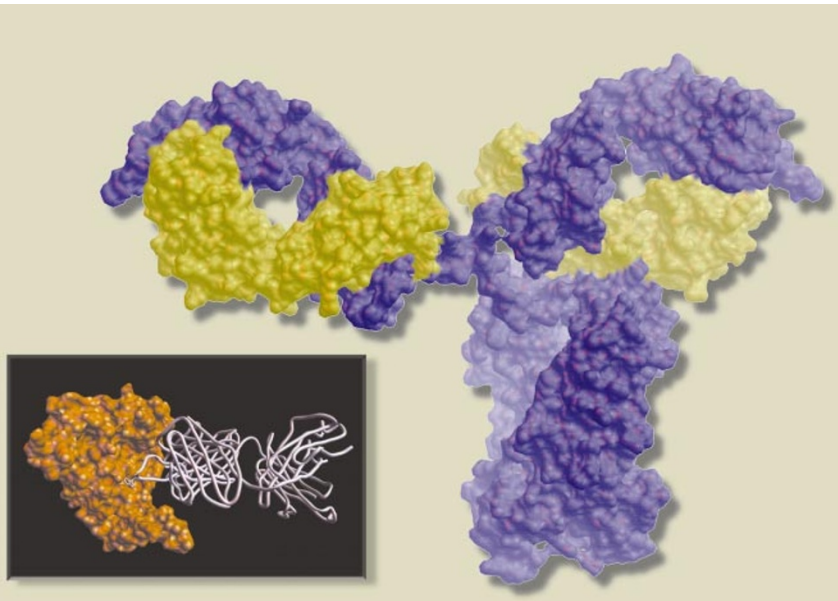
Back to 'plan A'

The received wisdom in AIDS vaccine research is that stimulating cellular immunity is more important than producing antibodies. But some experts are now reviving the antibody strategy, says Erika Check.



E. SAPHIRE, D. BURTON, I. WILSON

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Pointing the way: the antibody b12 can effectively neutralize HIV. The secret to its success is its structure, which features extended 'fingers' (shown in purple, top right and left) that can reach and dock with a recessed pocket in HIV's frequently mutating protein coat (inset, shown in orange).

Few clinical-trial results have had as much riding on them as those announced in February by a small biotech firm called VaxGen. The company, based in Brisbane, California, was the first to push a vaccine against HIV into 'phase III' clinical trials — the final tests that help regulators decide whether a drug should be put on the market. If the vaccine had worked, it would have been a watershed in worldwide efforts to defeat a disease that kills some 3 million people each year.

But when the numbers came in, they told a disappointing story. VaxGen gave its product, called AIDSVAX, to 3,330 high-risk volunteers — mostly homosexual men — from North America and Europe. Of those people, 5.7% contracted HIV, compared with 5.8% of the 1,679 volunteers who did not receive the vaccine. Despite efforts by VaxGen to argue that AIDSVAX performed better in certain ethnic groups, it was clear that the vaccine was a bust.

For many AIDS researchers , the vaccine's failure wasn't too surprising. VaxGen's charismatic co-founder, Don Francis, had raised the money to go ahead with phase III trials after the US National Institute of Allergy and Infectious Diseases (NIAID) decided not to bankroll the project. AIDSVAX consists of a protein called gp120, a major component of HIV's outer coat. It is designed to stimulate the production of antibodies that will prevent the virus from locking onto the immune cells that it infects, called helper T cells. Yet despite promising results from an early study in chimpanzees[1], most of the human data on gp120 were extremely discouraging. In particular, antibodies taken from people immunized with the protein showed little ability in lab tests to prevent HIV from infecting its cellular victims[2].

Given these results, most AIDS vaccine researchers long ago gave up on the idea of stopping HIV in its tracks by stimulating the production of antibodies. Instead, they began to design a second generation of AIDS vaccines that would spur the body to make armies of killer T cells — the foot soldiers of our cellular immune response. Whereas antibodies can in theory lock onto viruses in the bloodstream and prevent them from infecting their target cells, killer T cells search out and destroy those host cells that have been infiltrated by a viral invader.

Many of these second-generation vaccines are now working their way through clinical trials. But for the most part, they show no signs of being able to halt HIV completely. "People are increasingly realizing that they're not going to be able to do it all with cellular immunity," says Dennis Burton, an immunologist at the Scripps Research Institute in La Jolla, California.

