HIV 25 YEARS LATER

THE BIG CHALLENGES

EDITORS’ INTRODUCTION

In 1983 and 1984 scientists established that HIV (the human immunodeficiency virus) causes AIDS, which had recently begun cropping up in gay men in California and New York. The discovery quickly led to predictions that a preventive vaccine would soon be on tap. Similarly, in 1996, after powerful drug combinations began forcing HIV down to undetectable levels in the blood, prominent HIV researcher David D. Ho of the Rockefeller University voiced optimism that attacking the virus early and hard could prove curative.

Yet neither a vaccine nor a cure has materialized. Indeed, the most promising vaccine prospects have failed. And when aggressive treatment stops, the wily virus comes roaring back.

Where do we go from here? SCIENTIFIC AMERICAN asked two leading HIV researchers to address the biggest scientific challenges facing the field today: Is finding a vaccine even possible? And what, exactly, would it take to rid a person’s body of HIV and thus effect a cure? Their frank, thought-provoking answers follow.
Not long after the virus that causes AIDS was identified, Margaret Heckler, then the U.S. secretary of health and human services, told a group of reporters that the discovery would enable scientists to develop a vaccine to prevent AIDS. “We hope to have such a vaccine ready for testing in approximately two years,” she declared proudly. It was 1984.

Government officials have certainly been spectacularly wrong on other occasions but rarely has a large portion of the scientific community been so overly optimistic as well. Twenty-five years after isolating HIV, we still have no effective vaccine. One year ago a major clinical trial of a candidate made by Merck was shut down because it became obvious that the vaccine was not working and might even be doing harm. This past summer another vaccine hopeful was shelved and its trial canceled before it could begin because there was no reason to believe its results would be any better.

After decades of struggle to make a vaccine against HIV, these events plunged the effort into disarray. We in the field have realized that if none of the classical methods of making vaccines

KEY CONCEPTS

- HIV has so far defeated the best efforts of vaccine scientists because the virus evades and undermines the immune system.
- If HIV infection cannot currently be prevented, a second goal of vaccine makers is to reduce the virus’s spread and the severity of illness it causes.
- Researchers are already returning to basic science to follow new leads, and are far from giving up.

—The Editors
works against this virus, then we need a new one—some unusual creative approach that has yet to be imagined or some new insight into the virus itself that might reveal a vulnerability. We have to go back to basics, but that is not to say we have learned nothing of value over the past 25 years. Indeed, every failure has revealed tricks this virus uses, suggesting new ways to go after it. Those lessons are already spawning fresh ideas and bringing scientists together to attack remaining unanswered questions about this unique virus.

Why Vaccines Work—but Not against HIV
Understanding how to approach the problem of making a vaccine against HIV first requires an understanding of how vaccines normally function. Several different methods for manufacturing vaccines exist, but in each case a vaccine's effectiveness depends on the human body's natural immune responses. The annual influenza vaccine, for example, is made by inactivating that year's strains of influenza virus and administering the killed viruses to people through a
shot in the arm. Immune cells in the deep layers of the skin recognize the viral proteins as foreign and within a few weeks cause the body to manufacture tiny molecules called antibodies tailored to that virus strain. If the same virus enters the body again during flu season, the antibodies will “neutralize” the virus by attaching themselves to it, blocking its ability to infect the cells of the host.

In 1962 Albert Sabin licensed a successful vaccine made from live but attenuated (disabled) polioviruses. Because this live vaccine is able to infect cells to a limited degree, it induces not only antibodies but also a so-called cellular immune response from specialized cells known as T lymphocytes. Should someone vaccinated this way be exposed to polio, the T cells would quickly respond, destroying any host cells infected by viruses that eluded the antibodies. These two examples represent the basic principles underlying vaccines that have been the mainstay of defense against infectious agents over the past 50 years. Unfortunately, the standard methods of inducing antibodies and T cells have failed to protect against HIV. In essence, all vaccines imitate aspects of a natural infection, allowing the immune system to create a “memory” of the event and respond more aggressively the next time. Yet everything about HIV seems almost perfectly adapted to evading or disabling that very system of natural immune responses.

