Naked DNA Points Way to Vaccines

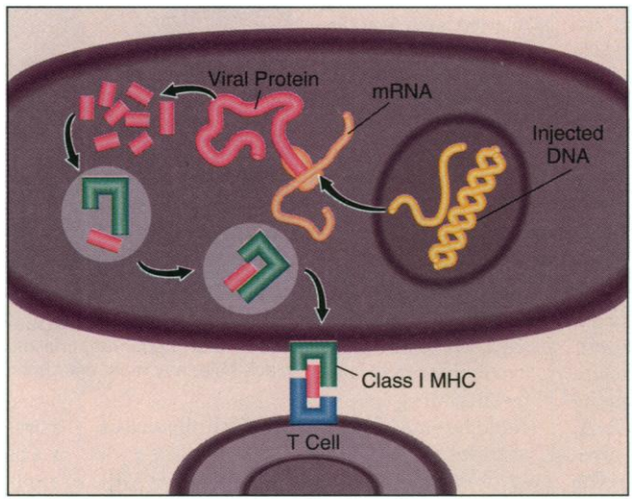
Direct injections of a gene from the influenza A virus can immunize mice, sparking hopes that this simple and cheap approach can trip up other cunning pathogens

Three years ago, researchers from Vical, a startup San Diego biotechnology company, presented some surprising data during a visit to Merck Research Laboratories in West Point, Pennsylvania—so surprising that one of those present wondered whether they were witnessing the "biological equivalent of cold fusion." Led by Philip Felgner, the Vical team revealed that the muscles of living mice would make foreign proteins if injected with naked genes. According to the gospel of biotechnology, that just wasn't supposed to happen: Elaborate genetic engineering tricks are needed to slip foreign DNA into cells. But here it appeared to sail right in and start producing proteins. If the finding were true, it could have enormous implications for the development of vaccines, which rely on foreign proteins to prime the immune system to recognize and attack invading pathogens.

Merck immunologist Margaret Liu, for one, was more intrigued than wary. "I was just really amazed," recalls Liu. Others at Merck were too, and a collaboration began. Merck quickly verified that Vical was on to something and now, on page 1745, they report that naked DNA can indeed work as a vaccine to protect mice from influenza virus.

Merck is not alone in being intrigued by the surprising finding about naked DNA, which Vical discovered in collaboration with Jon Wolff and colleagues at the University of Wisconsin, Madison. Indeed, while no one fully understands how it works, over the past year naked DNA has not only become one of the hottest areas of vaccine research but Wisconsin's Wolff has also been making steady progress in mice applying it as a gene therapy for Duchenne's muscular dystrophy. Harriet Robinson at the University of Massachusetts Medical Center in Worcester and Joel Haynes of Middleton, Wisconsin's, Agracetus Inc. have high hopes that naked DNA will be able to foil viruses as shrewd as HIV. It might even work as a gene therapy for diseases as baffling as cancer or as an "omnivax" that contains a cocktail of genes from different pathogens and protects against many diseases. And unlike recombinant proteins made in test tubes, proteins made in living animals don't have to go through expensive purification steps.

Naked DNA "is a very intriguing idea," says John La Montagne, chief of the Division of Microbiology and Infectious Diseases at the National Institute of Allergy and Infectious Diseases (NIAID). "Vaccines have to be cheap, safe, and effective. This promises to be all of those things." AIDS researchers are equally effusive. "It looks like a fantastic approach," says Alan Schultz, who heads the vaccine branch at NIAID's Division of AIDS. "It gives you a lot of additional options from traditional vaccines."



JEFFREY CAMPBELL

Naked came the DNA. Muscle cells allow foreign viral DNA in, make protein, and then display protein pieces on their surface, spawning legions of killer T cells.

As with many surprising discoveries, the finding that DNA injection could get the cells of living animals to produce proteins came serendipitously. Vical's Felgner and Robert Malone, working with Wolff's Wisconsin group, were attempting to engineer live mice to make new proteins by chemically coercing their muscle cells into taking up DNA. As a control, they left out the chemical and, confoundingly, the animals' muscle cells took up the DNA and produced even higher levels of the protein (*Science*, 23 March 1990, p. 1465).

