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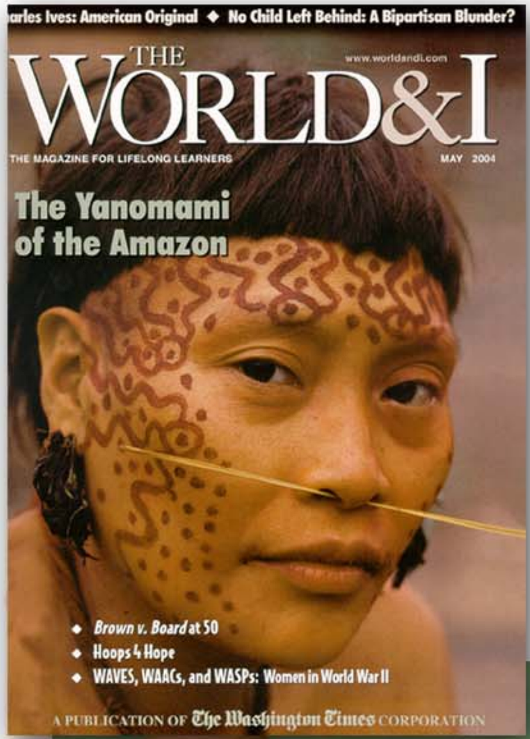

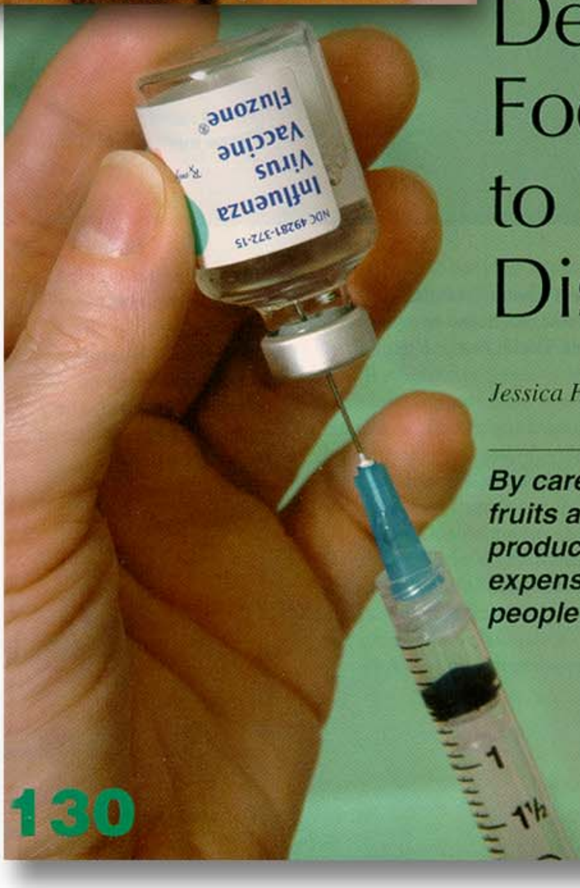

Designing Foods to Prevent Diseases

Jessica Hankinson

By careful genetic engineering of certain fruits and vegetables, scientists hope to produce vaccines that are less expensive and more easily available to people around the globe.

JIM GATHANY / COURTESY OF CENTERS FOR DISEASE CONTROL AND PREVENTION

It seems that nearly every day we are bombarded with messages about what foods are healthy for consumption, with the latest views of diet experts. As a result, some of us have chosen to go organic, others are on a low-carb or low-salt diet, and others have selected special combinations of foods. Yet, whatever diet we choose, one piece of advice seems to stay the same: eat your fruits and veggies.

Soon there could be even more reasons to eat your fruits and vegetables—or perhaps to swallow capsules containing special ingredients from them. In what may sound like a fad diet meets science fiction, a number of scientists are dedicated to designing foods that produce important vaccines. Combining their latest knowledge of molecular genetics with earlier studies of vaccines, they seek to alter the DNA of certain edible plants to produce foods that may help prevent deadly diseases.

■ *Opposite:* **Most traditional vaccines are expensive to produce and are delivered by injection.** ***Above:*** **By contrast, Professor Charles Arntzen of Arizona State University has been designing tomatoes and other edible plants to produce relatively inexpensive vaccines that can be taken orally.**

TIM TRUMBLE / COURTESY OF ARIZONA STATE UNIVERSITY

Why develop vaccine-producing plants?

