Vegetable Vaccines

By Diane Boudreau

banana, a day keeps the virus away
Everyone knows that eating a banana helps quell a case of diarrhea. But Charles Arntzen has taken this bit of folk wisdom to an extreme. The Arizona State University plant biologist has a vision of bananas preventing the millions of childhood deaths caused each year by diarrheal diseases such as cholera, Escherichia coli, and Norwalk virus. Of course, you cannot cure a severe case of cholera with a banana. Instead, Arntzen wants to prevent the illness by using fruits or vegetables engineered to contain vaccines. Such edible vaccines are far cheaper to produce than their traditional counterparts. ASU scientists say that they are also safer and more convenient.

Since their invention in 1998, vaccines have had a major impact on world health. The smallpox vaccine has helped to eradicate the disease—it exists only in laboratories today. Polio is rapidly disappearing as well. In developed countries, where childhood immunization is routine, parents no longer worry about the dangers of diphtheria, mumps, or measles. Unfortunately, vaccines do not exist for many diseases common in the developing world. Most poor countries cannot afford the investment required to produce traditional vaccines. Instead they rely on pharmaceutical companies in the developed world, which tend to overlook diseases like cholera because they rarely appear in wealthy nations.

Even when the right vaccines exist, developing countries often cannot afford them. And traditional vaccines must be kept refrigerated until they are used, which makes transport and storage difficult in remote, rural areas. Arntzen has pondered these problems for years. In 1990, the Children’s Vaccine Initiative put out a call for development of new vaccine technology. Traveling through Thailand the following year, Arntzen met with Mahoney, the master chef who is helping to guide the candidate plants through the review and regulatory processes that all drugs must follow.

"Because of this crossing of people with complimentary backgrounds, we’re able to pull together a proposal that no other institution has the capacity to do, which is to genetically redesign a tomato or potato so that the biological system is now a manufacturing plant," Arntzen explains. Arntzen and Mahoney’s efforts have started to bear fruit, literally. Early clinical trials have shown that eating plant-derived vaccines does produce an immune response in humans. "We have completed three human clinical trials. We feed people raw potatoes, and it worked. This is the first time the Food and Drug Administration allowed any research team to feed any transgenic food product to humans as a drug," says Arntzen.

Richard Mahoney

Most of the vaccines in use today contain actual viruses or bacteria that have been killed or weakened. When the human body detects a foreign organism it launches an immune attack on the invader. The body’s immune system produces antibodies designed to destroy specific proteins in or on the surface of viruses or bacteria. These proteins are called antigens. Antibodies produced to destroy specific antigens remain after the invader disappears. Vaccines prepare the human immune system for future battles. Later, when the vaccinated person comes into contact with the actual disease, the antibodies are already in place to fight it off.

On rare occasions, the dead or weakened viruses in a vaccine can cause the very disease they aimed to prevent. To avoid this reaction, scientists began to develop genetically engineered “subunit” vaccines in the 1980s.

Subunit vaccines are made from one or more genes instead of a whole pathogen. The genes produce proteins of the virus that trigger the production of antibodies, just like traditional vaccines do. However, because they contain only part of the virus, these vaccines cannot cause the illness in question. The trick in producing subunit vaccines lies in figuring out which proteins will produce an immune response, because not all of them do.

Edible vaccines are also subunit vaccines. They pose no risk to the person receiving them. However, they are even trickier to produce than injectable vaccines. For instance, the researchers need to know that the vaccines will not be destroyed by stomach acid before having a chance to take effect. And they have to ensure that the viral proteins will attract the attention of the immune system.

"We have to build this whole machinery one step at a time," Arntzen explains. "How do we put the gene in? How do we improve the gene? How do we get the gene in the fruit as opposed to the leaves?" He started working to put vaccines into food crops ever since. His work has focused on diarrheal diseases and hepatitis B, a virus that causes liver cancer. It has been a long journey from eureka to reality.

Fortunately, he has had a guide along that path.

"I started out with a rather naïve view that we would simply harvest, say, a banana," says Arntzen. "But you can’t just grow a crop and put it out into the public. You have to standardize doses and meet regulations." The ASU scientist realized he needed help turning his engineered plants into a usable product. Time to add a bit of luck to the expertise. While he was still tweaking genes, Arntzen met Richard Mahoney, a chemist with vast experience in bringing vaccines to developing nations. At the time, Mahoney was working with the International Vaccine Institute. Arntzen had recently accepted the Florence Ely Nelson Presidential Chair at ASU and the role as founding director of the Arizona Biodesign Institute. He convinced Mahoney to join him at the new institute.

"I deal with the translational research," explains Mahoney, using a buzzword created in cancer research. As more and more potential cancer treatments were developed, scientists realized they needed a system for taking candidate products through the regulatory and testing process in a systematic way.

"It was like having lots of chefs making the same cake. You need to have a master chef, and everyone needs to be using the same recipe," explains Mahoney. "Charlie knows how to do all you need to bring the vaccine to the candidate point. Mahoney’s talent is helping to guide the candidate plants through the review and regulatory processes that all drugs must follow.

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Phase I trials primarily test for safety, but these studies also demonstrated that the vaccines produced an immune response. The vaccines must undergo two more testing phases before gaining FDA licensing approval.

The next challenge is turning a bunch of produce into measurable doses of vaccine. "You aren’t going to slice a tomato and serve it for breakfast," says Mahoney. Instead, the researchers purify the fruit, removing seeds and skins. Then, they freeze-dry it, powder it, and put it into capsules.

This process has worked very well in a laboratory setting, where the research team grows small numbers of plants under highly controlled conditions. But how well will it work on a large scale?