In the current work, Merck and Vical focused on the nucleoprotein of influenza A virus. The researchers first stitched the DNA that codes for the nucleoprotein into a plasmid, a circular piece of bacterial DNA that serves as a vector. After injecting the plasmid carrying the viral gene into the muscles of mice, they found antibodies to the nucleoprotein, indicating that the gene was being expressed. They then "challenged" these animals with a lethal dose of influenza A, which kills mice within 2 weeks. A full 90% of the mice survived this challenge, while only 20% of the controls survived.

Not only did the vaccine work, but the Merck/Vical team has evidence suggesting it may be able to outwit the influenza virus's notorious ability to mutate its way around the immune system—and therefore vaccines. What Liu and her colleagues found is that the immunized mice remained healthy even though the virus used to challenge them surfaced 34 years after the strain of virus coded for by the naked DNA. The researchers suggest that they saw such powerful cross-strain protection because their naked DNA vaccine works in a different way from licensed flu vaccines. Ordinary influenza vaccines, made with chemically killed virus, aim to elicit antibodies against surface proteins on the virus. These "neutralizing" antibodies prevent free floating viruses from wiggling into cells and wreaking havoc. The problem with these vaccines is that the antibodies only work as long as the variable proteins on the viral surface don't change.

In contrast, core pieces of the virus—like the nucleoprotein that the Merck/Vical vaccine relies on—rarely change and can activate the arm of the immune system that produces killer cells, which, in turn, home in on virus-infected cells and destroy them. The idea is that the nucleoprotein is produced inside cells where pieces of it can be picked up by the Class I major histocompatibility proteins. These proteins, which are normal immune system components, then display the nucleoprotein fragments on the cell surface. Since the fragments are foreign to the body, T cells find them, latch on, and then teach killer T cells how to recognize this alien. Liu and co-workers report finding just such killer T cells in their vaccinated mice.

Some researchers familiar with the Merck/Vical work caution, however, that though they are impressed that the naked DNA protected the animals, they still have questions about the mechanism. Brian Murphy, a respiratory virologist at NIAID says there's "no evidence" that the killer T cells detected by the Merck/Vical team are

actually responsible for protecting against the disease. "It's all supposition," he maintains. What is more, Murphy says, there "is no data in humans that would suggest that [killer T cells] play an important role in protection and there's a tremendous amount of epidemiology that says otherwise." Though Merck's Liu takes issue with Murphy's arguments, she says the real point of the experiment is to prove that the concept—not this vaccine—is viable.

Merck's Maurice Hilleman, who has made several human vaccines, says a major value of naked DNA is that it can trigger immune responses against proteins without triggering one against the plasmid. Theoretically, this means the plasmid vector could repeatedly deliver DNA coding for different proteins and thus protect against a variety of diseases. "This is one of the most exciting things in modern vaccinology," says Hilleman.

Harriet Robinson at the University of Massachusetts wholeheartedly agrees. Robinson independently began making what she calls "gene vaccines" for influenza a few years ago with direct injections in chickens and mice of DNA coding for an influenza surface protein. Muscle, says Robinson, is but one cell type that can produce proteins this way. She has even had success with DNA nose drops, which may provide mucosal immunity, a powerful defense against respiratory viruses.

Robinson also has been collaborating with Joel Haynes from Agracetus, which has developed a way to coat DNA onto tiny gold beads. Haynes and Robinson shoot these coated beads into the skin with a "gene gun" to introduce the DNA into epidermal cells. With influenza, not only does this require three to four orders of magnitude less DNA, but, says Robinson, their technique gives "the best protection we've seen, with 100% survival" of the animals challenged. Haynes, Robinson, and colleagues now are beginning studies with an AIDS vaccine in monkeys. Merck and Vical are close-mouthed about their plans, but an AIDS vaccine is an obvious application.

Before a naked DNA vaccine is injected into humans, of course, it would have to clear safety hurdles. A chief question is what happens to the DNA and whether it can integrate with the host genome, potentially causing cancers. Though most researchers believe this risk is extremely low, NIAID's Murphy stresses that many safety tests would have to be completed first.