In 1980, the World Health Organization (WHO) declared that smallpox had been eliminated from the world. After it had killed more people than all the wars of the twentieth century combined, the virus that produced the dreaded disease had finally been confined to a few vials stored safely in laboratories. The conquest of smallpox could be counted as one of the greatest achievements of modern medicine.

Much of the credit for this success can be attributed to the worldwide vaccination campaign that WHO had conducted for more than a decade. Today, vaccines are available for a variety of diseases, including measles, mumps, influenza, cholera, rabies, typhoid, pneumonia, and polio. In the United States and many other countries, children get a battery of shots right from their preschool years. While they have to face their fear of injections, they avoid many of the diseases that were common in earlier generations.

Children in less developed nations have more to fear. Worldwide, only about 80 percent of children get basic vaccinations for diphtheria, whooping cough, polio, measles, and tetanus. The remaining 20 percent, concentrated in poverty-stricken regions, do not benefit at all from the vaccines in modern medicine's armamentarium. According to WHO, "Every year more than three million people die from vaccine-preventable diseases, most of them children in developing countries."

Several factors have hampered the distribution of traditional vaccines to all corners of the globe. They are expensive to produce and have to be kept refrigerated during storage. In addition, they need to be handled under sterile conditions and administered by highly skilled personnel. As a result, many have not benefited from them.

Given this situation, the primary motivation behind developing plant-derived vaccines is to make vaccinations affordable and accessible to people everywhere, even in remote parts of the world. "We are working on vaccines that poor people can afford," says Charles Arntzen, founding director of the Arizona Biodesign Institute at Arizona State University.

How are vaccines produced?

In the late eighteenth century, British physician Edward Jenner

One Type of Immune Response

To determine the strength of your immune system, doctors often count the number of white blood cells in your blood. White blood cells are essential components of the immune system. They defend your body against invading pathogens and also help it reap the benefits of vaccines. They are classified in several groups: B cells, T cells, and nonspecific effector cells. Let us consider one type of immune response, involving the work of the B cells.

The B cells may be compared to locksmiths—they produce antibodies, analogous to keys, that recognize and bind to specific foreign substances called *antigens*, analogous to locks. An antigen may be a specific molecule on the surface of an invading microbe. Each B cell is programmed to make only one type of antibody, carried on the cell surface, and each antibody recognizes one type of antigen. The body, however, contains an enormous number of B cells producing a large variety of antibodies, and these in turn bind to an equally large variety of antigens.

Like a locksmith's shop, the body cannot afford to have many copies of each antibody key. It waits to see which antibodies are actually needed. When a microbe (or a vaccine) enters the body, one or more of its molecules functions as an antigen that is recognized by an antibody on the surface of a B cell. The antibody binds to the antigen, and the B cell turns into a factory for producing more copies of that antibody. In addition, the B cell divides and produces clones of itself, each of which generates more such antibodies. When these antibodies bind to the corresponding antigens, some nonspecific immune cells (called *phagocytes*) receive a signal to come and break down the foreign substance or microbial cell.

A number of clones of that antibody-producing B cell remain in the body for years, in the form of "memory" cells. As a result, if the same type of pathogen (with the same antigens) invades the body at a later date, these B cells are quickly mobilized to produce more antibodies. Therefore, taking a vaccine means you are increasing the readiness of your body to produce a specific type of antibody.

—J.H.

observed that milkmaids who were exposed to cowpox (a disease similar to but much milder than smallpox) got blisters on their hands, but they seldom became ill with smallpox. He reasoned that exposure to cowpox somehow protects a person from smallpox.

To test his hypothesis, he conducted an experiment that would be considered highly unethical by today's standards. He extracted some fluid from blisters on the hand of a cowpox-infected milkmaid and injected the fluid into an eight-year-old boy. Two months later, he injected the boy with fluid from blisters on a person who had smallpox. Fortunately, the boy was resistant to smallpox, as Jenner had expected.

Jenner coined the term *vaccination* from *vaccinia*, the Latin word for cowpox. The fluid he had extracted from the milkmaid's hand contained cowpox virus, which served as a vaccine. When the boy was injected with this vaccine, it primed his immune system to build up its defenses against the related smallpox virus, making him resistant to smallpox infection.

