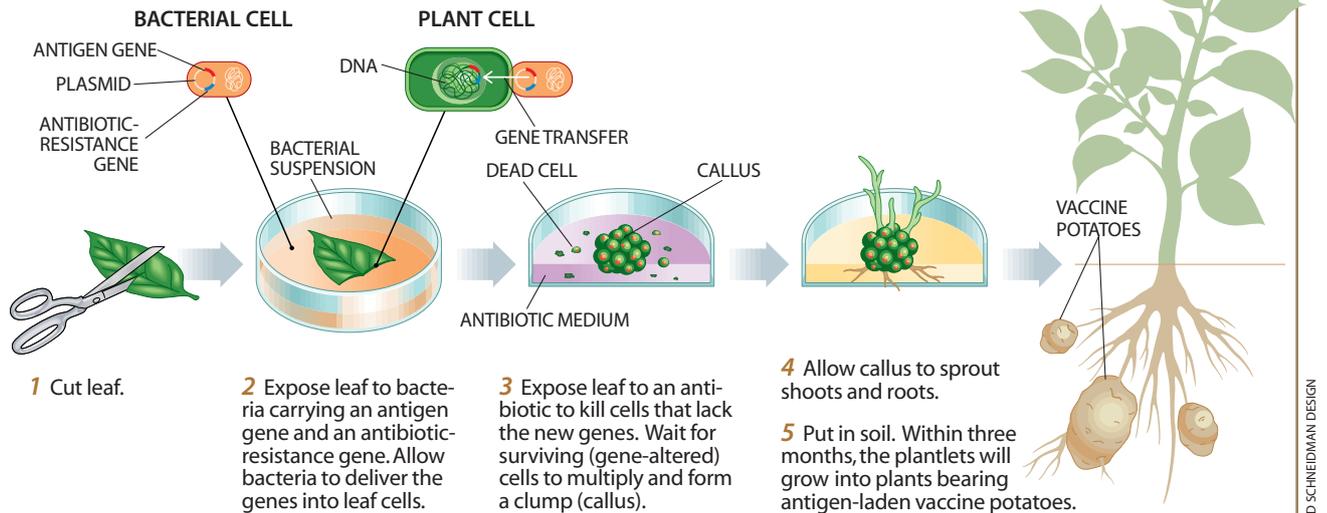
Food vaccines are like subunit preparations in that they are engineered to contain antigens but bear no genes that would enable whole pathogens to form. Ten years ago Arntzen understood that edible vaccines would therefore be as safe as subunit preparations while sidestepping their costs and demands for purification and refrigeration. But before he and others could study the effects of food vaccines in people, they had to obtain positive answers to a number of questions. Would plants engineered to carry antigen genes produce functional copies of the specified proteins? When the food plants were fed to test animals, would the antigens be degraded in the stomach before having a chance to act? (Typical subunit vaccines have to be delivered by injection precisely because of such degradation.) If the antigens did survive, would they, in fact, attract the immune system’s attention? And would the response be strong enough to defend the animals against infection?

Additionally, researchers wanted to know whether edible vaccines would elicit what is known as mucosal immunity. Many pathogens enter the body through the nose, mouth or other openings. Hence, the first defenses they encounter are those in the mucous membranes that line the airways, the digestive tract and the reproductive tract; these membranes constitute the biggest pathogen-detering surface in the body. When the mucosal immune response is effective, it generates molecules known as secretory antibodies that dash into the

HOW TO MAKE AN EDIBLE VACCINE

One way of generating edible vaccines relies on the bacterium *Agrobacterium tumefaciens* to deliver into plant cells the genetic blueprints for viral or bacterial

“antigens”—proteins that elicit a targeted immune response in the recipient. The diagram illustrates the production of vaccine potatoes.



JARED SCHNEIDMAN DESIGN

cavities of those passageways, neutralizing any pathogens they find. An effective reaction also activates a systemic response, in which circulating cells of the immune system help to destroy invaders at distant sites.

