The most surprising message from a major international AIDS meeting last month is that vaccine research is heating up, although many obstacles remain

AIDS Vaccines Show Promise After Years of Frustration

CHICAGO, ILLINOIS—When 3000 researchers gathered here last month for the largest annual AIDS conference* in the United States, most of the news was depressingly familiar: scant progress on new drugs and soaring infection rates in many parts of the world. But one big surprise at the meeting made few headlines: AIDS vaccine research is hot again, for the first time in years.

In session after session, AIDS researchers reported results from novel vaccine experiments that have worked to various degrees in monkeys. They range from clever new ways to make potent antibodies aimed at preventing HIV from infecting cells to new strategies to crank up the so-called cell-mediated arm of the immune system, which clears cells that HIV manages to infect.

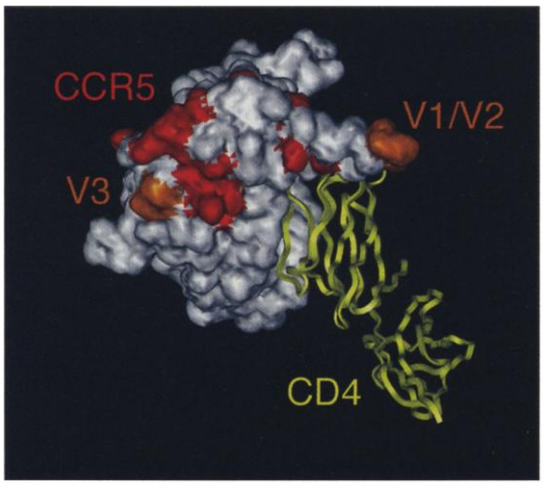
As AIDS researchers are painfully aware, results from animal experiments don't necessarily translate to humans. But this time around, a new element is lifting expectations: a flood of new AIDS vaccine projects sponsored by governments, nonprofits, and even the most elusive player to date, industry.

The U.S. National Institutes of Health (NIH) has revamped its AIDS vaccine program in the past few years and is aggressively helping move products into clinical trials. The International AIDS Vaccine Initiative (IAVI)—a New York City–based nonprofit that recently received a $100 million shot in the arm from the Bill and Melinda Gates Foundation (*Science*, 2 February, p. 809)—plans to announce in the next few weeks new large-scale trials for India and China.

Robert Gallo, director of the Institute of Human Virology in Baltimore, Maryland, is joining an AIDS vaccine effort called the Waterford Project, which will link his institution to leading researchers at Harvard University and the University of California, San Francisco (see sidebar). And the European Commission this week decided to enlarge a similar project, EuroVac, which links various AIDS research groups around that continent.

On the industrial front, although almost every large pharmaceutical company has avoided the field, Merck & Co., headquartered in Whitehouse Station, New Jersey, has quietly built a major AIDS vaccine program and plans next month to unveil a new strategy based on extensive in-house monkey studies. Moreover, *Science* has learned that Merck now owns a large colony of monkeys, which are in short supply (*Science*, 11 February 2000, p. 959), that it is using for AIDS vaccine studies.

All this has heartened investigators from the bench on up. "People are taking the bull by the horns now. ... These approaches will bring the field much further forward in a year than occurred over many years in the past," says Anthony Fauci, head of the NIH's National Institute of Allergy and Infectious Diseases (NIAID), the single largest funder of AIDS vaccine research in the world. David Baltimore, head of NIH's AIDS Vaccine Research Committee, says he, too, is excited: "Clearly, the field is energized."



Achilles' heel. Modifying the V1/V2 region of the AIDS virus's coat protein, gp120, triggers strong antibodies.

CREDIT: ROBERT DOMS

Antibody beautiful

In the 17 years since Gallo's lab proved that HIV causes AIDS, vaccine researchers have been thwarted by the virus's ability to dodge almost any antibody thrown at it. The quest has been so frustrating that many groups have shifted their focus from antibodies to cellular immunity. But antibodies regained center stage at the opening session of the Chicago meeting, when Ronald Desrosiers of the New England Regional Primate Research Center in Southborough, Massachusetts, presented promising new data.

Desrosiers has long prodded the field with provocative experiments that involve deleting genes from SIV, a simian cousin of HIV. These crippled strains, which mimic traditional attenuated vaccines, have created the strongest protection yet seen in monkey experiments. But many researchers, Desrosiers included, worry that a live HIV vaccine, no matter how attenuated, could theoretically pick up deleted genes or revert to virulence on its own. Desrosiers's latest approach might lessen those fears.

