# Is NIH Failing an AIDS "Challenge"?

*Some researchers think the government is responsible for the lack of a key reagent—a lack that is holding up trials of candidate AIDS vaccines*

CHIRON CORPORATION HAS AN AIDS VACCINE ready for the ultimate animal test: Chimps would be injected with Chiron's candidate vaccine, then challenged with virus to see whether the vaccine can indeed prevent infection. Virologists call this the chimpanzee challenge; if man's nearest relatives can resist infection, the next step would be human trials. But in spite of the high hopes of Chiron's researchers for their new vaccine, they can't get to the chimp challenge because the Emeryville, California, biotech company lacks a pure strain of the virus in the precisely measured form known as "chimpanzee challenge stock." And so the project is on hold.

Chiron is only one of several AIDS vaccine developers that now need—or will soon need—chimpanzee challenge stock. But various obstacles keep these firms from developing their own stock, and so their hopes rest on the federal government. Some of those hopes are being fulfilled: Officials at the National Institutes of Health say that in the next 6 months the stock Chiron needs should become available. Then again, some researchers think the snags exist in the first place only because of the government. They charge that NIH has failed to synchronize the needs of academia, industry, and government, creating lags that are holding up several AIDS vaccine trials.

If challenge stock is so crucial to the biotech companies, why do they rely on the government? The first—and main—reason is price. Although a company may need only a milliliter of challenge stock for a typical vaccine trial, it isn't practical to make the stock in such small quantities. And making a single large batch costs from $250,000 to $500,000. The reason for that high cost is the chimp shortage. Along with (the even rarer) gibbons, chimps are the only animals other than human beings that can persistently be infected with HIV. But chimps are an endangered species in the wild, and since 1976 it has been illegal to import them to the United States.

Scarcity, of course, drives the price up: Chimps cost a minimum of $15,000 apiece—and that's just for starters. Every U.S. primate center that houses chimps after they've been infected with HIV does so in special safety facilities. To pay for the special care, primate centers stipulate that researchers who infect chimps with HIV must establish an endowment for the animals—an endowment whose going rate is $30,000 per chimp. Add on maintenance charges, handler fees, and researchers' expenses, and it's easy to understand why the thought of making challenge stock can lead a biotech company to see red—red ink, that is.

Dennis Havel/SFBR

**Scarce resource.** *A shortage of chimps has contributed to delays in AIDS vaccine trials.*

## Finding the Right HIV Strain—Not an Easy Task

Making the right challenge stock (the carefully calibrated doses of AIDS virus used to test vaccine efficacy) is an important part of developing an AIDS vaccine. But there are many strains of HIV. How do researchers know which to choose for making challenge stock? The answer is important: It may help to determine how quickly a vaccine becomes available.

The right strain for a challenge experiment depends partly on the type of virus used to formulate the vaccine. For initial challenges, most researchers want the strain in the vaccine and the challenge stock to be the same, since this makes for an easier test of whether the vaccine is working. But choosing the strains for the vaccine itself is also proving to be a problem. Researchers want to use the ones that are the most common in the infected population—because it is those strains, above all, that vaccines must protect against. Remarkably, a decade or more into the AIDS epidemic, researchers don't yet have a full picture of which strains are the most common.

"The whole issue of what's the most important [strain of the] virus is open," says Phillip Berman, a molecular biologist who has been a central figure in the newly revitalized AIDS vaccine effort at Genentech.

The best information to date on the prevalence of HIV strains in the United States comes from a 1989 study by Repligen (a small Massachusetts biotech firm financially and scientifically backed by Merck), working with researchers at Duke University.