Injected vaccines initially bypass mucous membranes and typically do a poor job of stimulating mucosal immune responses. But edible vaccines come into contact with the lining of the digestive tract. In theory, then, they would activate both mucosal and systemic immunity. That dual effect should, in turn, help improve protection against many dangerous microorganisms, including, importantly, the kinds that cause diarrhea.

Those of us attempting to develop food vaccines place a high priority on combating diarrhea. Together the main causes—the Norwalk virus, rotavirus, *Vibrio cholerae* (the cause of cholera) and enterotoxigenic *Escherichia coli* (a toxin-producing source of “traveler’s diarrhea”)—account for some three million infant deaths a year, mainly in developing nations. These pathogens disrupt cells of the small intestine in ways that cause water to flow from the blood and tissues into the intestine. The resulting dehydration may be combated by delivering an intravenous or oral solution of electrolytes, but it often turns deadly when rehydration therapy is not an option. No vaccine practical for wide distribution in the developing na-

tions is yet available to prevent these ills.

By 1995 researchers attempting to answer the many questions before them had established that plants could indeed manufacture foreign antigens in their proper conformations. For instance, Arntzen and his colleagues had introduced into tobacco plants the gene for a protein derived from the hepatitis B virus and had gotten the plants to synthesize the protein. When they injected the antigen into mice, it activated the same immune system components that are activated by the virus itself. (Hepatitis B can damage the liver and contribute to liver cancer.)

Green Lights on Many Fronts

But injection is not the aim; feeding is. In the past five years experiments conducted by Arntzen (who moved to the Boyce Thompson Institute for Plant Research at Cornell University in 1995) and his collaborators and by my group at Loma Linda University have demonstrated that tomato or potato plants can synthesize antigens from the Norwalk virus, enterotoxigenic *E. coli*, *V. cholerae* and the hepatitis B virus. Moreover, feeding antigen-laced tubers or fruits to test animals can evoke mucosal and systemic immune responses that fully or partly protect animals from subsequent exposure to the real pathogens or, in the case of *V. cholerae* and enterotox-

igenic *E. coli*, to microbial toxins. Edible vaccines have also provided laboratory animals with some protection against challenge by the rabies virus, *Helicobacter pylori* (a bacterial cause of ulcers) and the mink enteric virus (which does not affect humans).

It is not entirely surprising that antigens delivered in plant foods survive the trip through the stomach well enough to reach and activate the immune system. The tough outer wall of plant cells apparently serves as temporary armor for the antigens, keeping them relatively safe from gastric secretions. When the wall finally begins to break up in the intestines, the cells gradually release their antigenic cargo.

Of course, the key question is whether food vaccines can be useful in people. The era of clinical trials for this technology is just beginning. Nevertheless, Arntzen and his collaborators obtained reassuring results in the first published human trial, involving about a dozen subjects. In 1997 volunteers who ate pieces of peeled, raw potatoes containing a benign segment of the *E. coli* toxin (the part called the B subunit) displayed both mucosal and systemic immune responses. Since then, the group has also seen immune reactivity in 19 of 20 people who ate a potato vaccine aimed at the Norwalk virus. Similarly, after Hilary Koprowski of Thomas Jefferson University fed transgenic lettuce carrying

a hepatitis B antigen to three volunteers, two of the subjects displayed a good systemic response. Whether edible vaccines can actually protect against human disease remains to be determined, however.

Still to Be Accomplished

In short, the studies completed so far in animals and people have provided a proof of principle; they indicate that the strategy is feasible. Yet many issues must still be addressed. For one, the amount of vaccine made by a plant is low. Production can be increased in different ways—for instance, by linking antigen genes with regulatory elements known to help switch on the genes more readily. As researchers solve that challenge, they will also have to ensure that any given amount of a vaccine food provides a predictable dose of antigen.

Additionally, workers could try to enhance the odds that antigens will activate the immune system instead of passing out of the body unused. General stimulants (adjuvants) and better targeting to the immune system might compensate in part for low antigen production.

