Innovations

Delicious vaccines

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The vaccine industry is turning babies into pin-cushions. Although some vaccines are combined, there is still more than one injection per baby, and plenty of screaming in pediatric offices. How soothing, then, to think of the injection being replaced by toothless munching on some mashed banana.

For developing countries, an edible vaccine could stop far more than a baby's screaming. If the transgenic plant containing the vaccinating material can be grown on-site, vaccination logistics could be simplified, and costs reduced.

Several large companies such as Dow and DuPont are moving into this area, but it is a small company named Axis Genetics, plc (Cambridge, UK) that is leading the way. The next few years will be crucial for Axis, as it moves beyond the promising early science, and into the real world of vaccine production.

First the virus

The quickest way to make lots of a foreign protein in a plant is to infect the plant with a virus. Axis started with the cowpea (known in the United States as the black-eyed pea) and its fast-growing virus CPMV (cowpea mosaic virus). The coat of CPMV is derived from the splice- and cleavage-products of a single gene, and up to 38 amino acids can be inserted into a loop of the coat protein with no adverse effects on coat assembly. Axis is using this system, dubbed Epicoat, for vaccines against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and cancer (the latter directed against a mucin expressed on breast cancer cells).

Epicoat is an alternative to peptide vaccines, which were first proposed in the 1980s and have struggled to establish themselves ever since. Axis CEO Iain Cubitt sees the lack of conformational stability in these peptides as a crucial flaw, and says that the Axis technology solves this by putting the peptide in a structured loop.

Epicoat is a purified injectable, but purified inhalant and nonpurified edible forms may follow. Inhaled or ingested vaccines encounter the sites that are used by most infecting pathogens, and these vaccines should induce more effective immunity — mucosal immunity and secretory IgA antibodies, in addition to serum antibodies. This approach is not practical for peptide vaccines made by chemical synthesis because of the large amounts of material necessary, but the quantity is reasonable for a plant-based vaccine.

If the developing world could grow vaccines they could afford them. But will edible vaccines work?

But with Epicoat the worst defect of peptide vaccines remains. "Epicoat can make a lot of material but you can only display a single epitope," says Hugh Mason of the Boyce Thompson Institute for Plant Research, Inc. (BTI; Ithaca, New York). "There is not a lot of data so far that single epitope vaccines will be very useful." With single epitope vaccines, it takes but a single mutation for the infectious agent to avoid the vaccine-induced immunity. This has led some investigators to string together several peptides in the same insertion site, an approach that is the subject of an Axis patent application. "It is likely that several peptides represents a more realistic approach than a single peptide," says Harry Greenberg of Stanford University (Palo Alto, California), who helped develop the newly released rotavirus vaccine.

I say potato, Dan says potatoe

If the limiting factor is the amount of protein sequence that can be inserted in the virus, why not insert the whole protein into the plant? This thought, or something similar to it, occurred to Roy Curtiss and Guy Cardineau (Washington University, St. Louis, Missouri) in 1990, and to Charles Arntzen and Mason of BTI a year later. Arntzen and Mason were heavily involved in subsequent developments in edible vaccine technology. In 1998 Axis concluded deals with both Mycogen (San Diego, California), who own the Curtiss patents, and BTI.

In the intervening years the plant vaccine field had demonstrated the successful production of foreign proteins in an immunogenic state (1992), the production of antibodies in mice fed with plant material (1995), protection against disease in animals fed plant material (1997), and finally the production of antibodies in humans fed plant material (1998). The latter trials involved the ingestion of "bite-sized chunks" of delicious raw potato from a plant producing LT-B — the binding subunit of a toxin from enterotoxigenic *Escherichia coli*. A pentamer of LT-B binds gut epithelial cells and allows the active LT-A toxin subunit to enter the cell. The trial participants ate 50 or 100g of raw potato on each of three occasions, for a mean of 0.75 mg of LT-B per dose. Ten of 11 showed a fourfold rise in serum antibodies, and six of 11 showed a fourfold rise in secretory (IgA) antibodies.

Potential pitfalls

The LT-B trials directed by Arntzen are encouraging, but no one is relaxing yet. "People said edible vaccines would never work," says Cubitt. "Then when LT-B elicited a good response they said of course it works because [the bacterium and the protein are] normally in the gut. For nonenteric diseases it is not so

clear. There it depends on how we present the protein."

