Genetic Vaccines

Vaccines crafted from genetic material might one day prevent AIDS, malaria and other devastating infections that defy current immunization technologies. They may even help treat cancer

by David B. Weiner and Ronald C. Kennedy

Vaccines arguably constitute the greatest achievement of modern medicine. They have eradicated smallpox, pushed polio to the brink of extinction and spared countless people from typhus, tetanus, measles, hepatitis A, hepatitis B, rotavirus and other dangerous infections. Successful vaccines have yet to be introduced, however, for too many deadly or debilitating disorders—among them, malaria, AIDS, herpes and hepatitis C. This gap exists because standard immunization methods work poorly or pose unacceptable risks when targeted against certain illnesses.

Clearly, alternate strategies are needed. One of the most promising creates vaccines out of genetic material, either DNA or RNA. In the past 10 years such vaccines have progressed from a maligned idea to entities being studied intensively in academia and industry and in early human trials.

Vaccines at Work

The merits of genetic immunization become most apparent when the actions of traditional vaccines are understood. Traditional preparations consist primarily of a killed or a weakened version of a pathogen (disease-causing agent) or of some piece (subunit) of the agent. As is true of most genetic vaccines under study, standard types aim to prime the immune system to quash dangerous viruses, bacteria or parasites quickly, before the pathogens can gain a foothold in the body. They achieve this effect by tricking the immune system into behaving as if the body were already beset by a microorganism that was multiplying unabated and damaging tissues extensively.

When responding to a real infection, the immune system homes in on foreign antigens—substances (usually proteins or protein fragments) that are produced uniquely by the causative agent and not by a host. Two major arms can come into play, both of which receive critical help from white blood cells known as helper T lymphocytes. The humoral arm, led by B lymphocytes, acts on pathogens that are outside cells. These B cells secrete antibody molecules that latch onto infectious agents and thereby neutralize them or tag them for destruction by other parts of the immune system. The cellular arm, spearheaded by cytotoxic (killer) T lymphocytes, eradicates pathogens that colonize cells. Infected cells display bits of their attacker’s proteins on the cell surface in a particular way. When cytotoxic T lymphocytes “see” those flags, they often destroy the cells—and the infiltrators within.

Beyond eliminating invaders, activation of the immune system against a specific pathogen leads to the creation of memory cells that can repel the same pathogens in the future. Vaccines confer protection by similarly inducing immune responses and the consequent formation of memory cells.

But standard vaccines vary in the kind and duration of security they provide. Those based on killed pathogens (such as the hepatitis A and the injected, or Salk, polio vaccines) or on antigens isolated from disease-causing agents (such as the hepatitis B subunit vaccine) cannot make their way into cells. They therefore give rise to primarily humoral responses and do not activate killer T cells. Such responses are ineffective against many microorganisms that infiltrate cells. Also, even when nonliving preparations do block disease, the protection often wears off after a time; consequently, recipients may need periodic booster shots.

Attenuated live vaccines, usually viruses, do enter cells and make antigens that are displayed by the inoculated cells. They thus spur attack by killer T lymphocytes as well as by antibodies. That dual activity is essential for blocking infection by many viruses and for ensuring immunity when investigators do not know whether a humoral immune response would be sufficient by itself. What is more, live vaccines—such as the measles, mumps, rubella, oral polio (Sabin) and smallpox types—frequently confer lifelong immunity. For those reasons, they are considered the “gold standard” of existing vaccines.

Live vaccines can be problematic in their own way, however. Even they can fail to shield against some diseases. Those that work can cause full-blown illness in people whose immune system is compromised, as in cancer patients undergoing chemotherapy, AIDS suf-
ferers and the elderly. Such individuals may also contract disease from healthy people who have been inoculated recently. Moreover, weakened viruses can at times mutate in ways that restore virulence, as has happened in some monkeys given an attenuated simian form of HIV, the virus that causes AIDS. For some diseases, the risks of reversion to virulence are intolerable.