> How do you design a vaccine that will elicit neutralizing antibodies instead of ineffectual molecules?

So antibodies are coming back into fashion — but with a twist. Virologists now believe that AIDSVAX and other first-generation vaccines failed because they produced the wrong kinds of antibody. But certain super-antibodies may provide more powerful weapons in the battle against HIV. By marrying traditional immunology with techniques from structural biology, Burton and other researchers hope to cook up a third generation of AIDS vaccines that will selectively elicit the production of these 'neutralizing' antibodies.

Moving target

HIV is good at eluding most antibodies, thanks to a series of subtle tricks. First, the virus is sloppy at copying its genetic material, which causes frequent mutations that subtly change the sequence, and hence the shape, of its proteins. So as soon as the immune system assembles a library of antibodies that can lock onto the virus, the target proteins have changed sufficiently for the antibodies to struggle to maintain their grasp.

What's more, HIV has ways of fooling the immune system into making useless antibodies. For instance, when the virus prepares to enter a host cell, its surface proteins temporarily join together to form more complex structures. But because the immune system usually sees these proteins as singletons, it tends to make antibodies against them individually. The trouble is that

antibodies against the individual proteins cannot stop the virus from successfully infecting helper T cells.

Neutralizing antibodies exploit weaknesses in these viral defence mechanisms. One example is an antibody called b12, which recognizes gp120 and was isolated by Burton's group 11 years ago from a man who had HIV for six years, but had not developed AIDS. When they tested b12 in culture dishes, Burton and his colleagues found that it stopped most of the major strains of HIV from infecting human helper T cells[3].

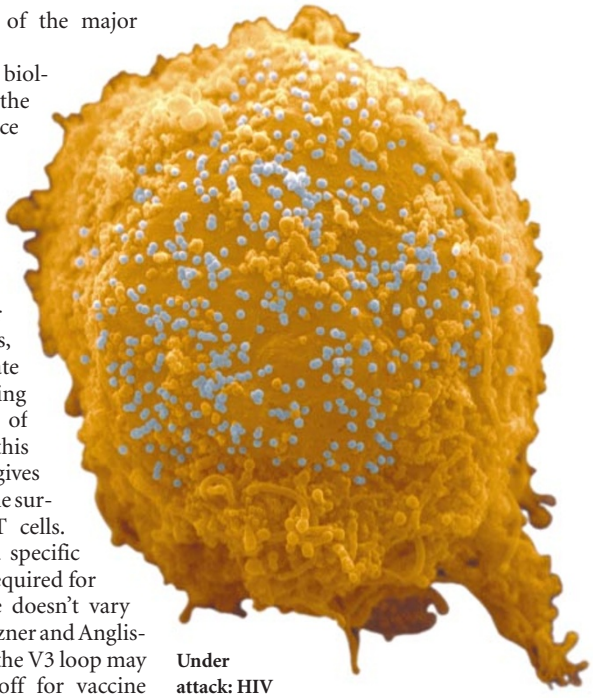
Perfect fit

More recently, Burton and structural biologist Ian Wilson, also at Scripps, have taken a closer look at the structure of b12, and have found out why it is so effective: the antibody has a special finger-like protrusion that fits neatly into a fold in the gp120 protein[4]. This fold can't mutate very much, nor can it really change shape when the protein forms an infectious complex, because it needs to retain its ability to link up with a receptor on the surface of HIV's T-cell victims.

Another potent antibody against HIV — called 447-52D — also exploits the fact that certain parts of HIV's outer coat cannot change too much without compromising the virus's ability to infect cells. Like b12, 447-52D was found in a blood sample from a patient with HIV who had not developed AIDS. It docks to a part of gp120 called the third hypervariable region — or the V3 loop, for short. As its full name suggests, the V3 loop frequently mutates, which would seem to give antibodies against the region little chance of success. Yet 447-52D neutralizes most of the major strains of HIV[5].

Working with structural biologist Jacob Anglister of the Weizmann Institute of Science in Rehovot, Israel, Susan Zolla-Pazner and her colleagues at New York University have revealed the secret of 447-52D's success. The structure of V3 mimics that of messenger proteins called chemokines, which help to regulate immune responses by binding to receptors on the surface of immune cells. Through this subterfuge, the V3 loop gives HIV another handhold on the surface of its target helper T cells. Again, 447-52D docks to a specific part of the V3 loop that is required for this binding, and therefore doesn't vary from virus to virus, Zolla-Pazner and Anglister found[6]. This means that the V3 loop may not, after all, be a write-off for vaccine development. "We've put it back on the map," Zolla-Pazner claims.

