FIG. 68.

DNA Drugs Come of Age

After years of false starts, a new generation of vaccines and medicines for HIV, influenza and other stubborn illnesses is now in clinical trials BY MATTHEW P. MORROW AND DAVID B. WEINER

IN A HEAD-TO-HEAD COMPETITION held 10 years ago, scientists at the National Institutes of Health tested two promising new types of vaccine to see which might offer the strongest protection against one of the deadliest viruses on earth, the human immunodeficiency virus (HIV) that causes AIDS. One vaccine consisted of DNA rings called plasmids, each carrying a gene for one of five HIV proteins. Its goal was to get the recipient's own cells to make the viral proteins in the hope they would provoke protective reactions by immune cells. Instead of plasmids, the second vaccine used another virus called an adenovirus as a carrier for a single HIV gene encoding a viral protein. The rationale for this combination was to employ a "safe" virus to catch the attention of immune cells while getting them to direct their responses against the HIV protein.

One of us (Weiner) had already been working on DNA vaccines for eight years and was hoping for a major demonstration of the plasmids' ability to induce immunity against a dreaded pathogen. Instead the test results dealt a major blow to believers in this first generation of DNA vaccines. The DNA recipients displayed only weak immune responses to the five HIV proteins or no response at all, whereas recipients of the adenovirus-based vaccine had robust reactions. To academic and pharmaceutical company researchers, adenoviruses clearly looked like the stronger candidates to take forward in developing HIV vaccines.

To DNA vaccine investigators, the results were not entirely surprising, because poor responses had been seen in some previous trials. Still, the failures were disappointing because we had good reasons for expecting the plasmid vaccine to be both safe and powerful. Convinced that the original concept was still strong, scientists went back to the drawing board to find ways to boost the effectiveness of the technology. Now these efforts are beginning to pay off. A new generation of plasmid-based vaccines is proving in human and animal trials that it can produce the desired responses while retaining the safety and other benefits that make DNA so appealing. The same DNA-based technology is also now expanding to other forms of immune therapy and the direct delivery of medicines. In their mature form, such DNA-based vaccines and treatments are poised to become a success story by addressing several conditions that now lack effective treatments.

KEY CONCEPTS

- Vaccines and therapies containing DNA rings called plasmids have long held promise for treating and preventing disease, but the plasmids made a weak showing in early tests.
- Improvements to the plasmids and new methods for delivering them have dramatically enhanced their potency.
- DNA vaccines and therapies now used in animals or in late-stage human trials demonstrate that plasmids are reaching their potential.