When given orally, a particulate virus or even bacterium is efficiently sampled by the immune system. Although LT-B may be a special case, single proteins are usually not efficiently taken up. One solution that the BTI is working on is to produce complete, empty virus-like particles in plants simply by expressing the coat protein. "We've shown that we can produce these structures with Norwalk virus in plants," says Mason. "The efficiency is questionable and variable, but I think it's largely a problem with the level of expression."

Virus-like particles or not, expression levels remain a challenge. The solution may be inducible expression at a particular time, to limit toxicity to the plant.

The flip side — containing expression — is also a concern. We do not make antibodies to all of our food because of tolerance — the damping down of the immune system in the face of overwhelming amounts of antigen. The escape of a vaccine plant into the general food chain could be a disaster if it induced tolerance to a major surface protein of a virus. "One can't say at this point that it's not a possibility," says William Langridge (Loma Linda University, Loma Linda, California). "We hope [Axis] will fund further research into this question."

Langridge stresses, however, that tolerance looks to be an unlikely prospect. His experience stems from the successful induction of tolerance to a diabetes autoantigen in mice (thus preventing further destruction of the pancreas). This was achieved with plant material, but only when the autoantigen was fused to LT-B, so that the complex entered gut epithelial cells efficiently. In earlier work an unfused autoantigen induced tolerance only after two months of continuous feeding.

The safety issue is particularly sensitive following the public uproar in Britain over genetically modified (GM) foods. This curious incident was sparked by one researcher's description of his unpublished and questionable results on a TV show. "It's bizarre what has happened in the UK," says Cubitt. "Science is being ignored in the formation of the public perception here." In the United States there is an equally distressing state of affairs — complete apathy — and it is in that country that Axis grows its crops (in greenhouses). But even in Britain, Cubitt does not anticipate any problems. "We are not involved because we are in pharmaceutical products not food products — we are already highly regulated and not planning to grow large areas of crops," he says. "There may be a moratorium on growing in the field or growing agricultural products, but we grow in the US and we don't do agriculture. It's nothing to do with us."

Business basics

Vaccines used to be a treacherous business, filled with interminable trials with thousands of participants, and endless lawsuits from the few of the vaccinated millions with an adverse reaction. No more.

"The vaccine field has changed out of all recognition in the last ten years," says Cubitt. "The reason it has changed is that, if you don't use the whole organism you can't cause the disease, so you get away from the liability issues."

"It's turned from being the Cinderella of the business to being very attractive," he says. "It's now one of the most profitable parts of the pharmaceutical industry."

Protection from liability has also come from legislation, and the size of some trials has been scaled back if the protective levels of antibody are known from an earlier vaccine (as is the case with Axis's hepatitis B vaccine). Axis is further protecting itself by drawing extensively on the expertise of academic collaborators. It uses this expertise, for example, to identify the proteins or epitopes for use in vaccines.

The promise to the developing world

The dream of a vaccine-laden banana tree in every backyard is not going to happen — for starters there is the containment issue. Cubitt says the final vaccine "won't be fresh material, it will be a powdered formulation that can be stored at room temperature." How the temperature stability will be achieved has not been disclosed but, he says, "we believe that our approaches will lead to temperature stability."

Could this material be produced in the countries that need it most? "The type of technology we are using is more related to food processing technology than pharmaceutical technology, because we are not taking out the food material or doing a purification," says Cubitt. "Theoretically this could be produced around the world, but we need to know that it is produced under pharmaceutical controls not agricultural controls. These are pharmaceutical products not agricultural products."

In the final stages of production, edible vaccines may resemble the pharmaceuticals that they most certainly are. But if the vaccines work, the wonder of their source will remain: protection from disease using only sunlight, dirt and water as the primary ingredients.

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No more fillings

The alternative to a vaccine is a bolus of ready-made antibodies. Making antibodies in cultured cells is prohibitively expensive, so Julian Ma at Guys Hospital in London has turned to plants.

Ma has tackled *Streptococcus mutans* – the primary cause of dental cavities – with an engineered IgA. Two topical applications a week for three weeks eliminated colonization for up to four months (published observations) or two years (unpublished observations). The technology is being commercialized by a company from Mountain View, CA, called Planet Biotechnology, Inc.