When HIV first infects a new host, the virus starts rapidly reproducing itself inside the host’s cells, and the new viruses move on to commandeer additional cells [see box on opposite page]. Viral replication is so intensive that sometimes infected individuals can have 100 million viral copies per milliliter of blood plasma within a month after infection. Normally the first line of natural immune defense is the innate, or “non-specific,” immune system, made up of cells that patrol the body for invaders. Some of these will destroy any virus-infected cell they encounter on the spot, although in most people this system is probably overwhelmed by the initial onslaught of replicating HIV. Innate immune cells known as antigen-presenting cells, however, are also busy engulfing some of the viral proteins so that they can later show them to more specialized immune system components with the aim of inciting a response.

Among these are the aforementioned T cells, which have two important types: “helper” and “killer.” The helper T cells play a critical role in sounding an alarm to engage the cellular immune system and in orchestrating its attack. Antigen-presenting cells first display the foreign proteins—antigens—they have sampled to the helper and killer T cells, using major histocompatibility complex (MHC) molecules to present the fragments. The T cells, in turn, use their T cell receptors to recognize the antigen-MHC complexes [see box on next two pages]. Once the killer cells have a description of the intruder and receive a chemical signal from helper cells, they multiply, then fan out on a seek-and-destroy mission. This killer T cell response kicks in approximately three weeks after infection, and it destroys most virus-infected cells, driving virus levels down. But the response is usually too little and too late, and lifelong chronic infection has been established.

The helper T cells may represent the body’s most important regulator of responses to infectious agents because of their pivotal role in directing the activities of other immune cells. Unfortunately, from the start, HIV targets helper T cells themselves, replicating inside them and destroying them in the process. In particular, HIV goes after so-called memory helper T cells, which serve as the immune system’s memory of past exposures to pathogens. Within a few weeks of the initial infection, the body’s supply of these memory helper T cells is so depleted that the entire immune system’s command-and-control system is crippled and never fully recovers.

At the same time, the virus gets better at evading the killer T cells. After entering a cell, HIV copies its RNA genetic material into DNA in a sloppy procedure prone to errors that result in mutations in the viral copy. These changes get passed along and added to every time the progeny viruses copy themselves. Moreover, if two virus copies infect the same cell, they can swap genetic material in a process called recombination, creating another virus variant. As a result of this growing diversity, viral proteins displayed by infected cells become increasingly unrecognizable to immune cells primed to remember the original version of the virus. As the killer T cells destroy all the cells displaying recognizable antigens, the virus-infected cells carrying mutant proteins take over. For much the same reason, antibodies produced by the immune system three to four weeks after the initial infection cannot recognize many of the virus particles in the host later in the infection.

This problem of immune defenses being unable to recognize variant versions of HIV is per-
An ideal vaccine would prime the body’s immune defenses to prevent HIV from infecting cells. A second-best solution would allow infection but prevent the virus from reproducing to high levels in the critical early stages of infection. Toward those ends, vaccines typically depend on stimulating some of the same immune responses provoked by natural infection to create a “memory” of the virus; however, HIV’s tremendous mutability often thwarts this approach because immune memory is not broad enough. The trick to making an effective vaccine is generating antibodies and killer T cells able to recognize HIV particles that may be as much as 20 percent different from the version used to make the vaccine.

**EARLY IMMUNE RESPONSE**

**NATURAL INFECTION**

Within **hours** of entering the body, HIV starts infecting helper T cells. Antigen-presenting cells (APCs) patrolling for invaders engulf any infected cells or viruses they encounter.

Within **days**, APCs display small pieces of virus (antigens) on major histocompatibility complex (MHC) molecules to uninfected helper T cells and killer T cells. In response, helper T cells release chemical messengers to activate B cells and the killer T cells. These, in turn, begin proliferating. Some of the resulting “memory” B and T cells are retained by the immune system to respond to future infections.