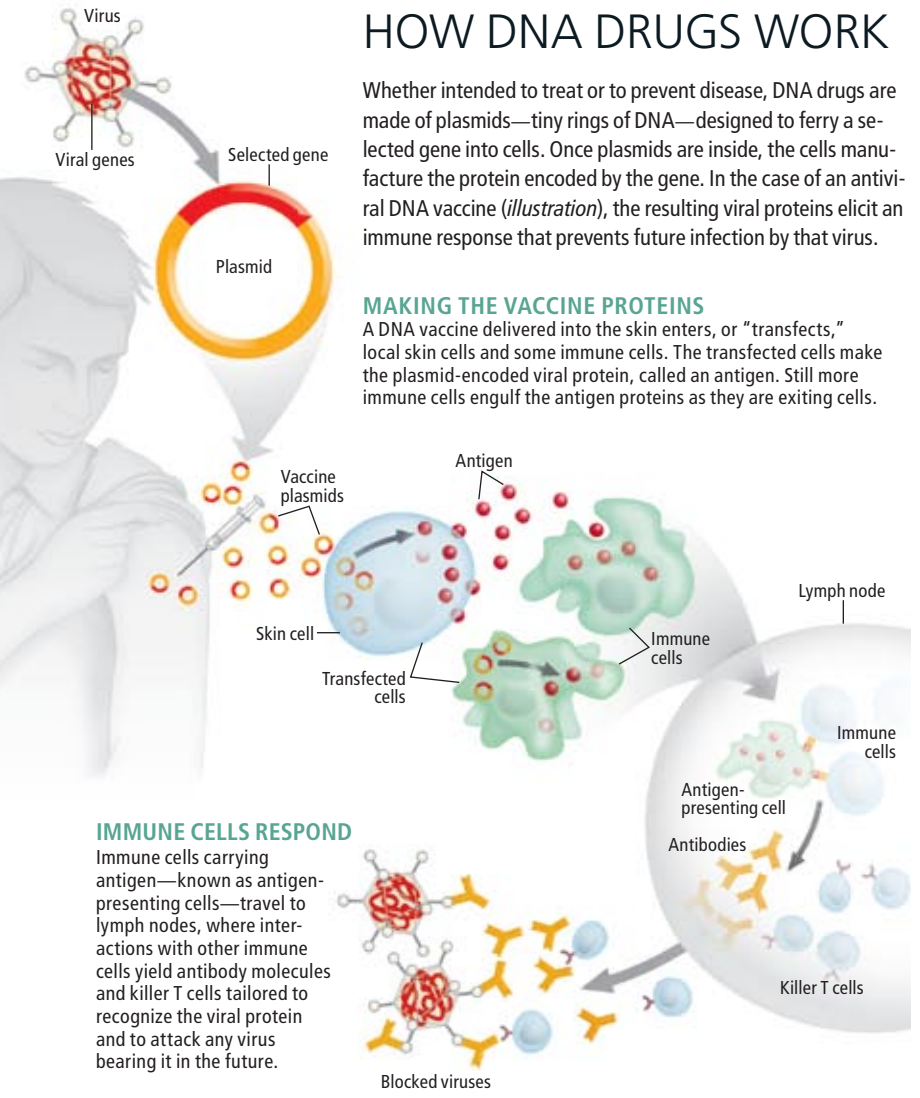
—The Editors

STUART BRADSHAW

[BASICS]

HOW DNA DRUGS WORK

Whether intended to treat or to prevent disease, DNA drugs are made of plasmids—tiny rings of DNA—designed to ferry a selected gene into cells. Once plasmids are inside, the cells manufacture the protein encoded by the gene. In the case of an antiviral DNA vaccine (*illustration*), the resulting viral proteins elicit an immune response that prevents future infection by that virus.



MAKING THE VACCINE PROTEINS

A DNA vaccine delivered into the skin enters, or "transfects," local skin cells and some immune cells. The transfected cells make the plasmid-encoded viral protein, called an antigen. Still more immune cells engulf the antigen proteins as they are exiting cells.

IMMUNE CELLS RESPOND

Immune cells carrying antigen—known as antigen-presenting cells—travel to lymph nodes, where interactions with other immune cells yield antibody molecules and killer T cells tailored to recognize the viral protein and to attack any virus bearing it in the future.

A GOOD IDEA, THEN AND NOW

WHEN THE CONCEPT of using DNA to immunize people began to gain traction in the early 1990s, its elegant simplicity was immediately apparent. The core components of the vaccine—the plasmids constructed to carry genes encoding one or more proteins from a pathogen—would induce the recipient's cells to make those proteins but would not carry instructions for making the entire pathogen, so the vaccine could not give rise to the pathogen itself.

When the plasmids enter a host cell, known as transfection, the machinery that normally decodes DNA starts reading the plasmid's gene and makes the desired protein, which is eventually released from the cell, much the way virus particles would be. Outside the cell the pathogen-specific proteins are recognized by immune cells as foreign to the body. The immune system should thus be tricked into thinking the body is infected, prompting long-term immune recognition and responses against the foreign protein. Just introducing a DNA ring carrying one gene could thereby induce immunity that protects against an entire pathogen.