Desrosiers is focusing on the surface protein of the AIDS virus, gp120. In the first step of the infection process, gp120 binds to CD4 receptors on white blood cells. That realization led to an obvious vaccine strategy: A shot of gp120 should stimulate production of antibodies that would prevent the protein from binding to CD4, "neutralizing" the virus. But gp120 vaccines have failed to raise potent neutralizing antibodies, prompting NIH in 1994 to abandon the two leading vaccines in human trials, both of which contained genetically engineered gp120. (One is now being tested using private funds.)

Desrosiers and his co-workers reasoned that gp120 may wear what amounts to bullet-proof vests to cover up regions of the molecule that are most vulnerable to antibodies. So they began deleting different portions of the gene that codes for SIV gp120 to expose underlying parts of the protein.

In one experiment, they produced a mutant SIV strain that makes a gp120 with deletions in the molecule's variable regions 1 and 2 (see illustration). When they injected this V1/V2 mutant into four monkeys, the animals all became infected, but their immune systems quickly knocked down the virus (which typically kills monkeys in about 1 year) to extremely low levels. When they re-

* 8th Conference on Retroviruses and Opportunistic Infections, 4–8 February, Chicago, Illinois.

Sublimating Egos for a Common Goal

Robert Gallo has long believed that AIDS vaccine research needs its own Manhattan Project. A few years ago, Gallo, who heads the Institute of Human Virology (IHV) in Baltimore, Maryland, mentioned this idea to one of IHV's board members, John D. Evans, a telecommunications entrepreneur who co-founded the cable TV station C-SPAN.

Evans thought the idea had some merit, especially if different institutions could collaborate through the latest Internet technology to form a "virtual lab." So, in July 1999, he invited Gallo and other leading AIDS scientists to Waterford Farm, his home in Middleburg, Virginia.

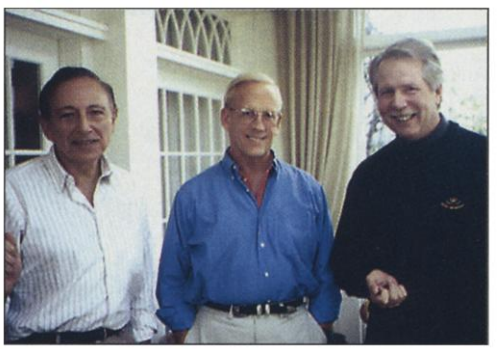
After the meeting, Evans wasn't sure the idea would fly. "Science is the most cut-throat business I've ever seen," he says. "Could we get these people to work together? Could we [get them to] sublimate their egos?" But after months of discussions, a newly configured group met at Evans's farm for 2 days before Thanksgiving last year and agreed to start a unique vaccine effort, dubbed the Waterford Project.

In addition to Gallo and his IHV colleagues, the Waterford Project will include Warner Greene and Tom Coates of the University of California, San Francisco, and Harvard University's Bruce Walker and Max Essex. "There's so much politics and struggle in academia, and it's not going to be easy for anyone to do this alone," says Gallo. "We need more allies and inputs."

With seed money from the John D. Evans Foundation, the Waterford Project hopes to raise at least $140 million over the next 10 years. The project will emphasize research, not development. And it will differ from the National Institutes of Health in the speed with which it can shift focus. "We can turn on a dime," says Evans.

To start with, the Waterford Project will pursue R&D on a baroque vaccine that combines the work of several IHV researchers. The core component will be a gp120 molecule linked to a CD4 receptor. Designed by IHV's Anthony Devico, this molecule theoretically exposes parts of gp120 that stimulate production of powerful antibodies (see main text).

The IHV team, which includes George Lewis and David Hone, will put the gene for this gp120/CD4 construct into a DNA vaccine. The researchers will then pack the DNA into a harmless version of *Salmonella typhi*, which will act as a Trojan horse and deliver the vaccine to the very cells that orchestrate an immune response.



The Waterford Project. Robert Gallo, John Evans, and Warner Greene (*l. to r.*) work on an allied attack.

Further jazzing up the vaccine, they plan to add an inactivated form of HIV's tat protein as a booster. This unusual component is based on findings that tat up-regulates chemokine receptors used by HIV during the infection process; an immune response against tat, they reason, should make it more difficult for HIV to enter cells.

This multilayered scheme is precisely the sort of approach that makes companies and granting agencies run for the hills. But tripping up HIV may require just this type of creativity. And the beauty of the Waterford Project is that the principal investigators can move it forward without having to convince a single outsider that it's worth trying.

–J.C.

moved antibody-producing cells from the monkeys, their SIV levels rapidly skyrocketed, suggesting that antibodies were playing a key role in controlling the infection.