Whole-organism vaccines, whether live or dead, have other drawbacks as well. Being composed of complete pathogens, they retain molecules that are not involved in evoking protective immunity. They can also include contaminants that are unavoidable by-products of the manufacturing process. Such extraneous substances sometimes trigger allergic or other disruptive reactions.

The Best of All Worlds

Genetic vaccines are quite different in structure from traditional ones. The most studied consist of plasmids—small rings of double-stranded DNA originally derived from bacteria but totally unable to produce an infection. The plasmids used for immunization have been altered to carry genes specifying one or more antigenic proteins normally made by a selected pathogen; at the same time, they exclude genes

MAKING OF A GENETIC VACCINE usually involves isolating one or more genes from a disease-causing agent (pathogen) and splicing those genes into plasmids (a), closed rings of DNA. The rings are then delivered into small groups of cells, often by injection into muscle cells (b) or by propulsion into the skin via a so-called gene gun (c). The chosen genes code for antigens—substances able to elicit an immune response—that are normally made by the pathogen.
that would enable the pathogen to reconstitute itself and cause disease.

The vaccines usually are delivered by injection or by a device known as a gene gun. Injection, commonly into muscle, puts genes directly into some cells and also leads to uptake by cells in the vicinity of the inserted needle. The gene gun propels plasmids into cells near the surface of the body—typically those of the skin or mucous membranes. Once inside cells, some of the recombinant plasmids make their way to the nucleus and instruct the cell to synthesize the encoded antigenic proteins. Those proteins can elicit humoral (antibody-type) immunity when they escape from cells, and they can elicit cellular (killer-cell) immunity when they are broken down and properly displayed on the cell surface (just as occurs when cells harbor an active pathogen).

Such features raise hopes that, once perfected for use in people, DNA vaccines will preserve all the positive aspects of existing vaccines while avoiding their risks. In addition to activating both arms of the immune system, they will be unable to cause infection, because they will lack the genes needed for a pathogen’s replication. As a bonus, they are easy to design and to generate in large quantities using now commonplace recombinant DNA technology, and they are as stable as other vaccines (perhaps more so) when stored. They should therefore be relatively inexpensive to manufacture and to distribute widely. Further, because they can be engineered to carry genes from different strains of a pathogen, they can potentially provide immunity against several strains at once, something that should be very helpful when the microorganism is highly variable, as in the case of influenza viruses and HIV.

Some investigators are testing vaccines composed of RNA, a single-stranded relative of DNA. RNA in cells leads readily to synthesis of any encoded proteins. RNA, however, is less stable than DNA, a property that can be problematic for vaccine manufacture and distribution. These difficulties are probably surmountable. Nevertheless, because RNA vaccines have been studied much less extensively than the DNA types, we will concentrate our discussion on DNA vaccines.

**Lemonade from Lemons**

The idea that genes might serve as vaccines grew in part out of research begun almost half a century ago. In the 1950s and 1960s experiments unrelated to vaccine development showed that delivery of genetic material into an animal’s cells could trigger some synthesis of the encoded proteins as well as of antibodies targeted against those proteins. Thereafter, workers occasionally assessed antibody manufacture as an easy way to demonstrate that a given gene was generating a protein.
In the 1970s and early 1980s the ability of inserted genes to prompt an immune response gained attention from other researchers, this time as a disappointing phenomenon. Scientists trying to develop gene therapy (the delivery of genes to correct inherited and other disorders) noted that proteins made from therapeutic genes were sometimes destroyed in animals receiving the genes. The reason: an immune reaction to unfamiliar proteins.

By the early 1990s a handful of laboratories had begun exploring whether the unwanted immune responses to the protein products of foreign genes might be put to good use—for vaccination. Many others were dubious at first, skeptical, for instance, that the immunity elicited would be strong enough to spare people from infection by a living pathogen.