In addition to their safety and simplicity, DNA vaccines offer a number of advantages over other types of vaccine. Their manufacture is considerably faster than some traditional vaccines, such as those for influenza that require handling and cultivating "live" viruses and a minimum four- to six-month production process. DNA is inherently stable at room temperature (luckily for our cells), so DNA vaccines should not require constant refrigeration, which is a concern during the transportation and storage of many vaccines.

From the standpoint of a vaccine designer, DNA has another plus, which in recent years played an important role in reopening the door to this technology. The immune system does not perceive the plasmids as foreign material—after all, they are made of DNA—so the vaccine itself technically does not provoke any immune response. Only the protein *encoded* by the plasmid gene, once manufactured by cells, garners the attention of immune sentinels, meaning that plasmids can be used over and over in the same recipient to deliver a variety of genes without fear that the body will develop immunity to the DNA carrier and attack the vaccine itself.

Unfortunately, in the early DNA vaccine tests the problem of weak immune responses was a significant pitfall. The main reasons for those failures seemed to be that vaccine plasmids were not getting into enough cells and, where they did penetrate, the cells were not producing enough of the encoded proteins. As a result, the immune system was not being sufficiently stimulated.

The rival technology would ultimately face a bigger problem, however. In 2007 pharmaceutical company Merck initiated a large trial of an HIV vaccine that used an adenovirus called AdHu5 to deliver HIV viral genes. In light of the potent immune responses seen in previous experiments with adenoviruses, great hope and excitement surrounded the beginning of this test, known as the STEP trial. In all, about 3,000 HIV-negative individuals received the vaccine or a placebo shot.

As the trial progressed, though, a disturbing difference between the two groups began to emerge: people who got the vaccine were no better protected than those who received the placebo, and eventually they appeared to be *more* vulnerable to being infected by HIV. An early

PRECISION GRAPHICS

tally found that 49 out of 914 men in the vaccine group became HIV-positive, whereas 33 out of 922 men in the placebo group did. With this realization, in the summer of 2009 the STEP trial was halted. The data are still being analyzed for clues to what happened, but some evidence is pointing to the AdHu5 carrier as one possible confounding factor. In people with preexisting immunity to AdHu5, a common cold virus, the immune system may have attacked the vaccine itself. Why some vaccine recipients seemed more susceptible to HIV infection remains unclear.

THE REBIRTH OF DNA

DURING THE YEARS leading up to the STEP trial, researchers still convinced of the DNA platform's potential had been working hard to develop solutions for the complex issues that handicapped the first generation of plasmid vaccines. These efforts focused on boosting all aspects of the plasmids' activity, including new methods of getting them into cells, new ways of increasing protein production once they were inside, and additions to the vaccines that enhance immune system responses to the vaccine-encoded proteins.

New vaccine delivery methods are among the most significant accomplishments to come out of this work, because they get considerably more cells—including immune cells themselves—to take up the plasmids. For instance, transdermal patches and other needle-free systems, such as Gene Gun and Bioject that use pressurized air to inject vaccine, deliver plasmids into the skin, where immune sentries called antigen-presenting cells are highly concentrated. These methods also physically force plasmids into more cells than needle injection would do. To achieve a similar result with vaccines delivered by needle into muscle or skin, the injection can be followed by electroporation, a series of electrical pulses that cause cell membranes to temporarily open pores that allow plasmids to enter more easily. Electroporation can increase cells' uptake of plasmids by as much as 1,000-fold.

The plasmid-gene constructs themselves have also been improved through several types of refinements to the DNA sequences of the genes they carry. Codon optimization, for instance, involves spelling out the gene's instructions in a way the cell will execute most readily. In the genetic code, the amino acid building blocks of proteins are specified by sets of three DNA "letters" that make up a codon. Certain amino acids are designated by more than one codon, but cells typically favor one of these synonymous codons and translate it more efficiently than the others. Choosing optimal codons thus increases the cell's production of the desired protein. Additional revisions to the gene sequence can improve the stability and accuracy of the messenger RNA gene transcripts that the cell actually reads to make the protein and can speed protein manufacture.