Within **weeks**, the trained killer T cells seek out infected cells displaying viral antigens to destroy them, while the antibodies block viruses from infecting new cells.

haps the greatest source of frustration for vaccine developers because it is equally true of antibodies and killer T cells generated by a vaccine. Even a strong vaccine-evoked memory response against one strain of HIV might be ineffective against the strain that later enters the body or might become useless as the virus mutates.

To get a sense of the scale of the challenge presented by the enormous diversity of HIV, note that manufacturers change the flu vaccine every year because the flu viruses in circulation around the world are continuously evolving, slightly changing their outer proteins just enough so that last year’s antibodies will not recognize and protect against this year’s flu strains. HIV mutates so rapidly that the diversity of proteins on the surface of HIV particles in a single person after six years of infection is estimated to be greater than the diversity of all the human flu virus strains worldwide in a given year. In effect, a vaccine that uses traditional methods to produce antibodies and other immune responses to HIV would have to be a vaccine against thousands, and perhaps hundreds of thousands, of different viruses, not just one.

**Shifting Goals, Litany of Failures**
The best long-term solution to the HIV pandemic would be a vaccine that prevents infection completely, providing “sterilizing immunity.” At
a minimum, that would probably require a vaccine able to induce broadly reactive neutralizing antibodies that can recognize HIV in all its forms and prevent it from infecting cells.

Once scientists discovered that to enter helper T cells HIV must attach to a CD4 receptor and usually to a co-receptor called CCR5 on the cells’ surface, blocking the ability of the virus to bind to those receptors became a major objective of vaccine research. One of the primary targets of that work is a glycoprotein on the virus’s outer shell that makes contact with the two receptors before the virus fuses with a cell. Known simply as Envelope, that protein is even more variable than the rest of the virus, however.

One of the first HIV vaccines to be tried in humans, called AIDSVAX, was designed to induce antibody responses against Envelope. After a five-year trial beginning in 1998, the vaccine was deemed a failure. Antibodies engendered by the vaccine did not prevent HIV from entering CD4+ T cells and thus did not prevent HIV infection in the people who received it.

To date, no HIV vaccine tried in humans has induced the kind of broadly neutralizing antibodies required to prevent HIV from entering cells. Because this neutralizing antibody problem remains the primary obstacle to a safe and effective vaccine, researchers are also now exploring the less desirable but still acceptable option of a vaccine that does not prevent infection but rather lowers the likelihood of getting sick or transmitting the disease.

Such a vaccine would aim to keep virus levels very low by inducing killer T cells that are primed and ready to destroy infected cells, thereby preventing viral levels from soaring in the early phase of infection. Suppressing HIV replication at this acute infection stage could help spare the body’s population of helper T cells. It could also reduce the risk of virus transmission to others. After the initial surge of viral replication, the virus levels in untreated HIV-positive subjects settle at a median of about 30,000 virus copies per milliliter of plasma, but in observational studies, those whose viral loads are less than 1,700 copies per milliliter had a substantially reduced risk of transmitting the virus to their HIV-negative partners. Any HIV vaccine that cannot provide sterilizing immunity should therefore aim to limit peak viral levels and to reduce chronic viral loads to 1,700 or less.

This approach has also been encouraged by data from studies of human HIV infections and of monkeys experimentally infected with a simian immunodeficiency virus (SIV), showing that killer T cells are important in controlling viral load. Furthermore, rare cases exist of both humans and monkeys whose bodies control replication of the AIDS virus with neither vaccines nor drugs. Most of these individuals possess particular variations in their genes encoding certain MHC molecules, which act as important intermediaries in priming killer T cells to respond to foreign antigens.

Such evidence formed a rationale to proceed with T cell-inducing vaccines, and researchers had high hopes for a recent trial of an HIV vaccine developed by Merck and aimed at inducing anti-HIV killer cells. The company had invested heavily in HIV vaccine research and tested many different methods for inducing the killer T cells. Ultimately, it settled on using a common cold virus known as adenovirus type 5 (Ad5) to carry three HIV genes into cells, expecting the cells to manufacture the HIV proteins. The immune system would then be tricked into thinking the body was infected with HIV and would mount a protective response. The proteins used, called Gag, Pol and Nef, are relatively conserved—meaning they tend not to vary much—across diverse HIV variants.