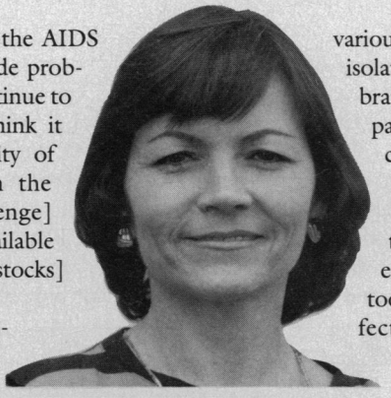
But that study was not a classical "seroprevalence" survey designed to determine the relative frequency of different strains of HIV in the populace. The Repligen/Merck investigators believe that making a successful AIDS vaccine depends largely on being able to evoke one particular element of the full immune response: antibodies capable of neutralizing various HIV strains. Therefore their prevalence study was tightly focused not on the entire AIDS virus

Even if a company like Chiron decides to funnel $250,000 or more into making challenge stock, there is another obstacle—consisting of red tape. There are only 2000 chimps in U.S. biomedical research facilities, less than a third of them available for AIDS work. The total includes some 800 chimps that are owned or leased by the federal government, and experiments on those animals require approval from NIH's Interagency Animal Model Committee, chaired by George Galasso.

Most biotech companies needing chimpanzees have perceivedthe Galasso committee—rightly or wrongly—as a bottleneck and opted to deal with private facilities, which can steer clear of the need for government approval. Yet even when companies turn to the private sector, it doesn't necessarily make their problems disappear. Many private groups are reluctant to use their chimps for challenge stock work. Jorg Eichberg, a veterinarian at the Southwest Foundation for Biomedical Research in San Antonio, Texas, who oversees one of the largest private chimpanzee colonies in the country, says he has turned down several requests to use Southwest's animals for making challenge stock.

Preparing a challenge stock "is scientifically unexciting work and you're really burning up animals," says Eichberg, who has performed more HIV-based chimp challenge experiments than any other researcher in the world. "But it's very important. It's a responsibility on a national basis and the government needs to take care of that."

Patricia Fultz, an associate professor at the University of Alabama at Birmingham who has done several AIDS vaccine challenges in chimps, agrees with Eichberg that the responsibility belongs at the national level. "Because of the nature of the AIDS epidemic and the worldwide problem it presents and will continue to present," says Fultz, "I think it would be the responsibility of government—not just in the U.S.—to generate [challenge] stocks or make funds available [for making new challenge stocks] as quickly as possible."



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—PATRICIA FULTZ

And, indeed, the challenge stock that has been used in almost all trials of AIDS vaccines in the United States has come from the government. But here's the rub: Until now, that stock has been based on only one strain of HIV, known as HIV-IIIB. That strain was among the first to be isolated by National Cancer Institute researcher Robert Gallo, co-discoverer of the AIDS virus, in 1984. Unfortunately, recent "prevalence" surveys indicate that HIV-IIIB is one of the least common strains of the virus present in the U.S. population, making it a less-than-ideal choice for firms like Chiron (see box on facing page).

In 1986, about a year and a half after Gallo first isolated HIV-IIIB, a contract researcher for NCI—Larry Arthur, an employee of Program Resources Inc. at the Frederick Cancer Research Facility—began working with his team to make a challenge stock based on IIIB.

The process, known in the scientific vernacular as "titration," is not particularly difficult. First, Arthur injected four chimps with various dosages of the Gallo isolate, which had been calibrated according to their capacity to infect cells in tissue culture. The three chimps receiving the higher doses became infected; the one receiving the lowest dose did not. So Arthur took two "naive" (never infected) chimps and tried again, infecting them with the two lowest dosages used in the previous experiment. This time both became infected. These results indicated that the lowest dose of the four was sufficient to infect a chimp about half the time.

And that dose—the one that is infective 50% of the time—was the aim of the work; it is known as 1 chimpanzee infective dose50, or $CID_{50}$. (It does not make sense to search for a dose that is infective 100% of the time, because an infinite number of doses above a threshold fulfill that requirement.) Having identified 1 $CID_{50}$, Arthur froze the stock in 500 one-milliliter vials that contained 4000 $CID_{50}$s each. A typical challenge uses between 10 and 100 $CID_{50}$s—so those one-milliliter vials can go a long way.