Next, the researchers injected a lethal strain of SIV into two animals that had carried the V1/V2 mutant for nearly 3 years and remained healthy. Neither monkey became infected with the new virus. "These animals were strongly protected—as well protected as we have seen with any live attenuated strains that we have studied," Desrosiers said. He is now working with Larry Arthur and Jeff Lifson at the National Cancer Institute to make a traditional whole, killed vaccine, which has obvious safety advantages over a live attenuated vaccine, with the V1/V2 mutant.

Desrosiers's talk bowled over many researchers. "I was jumping out of my seat," says Susan Barnett, the principal investigator on an AIDS vaccine project at Chiron, a biotech company in Emeryville, California. Barnett and her co-workers are exploring a similar strategy. Working with Leo Stamatatos of the Aaron Diamond AIDS Research Center (ADARC) in New York City, they have tested a vaccine that contains a V2-deleted gp120 in two monkeys. When injected with virulent SIV, the animals contained the infection, while two control animals did not.

CREDIT: HARVARD UNIVERSITY

Mix and match

Most vaccinemakers, Chiron included, are exploring a double-barreled approach: training the immune system both to make neutralizing antibodies and to launch a strong cell-mediated response. This has led to a raft of creative ideas for priming the immune system with one vaccine and boosting it with another.

Cell-mediated immunity kicks into action after HIV infects cells. To boost this response, AIDS researchers create what amounts to a mock HIV infection: They stitch HIV genes into a vector—usually a harmless virus or bacterium—that can infect a cell and then direct it to produce HIV proteins. To the immune system, this chimera looks like an HIV-infected cell.

The first AIDS vaccine tested in humans used vaccinia virus, the smallpox vaccine, as the vector. Several other investigators followed with monkey tests that explored other vectors, using modified vaccinia Ankara, various avian poxviruses, adenovirus, and members of the alphavirus family.

More recently, researchers have tried ferrying HIV genes directly into cells, using such vectors as circular pieces of bacterial DNA called plasmids. But the first human studies of these so-called naked DNA vaccines did not elicit strong cell-mediated immune responses (*Science*, 5 December 1997, p. 1711). Now, a new trick may boost their effectiveness.

Chiron's Barnett reported in Chicago that attaching an HIV DNA vaccine to microparticles of polylactide coglycolide (PLG)—a material used in resorbable surgical sutures—boosted cell-mediated immunity in mice 100-fold with just one shot. Even more impressive, this technique jacked up antibody levels by a factor of 1000. The company hopes to start human trials next year with a PLG-DNA prime and a V2-modified, gp120 boost.

John Rose of Yale University has been working with a weakened form of vesicular stomatitis virus (VSV), which causes disease in farm animals. Many vaccines require booster shots to achieve maximum impact; when repeatedly exposed to the same vector, people can develop immune responses against it, wiping it out before it can deliver its cargo. To get around this problem, Rose has stitched SIV genes into three different strains of VSV, which he delivers to monkeys in succession.

Working with ADARC's Preston Marx, Rose increased the animals' immune responses with each booster shot. When challenged with SIV, seven of seven vaccinated monkeys contained the infection, while four

AIDS VACCINE CLINICAL TRIALS

Sponsors	Vaccine	Location	Stage
VaxGen, U.S. Centers for Disease Control and Prevention	gp120	U.S., Canada, Netherlands	Phase III, interim results possible in November
VaxGen, Thai Ministry of Public Health	gp120	Thailand	Phase III, injecting drug users
Aventis Pasteur, VaxGen, NIH, Medical Research Foundation of Trinidad, National Laboratory of Research in Haiti, Federal University of Rio de Janiero, U.S. military	Canarypox–gag-pol-env, VaxGen gp120 boost	Thailand, Haiti, Trinidad and Tobago, Brazil, U.S.	Three separate phase II trials
IAVI, Oxford University, University of Nairobi	Modified vaccinia Ankara (MVA), DNA boost	U.K. and Kenya	Phase I
Italian NIH, Germany's Research Center for Biotechnology	Recombinant HIV tat protein	Italy	Phase I, fall 2001
AlphaVax, IAVI, NIAID, Johns Hopkins University, University of Natal	Venezuelan equine encephalitis–gag	U.S. and South Africa	Phase I, 2001
Therion, NIAID	Vaccinia-env-pol plus novel adjuvants	U.S.	Phase I, fall 2001
Chiron, NIAID	PLG-DNA, V2-deleted gp120 boost	U.S.	Phase I, 2002
Wyeth-Lederle, NIAID	DNA, peptide boost	U.S.	Phase I, 2002
Merck	DNA-gag, adeno-gag boost	U.S.	Phase I, spring 2001
French National Agency for AIDS Research	Lipopeptide	France	Phase I
NIAID, Protein Sciences	p55	U.S.	Phase I, end of 2001
NIAID, University of New South Wales, Australia	DNA, fowlpox boost	Australia	Phase I, 2002
EuroVax	NYVAC or MVA, gp120 boost	Switzerland and U.K.	Phase I, 2002
ABL, NIAID	DNA-env-tat	U.S.	Phase I, 2002
Institute of Human Virology, IAVI, NIAID	*Salmonella* delivering DNA vaccine	Maryland and Uganda	Phase I, 2002
Emory University, NIAID	DNA multigenes, MVA multigenes boost	U.S.	Phase I, 2002