In work that has yet to be published, Burton and Wilson have analysed the structure of another broadly neutralizing antibody — called 2G12 — characterized in 1996 after being isolated from a patient by a team led by Hermann Katinger, now at the Austrian Institute of Applied Microbiology in Vienna[7]. At the Keystone symposium on HIV vaccines, held in Banff, Canada, in April, Burton revealed that 2G12 is actually made up of two antibodies joined together in a structure no one had ever seen before — which his group has dubbed a 'domain-exchanged dimer'.



EYE OF SCIENCE/SPL

Under attack: HIV (grey) infects and destroys helper T cells (orange), which are an important component in the body's immune response.

This, says Burton, is an ingenious solution to one of HIV's notorious tricks. The virus cloaks itself in a coat of sugars that antibodies have a hard time distinguishing from those carried by the body's own cells. But the domain-exchanged dimer of 2G12 specifically recognizes the repeating pattern of sugars that is unique to HIV. "It's an ideal molecular solution to recognizing a tight cluster of repeating units," Burton says.

Reverse psychology

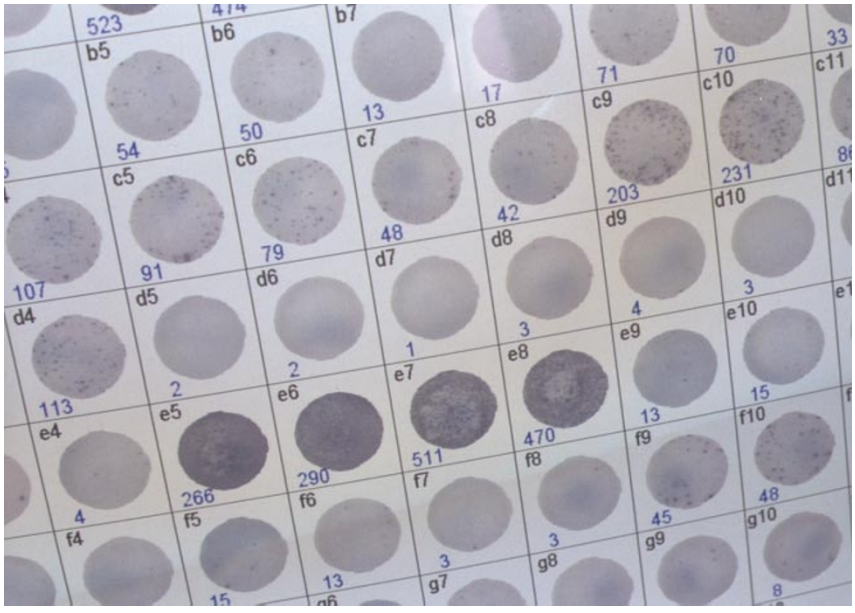
Understanding how neutralizing antibodies defeat HIV is important, but how do you design a vaccine that will specifically elicit their production instead of the usual panoply of ineffectual molecules? Burton calls the process "retrovaccinology", because it is working in the opposite direction to traditional approaches, which start with candidate vaccines and determine what antibodies they stimulate.

Retrovaccinology has never been tried before, and it won't be easy. But Burton is already thinking about how to provoke the selective production of 2G12. "We've dissected this antibody and shown exactly what it recognizes," he says. "So, if we immunize with a precise arrangement of sugars, we might be able to elicit an antibody like this."

Burton and Wilson have also engineered a gp120 protein that they hope will elicit the neutralizing antibody b12. The protein is capped by extra sugars in certain spots[8]. The idea is that these sugars will block the binding sites for the usual array of useless

P. KITTIWONGSAKUL/AFP PHOTO

VaxGen is continuing to test its AIDS vaccine in Thailand, although few expect it to prove successful.



VO TRUNG DUNG/CORBIS SYGMA

Screen test: results showing which antibodies were produced by a patient given a trial AIDS vaccine.

antibodies elicited by the protein, causing it to stimulate the production of b12.