A so-called leader sequence near the start of each gene is the first to be translated by the cell into the beginnings of a protein molecule, and optimizing a gene's leader sequence can improve the stability of the final protein molecules. Certain leader sequences can even mark a protein as one that the cell should secrete, which is desirable because it allows immune cells to encounter the foreign proteins both inside transfected cells and outside them. The two situations provoke slightly different types of immune

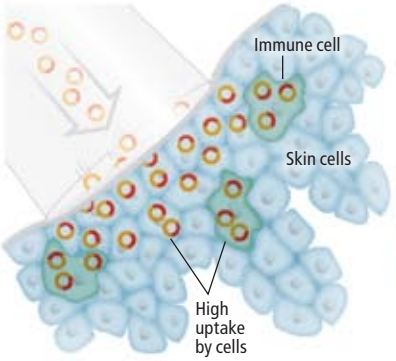
[PROGRESS]

BOOSTING DNA'S POWER

Technologies that increase the effectiveness of plasmid-based vaccines and therapies have renewed hope for the success of the DNA approach. The improvements raise cells' uptake of plasmids, augment their production of plasmid-encoded proteins and intensify immune system responses to those proteins.

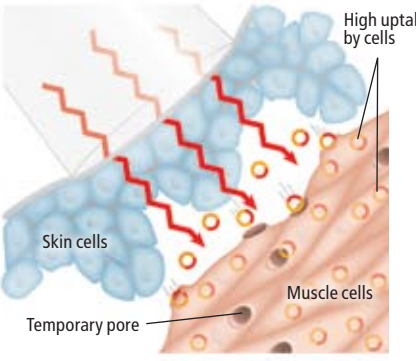
ENHANCED DELIVERY

Needle-free injection



Needle-free injection systems deliver vaccine into the skin, where immune cells are concentrated. The injectors push more plasmids directly into skin and immune cells than needle injections would.

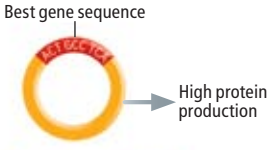
Electroporation device



Mild electrical stimulation called electroporation can boost cells' uptake of plasmids delivered by needle injection. The electrical pulses cause cells to briefly open pores that admit the plasmids.

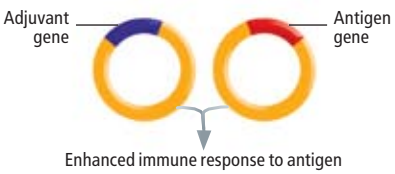
OPTIMIZED PLASMID DESIGN

Instructions for making a protein encoded by a plasmid gene can be spelled out using various sequences of DNA "letters," but choosing certain sequences can raise the amount of protein a cell generates.



IMPROVED IMMUNE STIMULATION

Immune cell–stimulating substances called adjuvants can be encoded by genes added to plasmids. The adjuvants manufactured alongside the antigens enhance immune responses to the vaccine antigens.



PRECISION GRAPHICS

response, and the combination enhances the overall immunity generated by the vaccine.

A final important improvement involves substances called adjuvants, which are typically added to traditional vaccines to boost immune system responses. In some cases, an adjuvant can even steer the immune system toward one form of response over another if desired, for instance, favoring greater production of T cells, which seek out and kill pathogen-infected cells in the body, as opposed to greater production of antibody proteins, which attempt to block pathogens from entering cells. A chemical compound called Vaxfectin, for example, has been shown to increase antibody responses to a DNA vaccine against influenza 200-fold. Another adjuvant—Resiquimod—is used with some DNA vaccines to provoke a strong immune reaction that includes both T cells and antibodies.