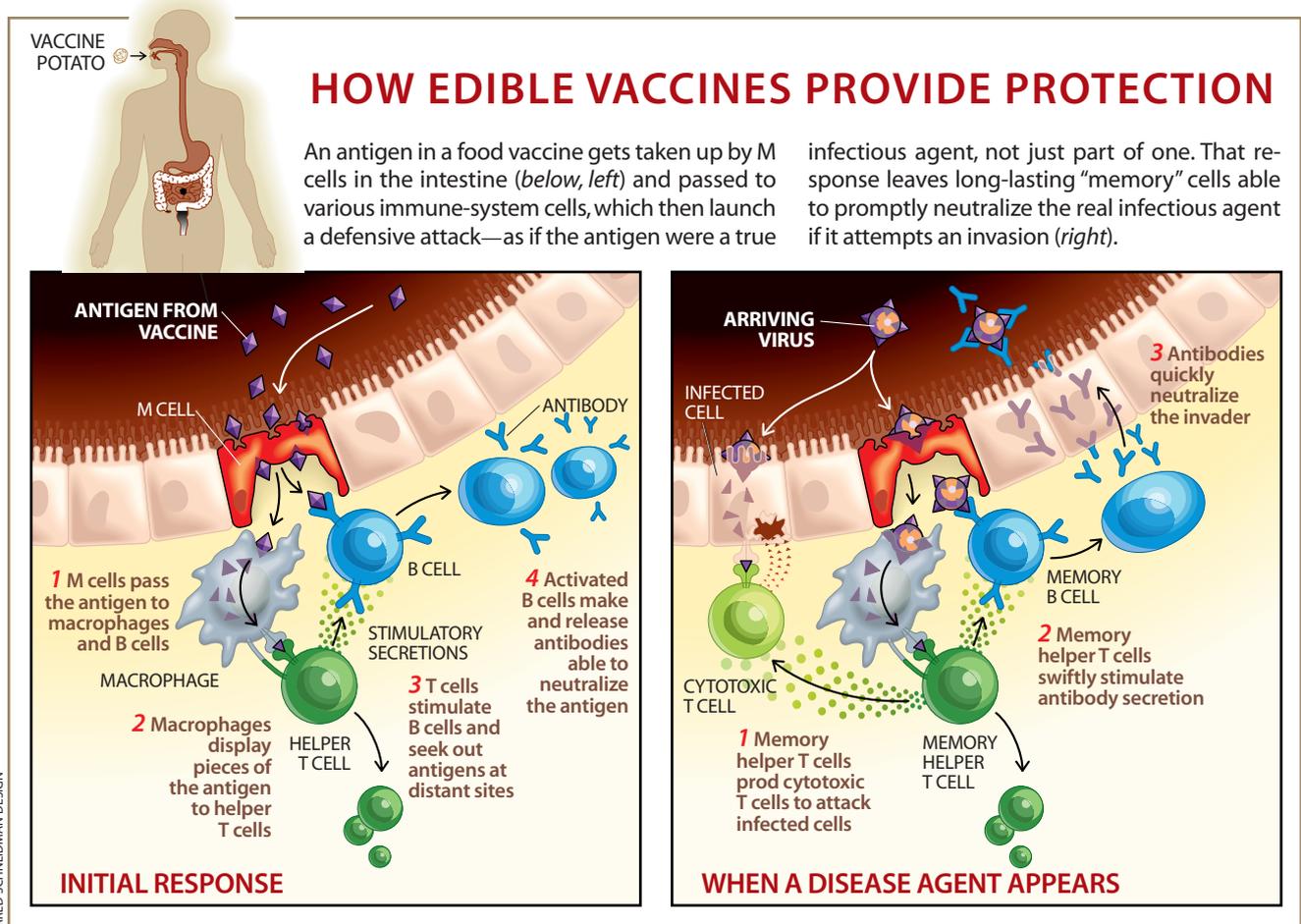
One targeting strategy involves linking antigens to molecules that bind well to immune system components known as M cells in the intestinal lining. M cells take in samples of materials that have entered the small intestine (including pathogens) and pass them to other cells of the immune system, such as antigen-presenting cells. Macrophages and other antigen-presenting cells chop up their acquisitions and display the resulting protein fragments on the cell surface. If white blood cells called helper T lymphocytes recognize the fragments as foreign, they may induce B lymphocytes (B cells) to secrete neutralizing antibodies and may also help initiate a broader attack on the perceived enemy.