Since that time, scientists have uncovered many secrets of how our immune system works and have learned various methods of preparing vaccines. In many cases, vaccines against viral and bacterial diseases consist of the attenuated (weakened) or heat-killed virus particles or bacterial cells. One risk of using a live but weakened pathogen is that it may occasionally cause disease, especially in people whose immune systems are challenged. For instance, the orally administered polio vaccine contains attenuated virus particles and has caused polio in rare instances.

To avoid this risk, a harmless component of the pathogenic microbe may be purified and used

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■ To introduce a vaccine-producing gene into a plant, graduate student Samuel Fletcher inoculates the plant with a bacterium containing the desired gene. When the plant produces its own proteins, it will also synthesize the protein encoded by the foreign gene.

as a vaccine. In other words, a vaccine could be a subunit of a bacterial toxin or a viral coat protein. Such vaccines, known as *subunit vaccines*, are sufficient to stimulate an immune response that protects the body against subsequent attack by the pathogen. Although more expensive to produce, subunit vaccines are safer to use than vaccines that contain attenuated viruses or bacteria.

A plant-derived vaccine is also a subunit vaccine. In other words, the plant is genetically engineered in such a way as to produce a harmless component of the human pathogen. This entails taking an appropriate gene from the microbe and inserting it into the plant DNA. The resultant plant is said to be *transgenic*.

To introduce a foreign gene into a plant, one common method is to first put the gene in a modified soil bacterium named *Agrobacterium tumefaciens*. This bacterium, when inoculated into the plant, facilitates insertion of the foreign gene into the nuclear DNA of the plant cells. Normally, this bacterium induces the growth of tumors in plants, but scientists have learned how to inactivate the tumor-inducing genes and make the microbe harmless.

Alternatively, a foreign gene may be inserted into plant DNA by bombarding the plant cells with tiny gold particles that carry copies of that gene. This method allows insertion of the gene into the DNA of chloroplasts as well as the DNA in cellular nuclei.

When the modified plant DNA (in the nuclei or chloroplasts) directs the synthesis of the plant's proteins, the foreign protein that will function as a vaccine is also produced. Normally, the vaccine is concentrated in the part of the plant that is high in protein content—such as the leaves of alfalfa or the seeds of legumes.

Regardless of how a vaccine is produced, the goal is the same: to use it to prime a person's immune system. When a vaccine is injected into an individual, his immune system detects the weakened pathogen or subunit and responds by developing weapons to fight and destroy it. Even after the immediate threat is neutralized, the immune system's memory cells stay on alert. If the fully functional pathogen were to infect the person at a later date, the immune system rapidly launches its preformed defenses and destroys the microbe before it can multiply

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Graduate student Mrinalini Muralidharan checks an electrophoresis gel to see if a genetically modified plant is producing a foreign protein that can function as a vaccine.

sufficiently to cause illness [see "One Type of Immune Response" on p. 132].

Benefits and drawbacks

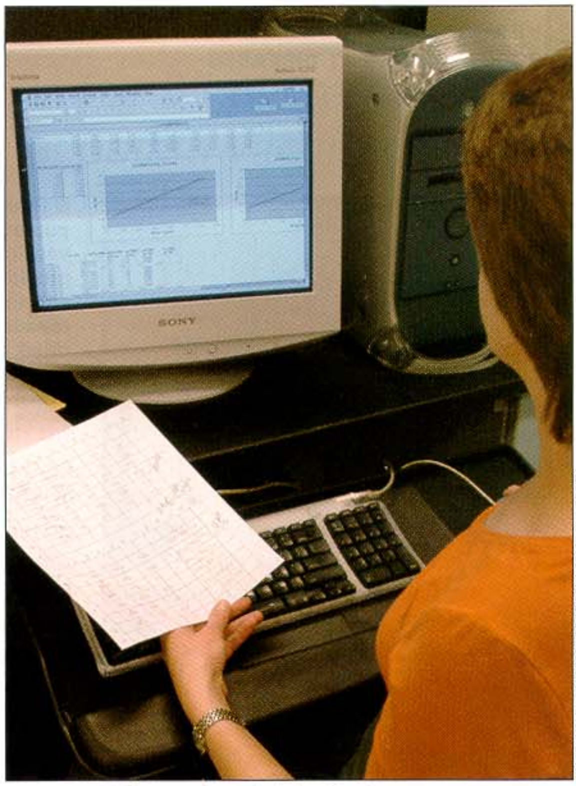
The genetic modification of edible plants to produce vaccines was initially undertaken for several reasons. One was that such a vaccine could be taken by simply eating the appropriate fruit or vegetable. Thus the vaccine could be administered relatively cheaply and easily, avoiding the need for injections by trained personnel.