Another compelling aspect of the DNA-based technology is that instead of adding adjuvants to the final vaccine formulation, which sometimes creates concerns about maintaining proper emulsification or stability of the formula, designers can incorporate the gene for an adjuvant molecule directly into a vaccine plasmid. Cells that take up the plasmids will then manufacture the encoded adjuvant alongside the vaccine proteins. When gene-encoded adjuvants are added to DNA vaccines, even when the plasmid has already been optimized, as described earlier, the adjuvant can further increase immune responses by fivefold or more.

These designer plasmid vaccines are a far cry from the simple protein-encoding constructs of the early years of the DNA platform. With optimized plasmids and improved delivery methods, the technology was ready to make a comeback by the start of the STEP trial. What is more, the DNA approach has begun to show promise for uses beyond classical vaccination, including plasmid delivery of some medications and of immune therapies targeted at cancers.

A MULTIPURPOSE TECHNOLOGY

THE ABILITY TO SAFELY deliver genes into cells and get those cells to efficiently manufacture the encoded proteins opens avenues to a host of potential treatments. Indeed, many of these DNA-based therapies are ahead of DNA vaccines in the race to widespread clinical use. Unlike classical drugs that often take the form of small chemical molecules, DNA therapies deliver a gene to treat an ailment. Unlike traditional gene therapy, however, the plasmid does not integrate permanently into the recipient's cellular genome or even remain permanently in cells, which avoids complications that have hampered progress in gene therapies.

As is often the case with new technologies, the earliest successes in plasmid-based therapies have been in animals. One example already licensed for use in pigs is designed to prevent fetal loss. Administered to pregnant sows along with electroporation, the plasmid enters the sow's cells, which then make a hormone (growth hormone–releasing hormone) that supports the gestating fetuses' survival. The success of this treatment is exciting in part because it requires only a single injection to work in such a large animal, which bodes well for human therapies.

Various large clinical trials for human DNA therapies are now under way [*see table on opposite page*], including one that delivers genes for proteins called growth factors that mobilize stem cells to treat congestive heart failure. Another employs a plasmid encoding a growth factor called IGF-1 to treat growth failure in patients with the disorder X-linked severe combined immunodeficiency. A third trial addresses a circulatory problem that can be notoriously hard to treat, called critical limb ischemia. This therapy delivers plasmid-encoded factors that induce new blood vessels to grow, in the hope of preventing the need for amputation.

A different category of treatments, known as DNA biological immunotherapy, combines the best aspects of DNA therapies and vaccines by delivering a gene that induces the body to mount an immune response to an existing disease, such as a tumor or a chronic viral infection. One early trial uses DNA encoding viral proteins to induce immune cell attacks on tumors caused by the human papillomavirus (HPV), for example. Initial results from this trial show that half of recipients muster T cell responses to the HPV proteins and that more than 90 percent generate high levels of antibodies. Another current trial is testing a DNA immunotherapy against the hepatitis C virus. Encouraging preliminary results in both these trials are significant because no effective immune therapies currently exist for either HPV tumors or hepatitis C.

In this arena, veterinary applications are once again even more advanced than human studies, and a successful DNA-based therapy for melanoma in dogs is exciting researchers who study human cancer. The dog melanoma treatment, made by Merial, increases the median survival time of dogs with advanced melanoma by sixfold compared with untreated dogs. This DNA

[THE AUTHORS]

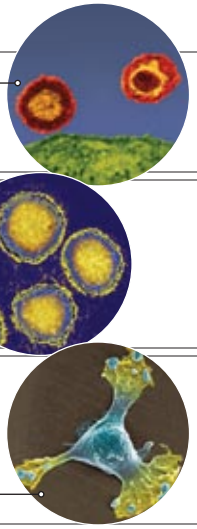

Matthew P. Morrow and **David B. Weiner** collaborate at the University of Pennsylvania, where Morrow is a postdoctoral research fellow. Investigating HIV for nearly 10 years led to his current focus on DNA vaccines and immune therapies. Weiner, a professor of pathology and medicine, is chair of the university's Gene Therapy and Vaccines graduate program. A pioneer of DNA vaccine technology, he brought the first plasmid-based vaccines to clinical trials and has been a consultant to the FDA and to many vaccine and pharmaceutical companies pursuing plasmid-based drugs.