Unfortunately, this most promising approach for inducing killer T cell responses tested in humans failed in the trial. On average, individual volunteer subjects mounted relatively weak T cell responses to the vaccine—between 10 and 20 percent of what is seen in HIV-infected individuals whose immune systems are controlling viral replication. Moreover, the cellular responses were specific to only three regions of the viral proteins. In contrast, HIV-infected patients who exhibit some measure of control over viral replication normally make between three and six specific responses against the Gag protein alone.

The Merck vaccine’s failure to suppress HIV replication may have been caused by the Ad5 vector or the choice of HIV genes it carried, or a combination of those factors. It is possible that Ad5 is inherently unable to stimulate cellular immune responses that are sufficiently potent or broad to control HIV infection. Many of us have been infected by this common cold virus and have already made immune responses to it. Pre-existing cold virus–specific responses against the Gag protein are.

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[VACCINE PLAN B]

Altering the Course of Infection

In the first 21 days after infection, HIV wipes out significant numbers of helper T cells, and memory helper T cells are the hardest hit. This cell population never fully recovers from the onslaught. Any vaccine that cannot prevent infection should aim to keep viral levels low early on. Sparing the memory helper T cells could prevent the sharp decline in overall immune competence that eventually leads to AIDS, the highly symptomatic end stage of HIV infection.

CRITICAL CLUES

Rhesus macaques provide a valuable model for AIDS research because they are susceptible to simian immunodeficiency virus (SIV), which is very similar to HIV.

Vaccines made from a disabled version of SIV completely protect the monkeys from SIV infection for years. Unfortunately, the weakened strain eventually repairs itself and the monkeys ultimately succumb to AIDS caused by the vaccine.

Understanding why the monkeys are protected for so long could reveal what immune responses need to be induced by an effective vaccine.

The Road Ahead

The Merck vaccine failure was a huge blow to the field, prompting open discussions about whether an effective vaccine against HIV will ever be possible. It has also led to a careful rethinking of current vaccine candidates. At present, a vaccine trial being conducted in Thailand and due to wrap up later this year is the only large-scale human test of a vaccine candidate under way, and none are expected to begin in the near future. A big international trial of a DNA plasmid vaccine developed at the National Institutes of Health had been scheduled to start this fall. In July, however, Anthony S. Fauci, director of the National Institute of Allergy and Infectious Diseases, canceled the trial, stating that the evidence did not support such a large test.

At the same time, Fauci announced that his agency would redirect its funding for HIV vaccine research efforts toward basic science to address fundamental questions about HIV and its behavior in the body that could reveal a new approach to disarming the virus. Developing the next generation of improved vaccine candidates will require that scientists tackle a number of important issues.

HIV diversity remains the great barrier to vaccine-induced antibodies or killer T cell responses able to mount an effective defense early in infection. As a result of mutation and recombination within each infected person, an individual vaccinee is likely to be exposed to a virus that differs by more than 10 percent from the virus used to make a vaccine. For instance, accumulated changes within the highly mutable env gene that encodes the viral Envelope glycoprotein are important in classifying HIV into different groups (labeled M, N and O) and then into subtypes, or clades. Analyses of the amino acid sequences that make up Envelope show that they can vary by up to 35 percent from one clade to another. Even within a clade, Envelope sequence diversity can reach 20 percent.

For this reason, many T cell–based HIV vaccine designs have abandoned the idea of using Envelope to induce a response by the immune system, focusing instead on more conserved regions of the virus, such as the Pol and Gag proteins. Relatively minor variations in those proteins may still have grave implications for vaccine efficacy, however. Single amino acid differences in a viral protein can impair or even eliminate the ability of vaccine-induced antibodies or killer T cells to recognize the virus. Figuring out how to make a broadly neutralizing antibody remains the most important goal of the HIV vaccine field.