In addition, the plant would supply only harmless subunits of the human pathogen for which the vaccine was designed. While other methods, such as mammalian cell culture, carry a risk of contamination by pathogens, plant-based production does not. As a result, this approach could avoid the costly purification process required for other methods of producing vaccines.

Moreover, it was considered likely that the plant's tough cell walls would protect the vaccine from breakdown in the person's digestive tract. At the same time, the vaccine could trigger what is called *mucosal immunity*—that is, the activation of immune cells in the lining of the gut—as well as the production of antibodies in the blood serum.

To determine which plants to engineer for the production of vaccines, scientists take into account a variety of factors. In particular, they consider those plants for which reliable and efficient techniques for inserting foreign DNA have been developed. In addition, they look for plants that give the best yields of vaccine.

Researchers also recognize that the costs of distribution and storage can be reduced by choosing plants that grow readily in those regions where the vaccines would be used. For long-term storage, it would be easy enough to save seeds or cuttings of the vaccine-producing plants. Traditional vaccines, on the other hand, must be transported long distances from their site of manufacture to their place of usage. In addition, they must be kept refrigerated during both transport and storage—a requirement that is difficult to meet in remote areas.



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■ **Research technician Barbara Gonzales analyzes the data on vaccine-producing plants.**

The choice of plants, however, is complicated by the fact that each one has a set of drawbacks as well as benefits. For instance, a new gene can be inserted into potatoes, tomatoes, and alfalfa at relatively high efficiency, while the genetic transformation of bananas and legumes tends to be inefficient. Potatoes can be stored for relatively long periods, but tomatoes and bananas rot more quickly. In addition, potatoes can be propagated easily, while banana trees take years to become established and produce fruit. On the other hand, the protein content of potatoes is relatively low, so the vaccine concentration is low.

Potatoes have been selected for several early animal and human trials. Even so, don't expect to have vaccine chips with your sandwich anytime soon. So far, volunteers in clinical trials have had to stomach their vaccinations as sections of raw potatoes. Although cooking makes potatoes more palatable, it also reduces or eliminates the vaccine's effectiveness.

Arntzen's research team has used several types of plants—including corn, banana, potato, tomato, and lettuce—to test the production of vaccines against such infectious agents as hepatitis B, Norwalk virus, cholera, and a harmful strain of *Escherichia coli*. They are currently using tomatoes to produce vaccines against cholera, hepatitis B, and Norwalk virus.

Recognizing the need to control the dosage of vaccines administered, and to use plant material that may or may not be palatable, these researchers have concluded that plant-derived vaccines will probably not be distributed to the public in the form of edible fruits and vegetables. It is more likely that plant tissue will be freeze-dried, powdered, and placed in capsules, much like herbal supplements. This approach will make it feasible to provide measured doses of each vaccine.

Some scientists are looking outside the plant kingdom entirely. At the National University of Singapore, for instance, Professor Gong Zhiyan and his research team are modifying the genes of zebrafish to produce hepatitis B vaccine. Fish is a high-protein food, suggesting that high doses of vaccine could be produced in muscles of the fish. These scientists also say that the technology can be easily transferred to species that are more commonly eaten, like salmon. Again, heat would damage the vaccine, so the fish would have to be eaten raw. In many cultures, sushi and sashimi are more appealing than raw potatoes.

Concerns about genetic engineering

The genetic engineering of plants, whether for the production of vaccines or foods, has raised a number of concerns. One concern

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Professor Arntzen holds a pack of pureed tomatoes containing a vaccine against Norwalk virus. To provide measured doses of the vaccine, an extract from the tomatoes will probably be freeze-dried, powdered, and supplied in capsules.

is that the modified plants might escape from their controlled environment and "invade" natural habitats. To prevent that, preliminary research is done in well-contained greenhouses. But what happens when these plants are eventually moved to the fields? Some worry that genetically modified plants might outcompete or breed with their relatives in the wild, so that foreign genes may spread and have unintended consequences.