COURTESY OF MATTHEW P. MORROW (*Morrow*); COURTESY OF DAVID B. WEINER (*Weiner*)

DEMONSTRATING THE POTENTIAL OF DNA

Plasmid-based vaccines and therapies are under study in humans for a wide range of disorders, and some are already approved for animals. The table below lists a selection of the disorders targeted by products in human clinical trials or already marketed for animals.

PRODUCT	DISORDER TARGETED IN HUMAN TRIALS	DISORDER TARGETED IN ANIMALS
Vaccines to prevent disease	■ HIV (3 vaccines) ■ Influenza (2 vaccines)	■ West Nile virus (horses) ■ Infectious hematopoietic necrosis virus (farmed salmon)
Immune-stimulating treatments for existing diseases	■ Hepatitis C ■ HIV ■ Human papillomavirus-induced tumors ■ Liver cancer ■ Melanoma	■ Melanoma (dogs)
Therapies that give rise to needed proteins	■ Congestive heart failure ■ Growth failure from X-linked severe combined immunodeficiency disorder ■ Limb circulatory disorders (3 treatments) ■ Melanoma	■ Fetal loss (pigs)



TOP TO BOTTOM: KLAUS BOLLER *Photo Researchers, Inc.*; JAMES CAVALLINI *Photo Researchers, Inc.*; STEVE GSCHMEISSNER *Photo Researchers, Inc.*

biological immunotherapy attests to the potential of the new-generation DNA platforms to succeed where previous approaches have not.

BACK TO THE FUTURE

DOZENS OF HUMAN clinical trials of DNA therapies and vaccines have been conducted in the past 10 years or are currently ongoing. Plasmid versions of flu vaccines exemplify some of the benefits the DNA approach has already demonstrated. A flu vaccine our research group developed, now in early human trials, was shown in animals to protect against common flu strains and against the highly lethal H5N1 avian flu that has infected several hundred people. The vaccine is able to provide this broad protection because its plasmids contain so-called consensus sequences of flu virus genes, meaning the resulting viral proteins resemble those of many different flu strains. Such vaccines might spell an end to mismatches between seasonal flu vaccines and the flu strains that emerge every year.

Of course, the novel H1N1 flu strain that appeared last year to produce a global pandemic highlights the urgent need for a new vaccine approach. An experimental DNA version of an H1N1 vaccine made by the pharmaceutical company Vical was completed in just two weeks in May 2009. Had it been tested and licensed in advance, such a vaccine could have been manufactured in large amounts at least two months sooner than the standard vaccines became available. It is now in early human trials with encouraging results.

The potential power of DNA vaccines and therapies to target diseases that have no other effective alternatives has also brought DNA back into the HIV vaccine race. One vaccine now in human trials, Pennvax-B, contains three HIV viral genes plus genes encoding adjuvant molecules and is delivered with electroporation. Two more vaccines are being tested in a strategy that uses plasmids to prime immune cells to recognize the HIV proteins followed by administration of another vaccine type to boost the early immune response to higher levels. One of these, GeoVax, is being given along with a vaccine based on a virus called modified vaccinia Ankara as the boost. And in an amusing irony, the NIH Vaccine Research Center is now testing a different DNA-based HIV vaccine with one of two adenovirus-based HIV vaccines as boosts.

The fact that several DNA vaccines and therapies are already used in animals and are in large, late-stage human trials involving hard-to-treat ailments attests to how far the plasmid technology has come. Dramatic progress in the field over the past decade has brought some of the most creative vaccines and therapeutics yet to clinical testing for human benefit. In this regard, those of us who have nursed this technology since its infancy cannot help but feel proud to see that it has emerged from a difficult childhood and can look forward to a bright future. ■

MORE TO EXPLORE

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