"With a typical drug, it’s very easy to produce large quantities. It’s a defined chemical. With vaccines, especially plant vaccines, you’re making a very complex product and you don’t even really know what the product is," says Mahoney. "To go from growing so plants in a lab up to a huge field full of plants is a major undertaking," he explains.

ASU has invested more than $1 million to construct a new greenhouse on the ASU East campus. The greenhouse will expose Arntzen’s crops to wider variations in light, water, and other variables. The setup will more closely resemble the real-life conditions under which vaccines will be produced. More importantly, it will allow for production of thousands of doses of vaccines while ensuring that the new plants do not mix with crops grown for food.

Developing the process is a difficult first step. That complete, the researchers will license the technology to a pharmaceutical company that can mass-produce vaccines. However, ASU will retain the right to transfer the technology to developing nations. This is a key component of the project.

"Normally, these companies would sell the vaccine to travelers from the United States for about $50 a dose, but they have no interest in selling them in Vietnam for 10 cents a dose," says Mahoney.

Because manufacturing plant-based vaccines is so cheap, developing countries can produce and distribute the products on their own without relying on pharmaceutical companies in Europe or the United States. "You don’t have to build a big, expensive factory to do what we do," explains Mahoney. "A modern vaccine factory costs from $80 million to $100 million to build. Plant vaccines, you’re talking about a greenhouse. We know what it costs to grow tomatoes. Even if the vaccine tomatoes cost ten times the price of regular tomatoes, it’s still just pennies a dose."

Besides being inexpensive, edible vaccines do not need to be refrigerated. This eliminates yet another cost and inconvenience.

Transferring vaccine technology is not simply a matter of handing off information and training workers, however. First, the researchers must build relationships with government and health care officials in the targeted countries. They also need to address people’s concerns about the new vaccines, such as fears about genetic modification. This is where Mahoney’s years of experience come into play. "We know for sure that we’re going to have to get a clear understanding of what people want to know about the vaccines. We want to learn in advance the issues that make people uncomfortable," Mahoney says.

The ASU scientists also need to learn the best ways to promote and deliver the new vaccines in each country. Approaches to delivering vaccines that would work in America might have little effect in another country.

To illustrate, Mahoney cites the results of a focus group conducted in Indonesia. The focus group consisted of many young mothers. The women were asked their opinions on the hepatitis B vaccine that first became available in 1986. "Hepatitis B is an infection that you get as a young child. However, the deadly disease can lead to liver cancer, which people usually don’t develop until they are 50 or 60 years old," Mahoney explains.

"While American mothers might easily agree to vaccinate their children against diseases that won’t appear until adulthood, mothers in other countries might not see the connection. The separation of medicine from benefit is not widely recognized in developing countries," says Mahoney. "They think, ‘Give me this medicine now and I’ll feel better in an hour,’ or ‘Give me this vaccine and I won’t get sick tomorrow.’"

The researchers wanted to know how to convince mothers to get hepatitis vaccines for their children given the long timeline of the disease.

"The conclusion from the focus group was, ‘If the village headman tells us it’s good for our children, that’s enough for us to get it,’” says Mahoney. "So we did that—explained the benefits to the village headman. That was easy. Every village headman had one or more friends who had died from hepatitis B. We learned the importance of asking people the best way to introduce things.”

Mahoney adds, "Science and medicine don’t just come out of a lab. And then the patient is told what to do. You can’t impose technological solutions on people.

"Now, ASU researchers need to convince decision makers in developing countries that they can shed their dependency on big pharmaceutical companies; that they can produce their own solutions on their own soil.

"No American or European company wants to make vaccines for cholera or dengue fever. Only poor people get those diseases. That’s their way of thinking," Arntzen says. "While there is no market incentive in the first world, there is a marvelous market possibility for companies in the developing world."

He continues, "We will provide a technological solution that will allow small companies in developing countries to create jobs while meeting pressing public health needs. Our goal is a sustainable vaccine production system for the next century."

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EXACTLY HOW DO YOU GET A VACCINE INTO A TOMATO? Arizona State University scientists are working on the recipe.

To turn a vegetable into a drug, scientists take a gene from the virus that causes a disease and insert it into a plant cell. The cell has to adopt the gene as part of its own DNA, the master molecule of the genetic code in every living thing. Each cell will pass on that inserted DNA when it divides into two new cells.

The first task is finding the right gene. Scientists use enzymes to chop up the viral DNA into manageable fragments. Each fragment is then spliced into bacterial DNA. The modified bacteria are allowed to multiply into cell colonies. The new “recombinant” DNA sequences are transmitted to their offspring. Only one bacterial colony will contain the viral DNA fragment the scientists want to use. To find it, the scientists use a gene probe to recognize which bacteria contain the DNA sequence needed to produce a vaccine. Once the colony is identified, scientists allow these bacteria to reproduce even further, creating millions of copies of the DNA fragment.

The most common method is to use a bacterium that naturally infects the plant. For example, Agrobacterium tumefaciens easily infects many plants. During infection, the bacterium transfers part of its DNA plasmid into the plant cell nucleus. In doing this, the bacterium causes the plant to grow tumors.

Scientists have figured out how to remove tumor-causing DNA from this bacterium. This makes it an effective vehicle for transporting DNA into plant cells without damaging the plant in any way. A newer technique for delivering genes is called the “gene gun.” Scientists load genes into microscopic gold particles. They accelerate the particles and shoot them into the plant cell. As the particles pass through the cell, some of the DNA is left behind to mix with the cell's own DNA.

Once the new DNA is embedded in the cell nucleus, the genetic blueprint for the new vaccine is replicated whenever the cell divides. When the plant reproduces, its offspring will contain the recombinant DNA as well.