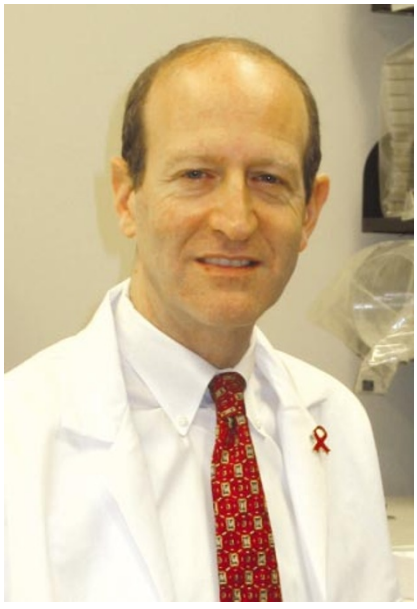
At the NIAID's Vaccine Research Center, which opened three years ago in Bethesda, Maryland, researchers are taking an approach that relies more on brute force. First, they induce mutations in HIV's coat proteins, then they analyse the structures of the mutated proteins to determine whether they are still likely to bind to known neutralizing antibodies. The idea is to produce a version of the proteins that won't induce the production of so many ineffectual antibodies, allowing the body's immune response to a vaccine to be dominated by neutralizing antibodies that could prevent infection. Gary Nabel, director of the Vaccine Research Center, says that this step-by-step approach has already yielded some good leads[9,10]. "But we're not where we need to be yet," he notes.

Defensive formation

Given previous disappointments, few researchers believe that vaccines that induce neutralizing antibodies, by themselves, will provide complete protection against HIV. "To put up a brick wall against incoming viruses, the antibody has got to be there, and for those viruses that get through, you'll need cellular immunity," says Zolla-Pazner.

A bevy of vaccines aimed at stimulating cellular immunity are working their way through clinical trials. These use various methods to deliver genes from HIV into people, either on circular pieces of DNA called plasmids, or in gutted viruses that have been modified so that they don't cause disease. The viral DNA enters human cells, which translate it into proteins and display the proteins on their surfaces. The immune system then sees the proteins and — it is hoped — responds by generating killer T cells primed to recognize and destroy any cells infected with HIV.

A diverse killer T-cell population has a better chance of recognizing the many possible forms of HIV. So a popular strategy for inducing cellular immunity these days is to use combinations of genes and delivery systems. Trials run by researchers at the University of Oxford, UK, working with colleagues in Kenya and Uganda, for example, are testing a vaccine that contains 20 different fragments of HIV genes. And a team based at Emory University in Atlanta, Georgia, has begun a trial in which a 'priming' shot of plasmid DNA will be followed by a 'booster' package of genes delivered in a modified vaccinia Ankara virus (MVA).



All change: Gary Nabel is creating mutant viral proteins in the hope of eliciting super-antibodies.

Pharmaceutical firms and vaccine manufacturers are also getting in on the act. Merck in Whitehouse Station, New Jersey, and Aventis Pasteur in Lyon, France, have joined forces to test a different combination strategy. Volunteers first get a shot of Merck's vaccine, which contains HIV genes in a modified adenovirus. They then get Aventis Pasteur's booster, based on a bird virus that causes canarypox. Meanwhile, Wyeth, based in Madison, New Jersey, has invested in vaccines that deliver the HIV genes in the vesicular stomatitis virus, which causes disease in livestock but is harmless to people. Wyeth is also exploring ways to boost the power of its vaccines by delivering them with immune signalling proteins called cytokines.

Cold war

Immunologists are still trying to assess the quirks of these various approaches. Plasmid DNA stimulates only weak immune responses by itself, for example. And because adenoviruses cause common colds, many people might simply respond to Merck's vaccine by reactivating their previous anti-cold defences, rather than launching a new immune response that incorporates cellular immunity against HIV. MVA could run into the same problem. It was used in Germany to vaccinate against smallpox during the 1970s, and the US government is considering using it in this capacity to respond to potential bioterrorist attacks, because it is safer than the alternative vaccines.

"Every one of the cell-based strategies has a potential downside," concludes Norman Letvin, an immunologist and vaccine researcher at Beth Israel Deaconess Medical Center in Boston. "But we have a much clearer idea than we did five years ago of what we need to accomplish, and any one of the strategies being pursued may help us get over the top."

Ultimately, many vaccine developers believe that the final leg-up may come from some combination of vaccines that stimulates both cellular immunity and the production of neutralizing antibodies. But even the most optimistic aren't bold enough to say that success is a sure bet. "Whether we can really come up with the magic bullet is still unknown," says Nabel. ■

Erika Check is *Nature*'s Washington biomedical correspondent.

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