Yet in 1992 a cluster of animal studies done by independent groups demonstrated resoundingly that the concept was sound. Those groups included teams led by Stephen A. Johnston of the University of Texas Southwestern Medical Center in Dallas; by Philip Felgner of the University of California in San Diego and Margaret Liu, then at Merck in West Point, Pa.; by Harriet L. Robinson, then at the University of Massachusetts; and by one of us (Weiner) at the University of Pennsylvania.

Collectively, those studies and a host of others conducted over the next few years revealed that DNA vaccines delivered into cells could stimulate the immune system of rodents and primates to generate B cell, cytotoxic T cell and helper T cell responses against many different pathogens and even against certain cancers. The research showed as well that immune responses and disease protection could be elicited when different routes of administration were used. The responses, moreover, could be enhanced by a variety of methods for facilitating DNA uptake by cells.

Since the mid-1990s many more researchers have turned their attention to DNA vaccines, and the technology has advanced to the first rung of human trials, focused on safety. The earliest trial began in 1995, when plasmids containing HIV genes were delivered to patients already infected by that virus. Bigger trials initiated in 1996 made history in another way. For the first time, physicians put new genes (coding for HIV or influenza proteins) into healthy people, instead of into those afflicted by some disorder.

So far human tests are examining vaccines designed to prevent various infections (by HIV, herpes, influenza, hepatitis B and Plasmodium—the parasite responsible for malaria), to bolster the impaired immunity of patients already infected with HIV and to treat a number of cancers (among them lymphomas and malignancies of the prostate and colon). Although cancer is not an infectious disease, much evidence indicates that harnessing the body’s immune defenses may help combat it.

The safety trials ask such questions as, are the plasmids toxic, and does DNA delivered as a drug incite an immune response against the body’s own DNA? Encouragingly, the studies have not identified any serious side effects to date.

Such trials do not assess disease prevention or amelioration, but many are monitoring the vaccines’ effects on the immune system. Preliminary findings hint that useful immune responses can be achieved. Notably, HIV vaccines have generated both humoral and cellular responses; plasmids bearing Plasmodium antigens have evoked significant cellular immune responses; and a vaccine against hepatitis B has resulted in levels of antibodies that should be high enough to prevent infection. In common with traditional vaccines, though, current genetic approaches will probably have to be combined in many cases with generalized immune stimulators (adjuvants) in order to elicit the strong immune responses required to shield recipients from future infections.

How Do the Vaccines Work?

As clinical trials continue, bench scientists are seeking deeper insight into exactly how genetic immunization stimulates immunity, especially by the often crucial cellular arm of the defensive system. A detailed understanding should offer clues to enhancing effectiveness.

In truth, for many years immunologists faced a paradox. DNA vaccines obviously activated killer T cells. Yet simply putting DNA into skin or muscle cells and prompting those cells to display fragments of the encoded antigens should not have produced that outcome. Before such display can activate cytotoxic T cells, the killers must be primed, or switched on, in part by interacting in a specific way with what are called “professional” antigen-presenting cells. In particular, the T cells must bind to the same antigenic fragments they will detect on inoculated nonimmune cells (such as muscle) and, simultaneously, to a second, co-stimulatory molecule (a “second signal”) ordinarily found only on antigen-presenting cells.

At one time, biologists thought DNA vaccines had no way of getting into antigen-presenting cells and therefore that those cells had no way of synthesizing and displaying the antigens encoded by those vaccines. Recent discoveries by several groups have shown, however, that the original view was mistaken. Some of the plasmids do in fact make their way into professional antigen-presenting cells. These cells then display antigens alongside the critical co-stimulatory molecules and help to prepare the T cells for action [see illustration on next two pages]. Such findings indicate that to induce a powerful cellular immune response, DNA vaccines must be deliv-
DNA vaccines elicit protective immunity against an infectious agent, or pathogen, primarily by activating two branches of the immune system: the humoral arm, which attacks pathogens outside of cells, and the cellular arm, which eliminates cells that are colonized by an invader. Immunity is achieved when such activity generates long-lasting “memory” cells—the sentries that stand ready to stop the pathogen from causing disease.