Scientists must consider several factors when determining the probability for the escape and proliferation of a genetically engineered plant. According to Jeff Tomkins, director of the Clemson University Genomics Institute, it is important to know how the crop is related to other species. As in the animal kingdom, breeding does not occur between distant species. For example, pollen from a genetically engineered tomato plant would be incompatible with a pumpkin flower, but it may easily affect the prize tomato plants in your backyard. It is therefore important to check whether there might be any wild relatives of the engineered crop near the growing site.

For some crops, outcrossing is simply not an issue. Potatoes, for instance, are unlikely to be affected by exposure to genetically altered pollen because they are reproduced without pollination. Instead of harvesting seeds from a cross, farmers simply cut the fleshy tubers into pieces and plant them for the next season's crop.

Plants can also be manipulated to prevent foreign genes from escaping. For example, Arntzen's group is currently planning on using sterile-male plant lines that are unable to produce viable pollen or seeds. Alternatively, foreign genes may be incorporated in chloroplast DNA, which is inherited maternally and does not spread through the pollen.

Even if outcrossing were impossible, there are concerns that a genetically modified organism might escape and become a weed on its own. When asked if

For 95 percent of those who ate the potato vaccine, the level of specific antibody-secreting cells had increased.

vaccine-producing plants might escape and become weeds in the wild, Tomkins replies, "To spread as a weed, the plant needs to have an advantage." An herbicide-resistant canola plant, for instance, would have a distinct advantage in farm areas where many doses of herbicides are sprayed every year. On the other hand, a vaccine-producing ability is unlikely to be advantageous for a plant's success in the wild. In any case, researchers emphasize the importance of containment of genetically modified plants.

The bottom line

One of the biggest hurdles for scientists performing research on plant-derived vaccines at university labs is to obtain financial backing from investors and pharmaceutical manufacturers. Given that such vaccines are targeted for use in developing countries, they are expected to be less profitable than established vaccines. For example, a reliable vaccine for hepatitis B already generates revenues of over $1 billion a year. A plant-based alternative could not come close to matching such figures. As a result, only a few small biotechnology companies have invested in the development of plant-derived vaccines.

So who are the main financial supporters of this research? Currently, most of the funds come from international aid organizations, national governments, and philanthropies. Nonetheless, research in this area remains underfunded. Admitting his frustration, Arntzen says, "Our problem is that we work on vaccines for poor people—and there is no profit in manufacturing drugs for poor people."

Despite these setbacks, Arntzen was able to obtain adequate funding to perform preliminary clinical trials in 2000. In one such trial, 20 volunteers ate sections of raw potatoes that were genetically engineered to produce a vaccine against the Norwalk virus. Four other persons (in a placebo group) were given unmodified potatoes. Blood tests showed that for 95 percent of those who ate the potato vaccine, the level of specific antibody-secreting cells had increased. Although the increase was modest, this result confirmed that plant-derived vaccines can trigger an immune-specific response.

In August 2002, another clinical trial was performed by a group led by Dr. Hillary Koprowski of Thomas Jefferson University in Philadelphia. Transgenic spinach, designed to provide a vaccine against rabies, was given as a salad to 14 human volunteers. While no one in the control group showed antibody production against the rabies virus, several of those who ate the transgenic spinach produced significant levels of antibodies against rabies. The researchers concluded that this type of vaccine could be used as an oral booster for conventional vaccinations against rabies.

While scientists believe that the technology is nearly ready to move from the research stage to clinical trials and production, adequate funding remains an issue. "We have not yet done a challenge trial, which is needed to prove that the vaccines prevent disease," notes Arntzen. "Such trials are extremely expensive, often costing $50–100 million or more. We need a big partner to do that." Heribert Warzecha and Hugh Mason, two of Arntzen's collaborators, remain optimistic. Expressing their views in a review article, they say, "Even if it takes another decade before we see the first plant-derived vaccine on the market, the advantages of plants over other systems are convincing."■

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