It turns out that an innocuous segment of the *V. cholerae* toxin—the B subunit—binds readily to a molecule on M cells that ushers foreign material into those cells. By fusing antigens from other pathogens to this subunit, it should be possible to improve the uptake of antigens by M cells and to enhance immune responses to the added antigens. The B subunit also tends to associate with copies of itself, forming a dough-

nut-shaped, five-membered ring with a hole in the middle. These features raise the prospect of producing a vaccine that brings several different antigens to M cells at once—thus potentially fulfilling an urgent need for a single vaccine that can protect against multiple diseases simultaneously.

Researchers are also grappling with the reality that plants sometimes grow poorly when they start producing large amounts of a foreign protein. One solution would be to equip plants with regulatory elements that cause antigen genes to turn on—that is, give rise to the encoded antigens—only at selected times (such as after a plant is nearly fully grown or is exposed to some outside activator molecule) or only in its edible regions. This work is progressing.

Further, each type of plant poses its own challenges. Potatoes are ideal in many ways because they can be propagated from “eyes” and can be stored for long periods without refrigeration. But potatoes usually have to be cooked to be palatable, and heating can denature proteins. Indeed, as is true of tobacco plants, potatoes were not initially intended to be



used as vaccine vehicles; they were studied because they were easy to manipulate. Surprisingly, though, some kinds of potatoes are actually eaten raw in South America. Also, contrary to expectations, cooking of potatoes does not always destroy the full complement of antigen. So potatoes may have more practical merit than most of us expected.

Bananas need no cooking and are grown widely in developing nations, but banana trees take a few years to mature, and the fruit spoils fairly rapidly after ripening. Tomatoes grow more quickly and are cultivated broadly, but they too may rot readily. Inexpensive methods of preserving these foods—such as drying—might overcome the spoilage prob-

lem. Among the other foods under consideration are lettuce, carrots, peanuts, rice, wheat, corn and soybeans.

In another concern, scientists need to be sure that vaccines meant to enhance immune responses do not backfire and suppress immunity instead. Research into a phenomenon called oral tolerance has shown that ingesting certain proteins can at times cause the body to shut down its responses to those proteins. To determine safe, effective doses and feeding schedules for edible vaccines, manufacturers will need to gain a better handle on the manipulations that influence whether an orally delivered antigen will stimulate or depress immunity.

A final issue worth studying is whether food vaccines ingested by mothers can indirectly vaccinate their babies. In theory, a mother could eat a banana or two and thus trigger production of antibodies that would travel to her fetus via the placenta or to her infant via breast milk.

Nonscientific challenges accompany the technical ones. Not many pharmaceutical manufacturers are eager to support research for products targeted primarily to markets outside the lucrative West. International aid organizations and some national governments and philanthropies are striving to fill the gap, but the effort to develop edible vaccines remains underfunded.

In addition, edible vaccines fall under the increasingly unpopular rubric of “genetically modified” plants. Recently a British company (Axis Genetics) that was supporting studies of edible vaccines failed; one of its leaders lays at least part of the blame on investor worry about companies involved with genetically engineered foods. I hope, however, that these vaccines will avoid serious controversy, because they are intended to save lives and would probably be planted over much less acreage than other food plants (if they are raised outside of greenhouses at all). Also, as drugs, they would be subjected to closer scrutiny by regulatory bodies.