A simplified description of how the vaccines induce immunity begins at the far left of the diagram, with entry of a DNA vaccine into a targeted cell, such as muscle, and the cell’s subsequent production of antigens normally found on the pathogen of interest. In the humoral response (top unboxed sequence), white blood cells called B cells bind to released copies of antigenic proteins and then multiply. Many of the progeny secrete antibody molecules that during an infection would glom onto the pathogen and mark it for destruction. Other offspring become the memory cells that will quell the pathogen if it circulates outside cells.

Meanwhile display of antigenic protein fragments, or peptides, on inoculated cells (within grooves on MHC class I
in a way that will yield good uptake by antigen-presenting cells, not only by other cell types.

Separate work suggests that the plasmid DNA surrounding antigenic genes is more than a mere gene-delivery vehicle; it strengthens the immune response evoked by the antigens. This effect apparently stems from the high frequency of CG sequences in plasmids. Each strand in the DNA double helix is built from units called nucleotides that are distinguished by the bases they contain—either adenine (A), cytosine (C), guanine (G) or thymine (T). Plasmid DNA, derived from bacteria, has a greater frequency of CG sequences than does the DNA in vertebrates. Moreover, the CG units in bacterial plasmids tend to have no methyl group attached, whereas those in vertebrates generally are methylated.

Investigators have proposed that the vertebrate body interprets a high frequency of unmethylated CG pairs as a danger signal. In response, a relatively primitive part of the immune system (one not dependent on antigen recognition) attempts to destroy or wall off the foreign intruder.

**Engineering for Optimal Effect**

Along with analyzing the natural behavior of genetic vaccines in the body, immunologists are looking ahead, exploring ideas for increasing overall immune reactivity and for optimizing the ratio of cellular to humoral responses.

One proposal for amplifying responsiveness has emerged from studying the DNA around CG sequences. Researchers have demonstrated that plasmid DNA yields the most potent immune response when CG sequences are flanked by two purines (adenine or guanine) to their “C” side and two pyrimidines (thymine or cytosine) to their “G” side. In mice, plasmids containing such “immunostimulatory sequences” induced more vigorous antibody and cytotoxic T cell activity than did an otherwise identical vaccine. Hence, increasing the number of immunostimulatory sequences in plasmids might well amplify the immunogenicity of the antigenic codes in a DNA vaccine.

A different approach is incorporating genes for signaling molecules called cytokines into antigen-carrying plasmids or into separate plasmids. Cells of the immune system release these molecules to regulate their own, and one another’s, activities. As an example, a molecule named granulocyte-macrophage colony-
stimulating factor stimulates the proliferation of antigen-presenting cells, among other actions. Inclusion of its gene has been shown to boost overall responses to DNA vaccines.

To ensure that genetic vaccines trigger a strong cellular response when needed, researchers are experimenting specifically with genes for cytokines that are known to promote killer-cell activity. In mice, scientists have found that helper T cells called Th1 cells secrete cytokines that favor cellular responses at the expense of humoral (antibody) ones, whereas other helper cells (Th2 cells) secrete cytokines that favor humoral activity. In humans, helper T cells seem to come in more varieties, but a preponderance of Th1-type cytokines still promotes a cellular response, and a preponderance of Th2-type cytokines stimulates a humoral response.

One such project showed that a vaccine including genes for HIV antigens and for interleukin-12 (a classic Th1 cytokine) reduced production of anti-HIV antibodies in mice and markedly enhanced the responsiveness of cytotoxic T cells to HIV antigens. This bias toward a cellular response is particularly encouraging, because recent findings by HIV researchers indicate that a potent killer T cell response to HIV is critically important for combating HIV replication.

Genes for substances known as chemokines might be incorporated as well. Chemokines are small molecules that attract both antigen-presenting cells and T cells to damaged or infected tissues. Like cytokines, these substances differ in the mix of cells on which they act and in the precise effects they exert. As their individual actions are better understood, carefully combining specific chemokine genes with selected cytokine genes could go far toward customizing both the type and the extent of immune responses elicited.