SOURCE: NIH, WALTER REED ARMY MEDICAL CENTER, UNIVERSITIES, AND COMPANIES

of eight controls quickly developed AIDS. Wyeth-Lederle now hopes to develop this for human tests.

Merck is also trying to boost cell-mediated immunity. Although the company did not present any vaccine data at the conference, several researchers who had heard confidential presentations by the company said Merck has combined a DNA-based approach with an adenovirus vector boost. Merck's Emilio Emini, the lead scientist on the project, says the company plans to present its data at an AIDS vaccine meeting in Keystone, Colorado, next month.

The reappearance of Merck, which all but abandoned its AIDS vaccine program in the early 1990s, has heartened many researchers. "I'm really delighted," says Peggy Johnston, head of NIAID's AIDS vaccine program. "We need to engage the large players more and more."

Clinical concerns

Big money—if not big pharma—will be needed to move candidate vaccines through clinical trials. To date, three dozen vaccines—most manufactured by small biotechs—have made it into human tests, but only two have advanced beyond small-scale, phase I experiments that gauge safety and immune responses. Only one of those has made it into large-scale efficacy trials to determine whether it actually works. Doubting its effectiveness, NIH declined to fund this genetically engineered gp120 vaccine in 1994. Originally made by Genentech of South San Francisco, California, the vaccine is now being tested by VaxGen—a spin-off company formed solely to stage the efficacy trials—in 5400 people (mostly gay men) in the United States, Canada, and the Netherlands.

A second trial involving 2500 injecting drug users is under way in Thailand. Donald Francis, VaxGen's president and co-founder, says a safety and monitoring board will take a first look at the larger trial's results in November and could halt it if the panel finds that the vaccine clearly works (defined as at least 30% efficacy), or is worthless.

Aventis Pasteur, a French-German company, has the second most developed vaccine, which relies on a canarypox vector. It could move into phase III trials next year (see table).

Big pharma has shied away from vaccines both because of scientific uncertainties and because vaccines do not make anywhere near as much money as drugs. IAVI, which has raised a war chest of $335 million from governments and foundations, hopes to overcome this market failure by linking scientists from poor and wealthy countries with biotechs and helping underwrite clinical trials. It has already moved a DNA vaccine into human trials in Oxford and Kenya and is funding four other projects.

In the next few weeks, IAVI hopes to win approval from Chinese officials for a collaboration between researchers there and ADARC director David Ho, and, separately, Hans Wolf of Regensburg University in Germany. IAVI is also negotiating with officials in India to start producing a vaccine for trials there.

NIH is stepping up its support for clinical trials, too, funding companies such as Chiron and Wyeth-Lederle to move vaccines into the clinic and offering contracts to companies to manufacture some vaccines. "The pipeline has grown enormously," says NIAID's Johnston. "Two and a half years ago, there were two products that NIAID was supporting the development of; now there are close to two dozen." NIH's new Vaccine Research Center will also soon have its own in-house capability to make vaccines for small clinical trials.

Finally, the European Commission has linked a far-flung group of researchers called EuroVac to compare HIV vaccines that rely on various weakened vaccinia vectors plus gp120 boosts, as well as a vector called Semliki Forest virus (an alphavirus) with a DNA boost. EuroVac, which now has roughly $11 million, hopes to have its first vaccine ready for human trials next year.

Jaap Goudsmit of the University of Amsterdam, who co-launched EuroVac and also chairs IAVI's scientific advisory committee, recognizes that major problems lie ahead. Still, Goudsmit is encouraged by the number of scientists now reshaping themselves into AIDS vaccine researchers. "There's a lot more energy in the field, and a lot of new people have moved into it," says Goudsmit. "I'm quite optimistic that something smart will come out of this." **–JON COHEN**

Jon Cohen is the author of *Shots in the Dark: The Wayward Search for an AIDS Vaccine.*