Fighting Autoimmunity

Consideration of one of the challenges detailed here—the risk of inducing oral tolerance—has recently led my group and others to pursue edible vaccines as tools for quashing autoimmunity. Although oral delivery of antigens derived from infectious agents often stimulates the immune system, oral delivery of “autoantigens” (proteins de-

Moving against Malnutrition

As research into edible vaccines is progressing, so too are efforts to make foods more nutritious. A much publicized example, “golden rice,” takes aim at vitamin A deficiency, rampant in many parts of Asia, Africa and Latin America. This condition can lead to blindness and to immune impairment, which contributes to the death of more than a million children each year.

Rice would be a convenient way to deliver the needed vitamin, because the grain is a daily staple for a third or more of all people on the earth. But natural varieties do not supply vitamin A. Golden rice, though, has been genetically altered to make beta-carotene, a pigment the body converts to vitamin A.

A team led by Ingo Potrykus of the Swiss Federal Institute of Technology and Peter Beyer of the University of Freiburg in Germany formally reported its creation this past January in *Science*. In May an agribusiness—Zeneca—bought the rights and agreed to allow the rice to be donated to facilities that will cross the beta-carotene trait into rice species popular in impoverished areas and will distribute the resulting products to farmers at no charge. (Zeneca is hoping to make its money from sales of the improved rice in richer countries, where beta-carotene’s antioxidant properties are likely to have appeal.)

Golden rice is not yet ready to be commercialized, however. Much testing still lies ahead, including analyses of whether the human body can efficiently absorb the beta-carotene in the rice. Testing is expected to last at least until 2003.

Meanwhile scientists are trying to enrich rice with still more beta-carotene, with other vitamins and with minerals. At a conference last year Potrykus announced success with iron; more than two billion people worldwide are iron deficient.

Investigators are attempting to enhance other foods as well. In June, for instance, a group of British and Japanese investigators reported the creation of a tomato containing a gene able to supply three times the usual amount of beta-carotene. Conventional breeding methods are being used, too, such as in an international project focused on increasing the vitamin and mineral content of rice and four other staples—wheat, corn, beans and cassava.

Not everyone is thrilled by the recent genetic coups. Genetically modified (GM) foods in general remain controversial. Some opponents contend that malnutrition can be combated right now in other ways—say, by constructing supply roads. And they fear that companies will tout the benefits of the new foods to deflect attention from worries over other GM crops, most of which (such as plants designed to resist damage from pesticides) offer fewer clear advantages for consumers. High on the list of concerns are risk to the environment and to people. Supporters of the nutritionally improved foods hope, however, that the rice won’t be thrown out with the rinse water.

—Ricki Rusting, staff writer



“GOLDEN RICE” contains beta-carotene, which adds color as well as nutrition to the grain.

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rived from uninfected tissue in a treated individual) can sometimes suppress immune activity—a phenomenon seen frequently in test animals. No one fully understands the reasons for this difference.

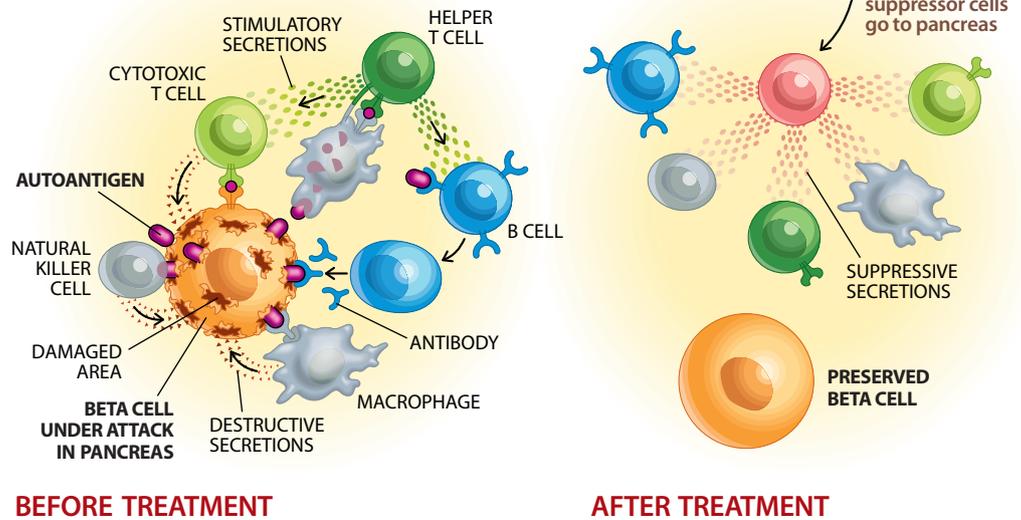
Some of the evidence that ingesting autoantigens, or “self-antigens,” might suppress autoimmunity comes from studies of type I diabetes, which results from autoimmune destruction of the insulin-producing cells (beta cells) of the pancreas. This destruction progresses silently for a time. Eventually, though, the loss of beta cells leads to a drastic shortage of insulin, a hormone needed to help cells take up sugar for energy. The loss results in high blood sugar levels. Insulin injections help to control diabetes, but they are by no means a cure; diabetics face an elevated risk of severe complications.