DNA vaccines could in theory even sidestep the need for classical antigen-presenting cells to prime cytotoxic T cells. If a gene for an antigen were bundled with a gene for a co-stimulatory molecule normally made by an antigen-presenting cell, then inoculated skin, muscle or other cells would themselves display both the antigen and the crucial “second signal,” thereby facilitating both the priming and the activation of cytotoxic T cells.

Getting from Here to There

If first-generation genetic vaccines do well in clinical trials, they may sometimes be combined initially with more traditional vaccines to achieve even better effects. Let us say, for example, that a subunit vaccine (consisting of a protein) evoked a good antibody response against a pathogen but that a cellular response was needed as well. Meanwhile a new DNA vaccine proved able to induce a cellular response but did not excite an ideal antibody response. In a so-called prime-boost strategy, physicians might deliver the DNA vaccine and then boost the antibody response by later delivering the subunit vaccine as well. Eventually, though, as vaccine makers learn how to optimize responses to genetic immunization (such as through the techniques described above), manufacturers may be able to achieve the needed effects by constructing genetic vaccines alone.

As the exciting, futuristic possibilities of genetic immunization are being considered, those of us who are captivated by this technology also have to roll up our sleeves and grapple with a great many details. For instance, most DNA vaccines stop yielding much protein after about a month. Would finding a way to extend plasmid survival lead to stronger immunity, or would it backfire and encourage attacks against unvaccinated, healthy tissue? How long does immunity last in human beings? How much do people vary in their responses? Which doses are most effective and what kinds of delivery schedules are best? We also need to know which substances are most useful for targeting genetic material to specific cells (including to antigen-presenting cells) and for enhancing the cellular uptake of plasmids. And which genes, out of the sometimes thousands, in a given pathogen should be selected for maximal power?

Clinical trials answering these questions and assessing the effectiveness of the first generation of DNA vaccines may not be completed for five or 10 years. Whether those specific versions reach the market, though, genetic immunization technologies are likely to prove extremely valuable for research into the basic biology of the immune response and for the design of even better vaccines.

Vaccine makers today often have little idea of which components of the immune system need to be activated most strongly against a given pathogen and which antigens and other substances can achieve that stimulation. Now, however, they can readily mix and match antigenic and other genes (such as those for uptake of DNA vaccines by antigen-presenting cells, a crucial event in the induction of immunity, has now been demonstrated by several groups. In one approach, scientists added two kinds of labels to cells in a snippet of tissue that was exposed to a DNA vaccine—one tag (red at left) marked antigen-presenting cells; another (green at center) denoted any cells making an antigen specified by the vaccine. When images of the cells were superposed, the appearance of an orange color (right) signified the presence of antigen-presenting cells that had taken up the vaccine and produced the encoded protein.
cytokines and chemokines) in experimental DNA vaccines and compare the success of different combinations in small animals quite quickly. In that way, they can simultaneously gain a handle on the immune responses that are needed for protection and on the antigens and other proteins that can generate them.

As part of this testing, some researchers are creating “libraries” of a pathogen’s genes; an individual library contains every gene in the organism, with each gene spliced into its own plasmid. They then deliver subsets of such libraries to animals, which are also exposed to the live pathogen. Next, they identify the subsets that work best, further subdivide the groups and do more testing, until the most useful mix of antigens emerges.

As the years go by, the inherent manipulability of DNA should make it a vehicle of choice for teasing apart the body’s complex immune responses to different disease-causing agents. With such information in hand, vaccine makers should be able to design vaccines that will channel immune responses down selected pathways. In the past, manufacturers had no way to custom-tailor their products easily and inexpensively. In the future, such “rationally” designed genetic vaccines are likely to provide new immune therapies for cancer and powerful ways to prevent or minimize any number of devilish infections that elude human control today.

### The Authors

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### Further Reading