A related question has to do with host killer T cell responses to HIV during natural infection: Should we be seeking to emulate or boost all of them, or should we focus on just certain kinds? Killer T cells select various parts of HIV to target, and the regions of the virus they exploit are essentially distinct from those used by the vaccine. This vaccine’s ability to protect a host against infection, even when the immune system is challenged by diverse versions of the virus, is, of course, the goal of HIV vaccine researchers. But the experiments also show that the disabled vaccine virus eventually repairs itself and goes on to create a full-fledged SIV infection that kills the monkeys. Furthermore, the vaccine and experimental challenge viruses can combine, producing a deadly new strain. For safety reasons, therefore, a live, attenuated HIV is unlikely to ever be used as a vaccine in humans.
**Vaccine Timeline**

Only a handful of vaccine candidates made it to large-scale human trials in the past decade. So far traditional vaccine-making methods for inducing antibodies or mobilizing immune T cells have failed to produce a vaccine that protects against AIDS. In light of these disappointments, the National Institute of Allergy and Infectious Diseases (NIAID) announced in August it would refocus on fundamental HIV research.

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<tr>
<th>Year</th>
<th>Event</th>
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<td>1984</td>
<td>April 23: Margaret Heckler, U.S. secretary of health and human services, and Robert Gallo of the National Cancer Institute announced the discovery of a virus believed to be the cause of AIDS. With the infectious agent known, Heckler said that a vaccine could be ready for trials in two years.</td>
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<td>1998</td>
<td>VaxGen's AIDSVAX was the first vaccine to enter phase III testing. After international trials, the vaccine—designed to stimulate antibodies to HIV's outer envelope—was declared a failure in 2003. It provided no greater protection against infection than a placebo.</td>
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<td>2003</td>
<td>The U.S. and Thailand launched a large trial of a vaccine designed to elicit T cell responses to the Envelope glycoprotein by first priming the immune system with canarypox virus. Many scientists publicly opposed the trial at its outset because smaller studies showed only weak responses to the vaccine. Final results are expected in 2009.</td>
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<td>2004</td>
<td>Merck's STEP trial tested a vaccine comprising three HIV genes within the Ad5 cold virus. Also designed to induce T cells, the vaccine generated robust immune responses in recipients. Nevertheless, the trial was cut short in 2007 when monitoring showed that more vaccinees than placebo recipients had become infected with HIV. Analyses of the vaccine failure are ongoing.</td>
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<td>2008</td>
<td>An international trial set to start in September of a vaccine that delivered HIV genes packaged in naked DNA, followed by Ad5, was canceled in July by NIAID director Anthony S. Fauci. The PAVE 100 trial would have included 2,400 men. Immune responses produced by the vaccine in smaller tests were not substantially different from those produced by the Merck vaccine, and Fauci called the trial’s size unwarranted.</td>
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In these cases, the suppression of viral replication is currently being assembled for study, and extensive genetic, immunological and virological analyses will likely yield important clues as to why these individuals are able to suppress virus replication. These discoveries, in turn, will give rise to new vaccine concepts that can be directly tested in monkeys.

Further studies of monkey responses to attenuated, live SIV vaccines will also be valuable because these potent vaccines enable the monkeys to fend off highly pathogenic viruses, even those that differ significantly from the vaccine strain, for considerable amounts of time. Although safety concerns mean the attenuated virus approach will never be used in humans, understanding exactly why it is so effective could yield new insights.

Finally, scientists' ability to find a new approach to creating an HIV vaccine will also benefit from our taking a new approach in our work. For the first time, groups of researchers have assembled in consortia to address these key issues, and funding for these collaborative efforts is coming from the Bill & Melinda Gates Foundation, the International AIDS Vaccine Initiative and the NIH. Working together, these consortia have a stronger chance than ever before of finding the all-important clues that facilitate the discovery of an HIV vaccine.

Far from giving up, HIV vaccine researchers are gearing up for a renewed fight. We could never have imagined in Margaret Heckler’s day how stubbornly this virus would resist traditional vaccine techniques, but we are a stubborn bunch, too, and given time, science will find a way to defend against HIV.