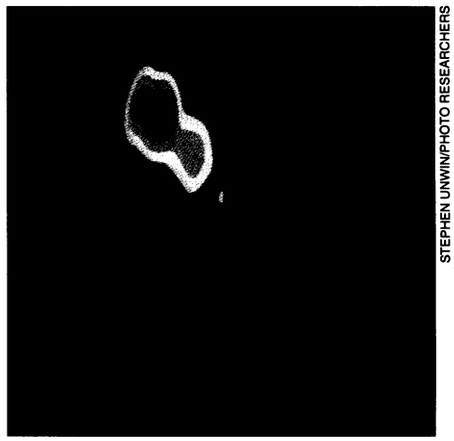
Researchers may be facing these safety questions sooner rather than later. As Vical's Felgner notes, naked DNA has progressed more quickly than anyone imagined. "Almost every experiment has worked the first time," says Felgner. "It's remarkable and you get suspicious, but it's clearly real and well beyond the stage of phenomenology." And well beyond analogies to cold fusion.

–Jon Cohen

ASTRONOMY

Could Quasars Get Their Shine From Stars?

Ever since they were discovered 30 years ago, quasars have been an enigma. Shining like beacons from the very edges of the universe, they emit such an astounding amount of energy—the equal, in some cases, of the combined brilliance of 100,000 ordinary galaxies—that scientists have turned to even more enigmatic constructs to explain them. The leading theory is that their extraordinary brightness is powered by black holes as massive as millions of suns, dragging material into the centers of newborn galaxies and heating it to incandescence. But if Roberto Terlevich of the Royal Greenwich Observatory and Brian Boyle of Cambridge University are right, the puzzle of quasars' power source may have a more prosaic solution: Synchronized bursts of starbirth and death in the cores of young galaxies, they argue in a forthcoming paper, could be enough to explain the brilliance of at least some quasars.



STEPHEN UNWIN/PHOTO RESEARCHERS

A monster's tongue. The radio-emitting jet of this quasar *(upper left)* implies a massive black hole, say most astronomers.

Terlevich and Boyle are reviving an idea that was first proposed soon after the discovery of quasars themselves. This "starburst" model fell out of favor after 1969, when Donald Lynden-Bell of Cambridge proposed that quasars and their dimmer cousins, active galaxies, could be powered by black holes—far more efficient and compact powerhouses. Most quasar experts still doubt that starbursts are potent enough to explain the very brightest quasars. But, by showing that the number of quasars at different brightnesses matches what would be expected from starburst galaxies, Terlevich and Boyle have given the model a boost, notes one supporter, Jorge Melnick of the European Southern Observatory.

Where the black hole model explains a quasar's brilliance with a single display of brute force, the starburst model does it with many precisely timed smaller events. As dust and gas collapse to form a new galaxy, say Terlevich and Boyle, the dense core of the newborn galaxy would give birth to a large litter of hot, massive stars, all at roughly the same time. The biggest of them, stars 25 to 60 times as massive as the sun, would burn up all their hydrogen fuel within 3 million or 4 million years. As these aging, supermassive stars bloated in their old age and expelled their outer layers in strong stellar "winds," the stage would be set for the first phase of quasar activity.

The starburst galaxy would become a quasar as the massive stars entered their death throes, exploding as supernovae and creating a burst of brilliance. What's more, the explosions together with the winds from surviving stars would carve out a hot cavity in the interstellar gas. Ionized gas within the cavity could generate radio waves, accounting for the radio emissions broadcast by some active galaxies.

Following this first wave of spectacular deaths, less massive stars, eight to 25 times the mass of the sun, would start to expire as supernovae. The explosion of these stars—more numerous than their supermassive brethren—would drive the galaxy to maximum brilliance about 10 million years after its birth, reckon Terlevich and collaborators. Afterward, the young galaxy would dim and continue a quieter evolution.

One of the strongest objections to that model has been that starbursts simply cannot produce enough energy to account for a quasar's brilliance; after all, present-day starburst galaxies are much fainter. But Terlevich and Boyle now say they have indirect evidence showing that starbursts could do the job. Recent quasar surveys by Boyle and others have given investigators a sharper picture of the distribution of quasar brightness. To see whether starburst galaxies could match it, Terlevich and Boyle looked at the brightness of present-day elliptical galaxies—usually considered to be the oldest galaxies—and extrapolated backward to the early universe, calculating how bright the galaxies' cores would have been if they had undergone a spasm of starbursts early in their formation. The result: a predicted distribution of brightness that closely matches that of quasars, even the most brilliant of them.

The finding hasn't won over skeptics of