Hunting Livestock Parasites With a Gene Gun

The news seemed too good to be true—and, in a sense, it was. After years of painstakingly moving genes, embryo by embryo, to create a transgenic animal with functioning genes other than the ones nature granted it, the scientific community was rocked in 1990 by the revelation that new genes could be injected into an animal after birth as simply as giving a vaccination.

In fact, the equipment being used to create this apparent miracle was a vaccination tool: a commercially available, hydraulically powered device originally designed for mass inoculations and dating to World War II. For the world's gene jockeys, this was akin to the cat burglar risking life and limb to scramble into an open third-floor window, only to discover that the front door had been unlocked all along.

Then came the catch. Yes, new genes could be injected into an animal in a split second using the gene gun. And yes, by some incredible process still not totally understood, the new genetic material would be taken into the nucleus of the animal's own cells, there to function as though it had always been there. But it could persist only until the animal's immune system got wind of the invader and swept in to destroy the cells that were playing host to the new DNA and producing the foreign protein.

Now, in a classic example of taking life's lemons and making lemonade, the scientific community has found a way to put this inhospitable trait of nature to work.

"The idea of creating vaccines with DNA began to take shape," explains geneticist Robert J. Wall at the ARS Gene Evaluation and Mapping Laboratory in Beltsville, Maryland. "Different genes from a parasite such as *Cryptosporidium parvum* produce different proteins, and some of those proteins stimulate a greater protective response from an animal's immune system. The idea was to figure out which protein caused the greatest immune response, inject the animal with the gene that produced that protein, and let the animal's immune system do the rest."

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With 2,000 pounds per square inch of pressure, a sapphire-tipped gene gun can shoot atomized droplets of genetic material through the skin and directly into test animals quickly and almost painlessly. (K7516-1)

The advantages of these so called nucleic acid vaccines, versus the typical vaccine, are numerous.

For one thing, the animal is not exposed to the whole parasite, but only to a fraction of its genetic code. And the immune system perceives the threat as coming from within the injected animal's own cells, rather than merely circulating in the bloodstream. This internal threat warrants a marshaling of the immune system's biggest guns, the more comprehensive cell-mediated response, instead of relying on mere antibodies to fend off the foe.

In experiments in 1993-94, Wall and molecular biologist David E. Kerr teamed up with fellow ARS scientists Mark C. Jenkins and Ronald Fayer to demonstrate the effectiveness of a nucleic acid vaccine against *C. parvum*, an apparently ubiquitous parasite that infects an amazing range of creatures—from humans and horses to rabbits and raccoons. Fayer and Jenkins are at the Immunology and Disease Resistance Laboratory in Beltsville.

The ARS researchers injected a single protein-producing gene from *C. parvum* into the mammary glands of pregnant sheep, hoping to harvest the resultant antibodies from the animals' milk. These antibodies could be used to immunize humans whose own immune systems are impaired and unable to protect them against the parasite. The experiment was a success, Kerr reports.

"One of the joys of the cryptosporidium project was that it was easier than standard vaccines against this parasite. They require growing the parasite in culture, and that's very difficult to do," he says. "Otherwise, we have to collect the parasite from feces of infected calves, grind it up, and make a paste for a vaccine.

"With the nucleic acid vaccine, DNA is very easy to grow in culture. Then we take a tiny bit of that DNA and inject it in the animal, and the animal makes the protein for us."

The scientists did not exactly send the gene naked into battle. Instead, it was tailored with a regulatory element borrowed from a gene of a common virus. This regulatory element, not unlike an on-off switch, enables the gene to be active in a wide range of tissues, rather than at a specific site such as the liver, and to produce large quantities of immunity-stimulating protein.

"You have to add the regulatory element to each gene before you inject that gene," says Wall. "But with common genetic engineering tools, that's a trivial task and goes very quickly."

Delivered by the sapphire-tipped gene gun at a flesh-piercing but surprisingly painless 2,000 pounds per square inch of pressure, the DNA dissolved in salt water is atomized into fine droplets that find their way into the cells. But where they land is not necessarily where they all stay, as Kerr was the first to discover in follow-up checks on the vaccinated Beltsville sheep.

"We thought a limited number of cells were taking up the injected genetic material, and we wanted to check on that by doing a biopsy of a sheep's tissue a few days after injection," Wall recalls. "But since the gene gun leaves no traces where the material was injected, David tried adding some india ink to the DNA so we could track it in animal tissue.

"When we checked a few days later, not only could we see the black streak where the DNA went into the tissue, but we also noted that the lymph node near the mammary gland had turned black from the ink. We found that the injected DNA was being expressed in that lymph node. Yet the lymph node was half a foot away from where we injected."

Therein lies a mystery, according to Wall. Did the ink-stained lymph node mean simply that DNA that didn't embed in a specific cell drained to the lymph system? Or was it picked up by immune cells circulating on their way to their home base in the lymph system? Either way, the results suggest that if foreign DNA is injected into an animal in hopes of jump-starting that animal's immune system to mount a defense, it shouldn't take long for the immune system to notice the invader.

Wall and Kerr envision dazzling possibilities for the process they've dubbed "somatic cell engineering," putting genes and their proteins to work with the flick of a trigger.

"We're looking next at a strategy to counteract the hormones that regulate animals' food intake," says Kerr. "Leptin is a hormone discovered a few years ago; if mice have a defective leptin gene, they become obese.

"We're hoping that if we inject a pig with the gene for leptin, only slightly altered, its immune system will perceive that altered gene as an invader and make antibodies against it. And since antibodies aren't all that precise, they might also neutralize the animal's own natural leptin molecule and increase the animal's appetite. This could lead to faster growing pigs going to market."—By **Sandy Miller Hays**, ARS.

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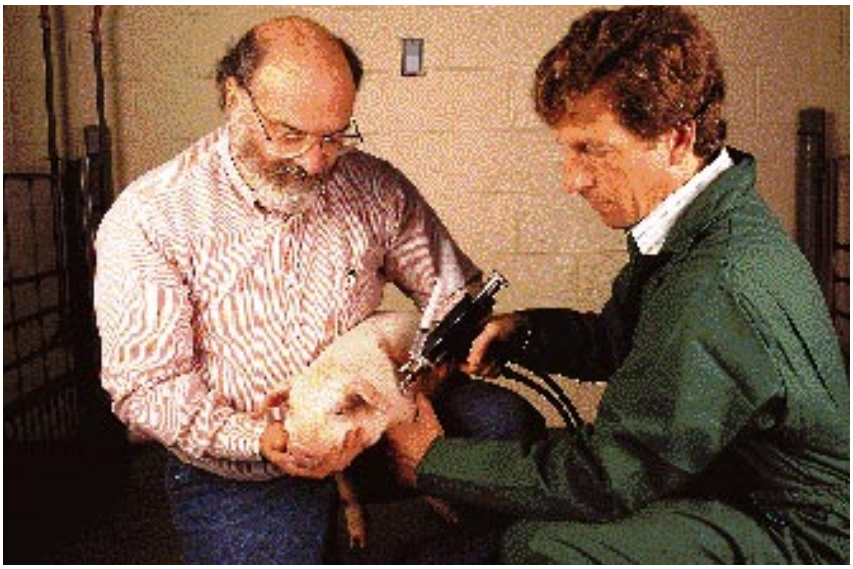

Using the jet-injection gene gun, molecular biologist David Kerr (right) and geneticist Robert Wall insert DNA solution into a 3-week-old piglet. They call the process somatic cell engineering. (K7517-1)

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