In the past 15 years, investigators have identified several beta cell proteins that can elicit autoimmunity in people predisposed to type I diabetes. The main culprits, however, are insulin and a protein called GAD (glutamic acid decarboxylase). Researchers have also made progress in detecting when diabetes is “brewing.” The next step, then, is to find ways of stopping the underground process before any symptoms arise.

To that end, my colleagues and I, as well as other groups, have developed

STOPPING AUTOIMMUNITY

The autoimmune reaction responsible for type I diabetes arises when the immune system mistakes proteins that are made by pancreatic beta cells (the insulin producers) for foreign invaders. The resulting attack, targeted to the offending proteins, or “autoantigens,” destroys the beta cells (*below, left*). Eating small amounts of autoantigens quiets the process in diabetic mice, for unclear reasons. The autoantigens might act in part by switching on “suppressor” cells of the immune system (*inset*), which then block the destructive activities of their cousins (*below, right*).



plant-based diabetes vaccines, such as potatoes containing insulin or GAD linked to the innocuous B subunit of the *V. cholerae* toxin (to enhance uptake of the antigens by M cells). Feeding of the vaccines to a mouse strain that becomes diabetic helped to suppress the immune attack and to prevent or delay the onset of high blood sugar.

Transgenic plants cannot yet produce the amounts of self-antigens that would be needed for a viable vaccine against human diabetes or other autoimmune

diseases. But, as is true for infectious diseases, investigators are exploring a number of promising schemes to overcome that and other challenges.

Edible vaccines for combating autoimmunity and infectious diseases have a long way to go before they will be ready for large-scale testing in people. The technical obstacles, though, all seem surmountable. Nothing would be more satisfying than to protect the health of many millions of now defenseless children around the globe.

The Author

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Further Information

ORAL IMMUNIZATION WITH A RECOMBINANT BACTERIAL ANTIGEN PRODUCED IN TRANSGENIC PLANTS. Charles J. Arntzen in *Science*, Vol. 268, No. 5211, pages 714–716; May 5, 1995.

IMMUNOGENICITY IN HUMANS OF A RECOMBINANT BACTERIAL ANTIGEN DELIVERED IN A TRANSGENIC POTATO. C. O. Tacket et al. in *Nature Medicine*, Vol. 4, No. 5, pages 607–609; May 1998.

A PLANT-BASED CHOLERA TOXIN B SUBUNIT-INSULIN FUSION PROTEIN PROTECTS AGAINST THE DEVELOPMENT OF AUTOIMMUNE DIABETES. Takeshi Arakawa, Jie Yu, D. K. Chong, John Hough, Paul C. Engen and William H. R. Langridge in *Nature Biotechnology*, Vol. 16, No. 10, pages 934–938; October 1998.

PLANT-BASED VACCINES FOR PROTECTION AGAINST INFECTIOUS AND AUTOIMMUNE DISEASES. James E. Carter and William H. R. Langridge in *Critical Reviews in Plant Sciences* (in press).