"The intent was to make the equivalent of a national repository," says the man behind the project, Peter Fischinger, then NCI's associate director and now a vice president at the Medical University of South Carolina. Fischinger's foresight paid off. More than twenty research groups have made use

but on one region of the virus' envelope protein, the part that elicits the neutralizing antibodies, which is known as the principal neutralizing determinant, or V3 loop.

By sequencing 40 amino acids from the V3 region of 245 different HIV isolates, the researchers arrived at a "consensus sequence" made up of the most common amino acid in each of the 40 slots. They then estimated the prevalence of each of the 245 isolates by comparing their sequence with the consensus sequence. The isolate known as MN, isolated in the laboratory of Robert Gallo, was considered "more prevalent" by this criterion than the isolate SF2, found by Jay Levy of the University of California at San Francisco. Both were more prevalent than IIIB—one of the first Gallo isolates—or RF, another strain from the Gallo lab.

Researchers already recognize that the Repligen/Merck data, while very helpful, are not adequate, and Peter Nara of the National Cancer Institute (NCI) plans a more extended seroprevalence study. Nara contends that simply comparing amino-acid sequences overlooks one key aspect of the way a particular strain elicits antibodies: its conformation, or three-dimensional shape.

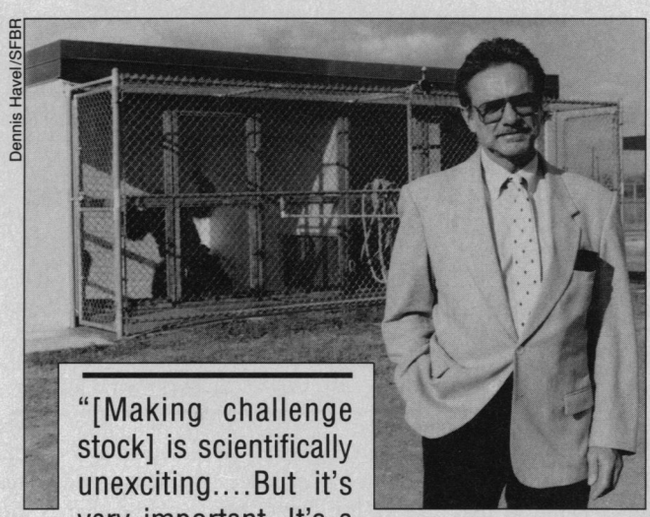
For example, Nara says, it's possible to have two MN isolates, with the same amino-acid sequence in the V3 loop, that have different conformations and therefore will be vulnerable to different neutralizing antibodies. If a vaccine were designed to stimulate neutralizing antibodies against the less prevalent conformation, the vaccine would presumably be less effective.

Teaming up with researchers at the Walter Reed Army Institute of Research and Jaap Goudsmit of the Academic Medical Center in Amsterdam, Nara is going to do a "neutralization analysis" that explicitly takes into account the three-dimensional shape along with amino-acid sequence. The eventual goal is to classify strains into families and sub-families. "It's a functional analysis," says Nara. "That's the only way we'll get a true sense of what's going on."

To Alan Schultz of the NIH Division of AIDS, it's high time for just such an experiment. "What Pete's proposing to do is desperately needed. We've gathered all this sequence data, and we need to get some functional sense of what it all means." That functional sense of different HIV strains could be a vital step in coming up with an AIDS vaccine. ■ **J.C.**

of those warehoused vials.

But the potential usefulness of the IIIB stock to the field of AIDS vaccine research in general wasn't the reason that NCI began by making a IIIB-based challenge stock. The institute was making challenge stock primarily for its own researchers, several of whom were fashioning vaccines from IIIB, which had been isolated in-house. And although the final AIDS vaccine will certainly need to protect against all the many strains of HIV, demanding broad protection from a prototype vaccine would be akin to having required the Wright brothers' biplane to make its first flight at 35,000 feet. Hence vaccinologists typically begin by challenging animals with the same strain of the virus used in the vaccine.



Dennis Havel/SFBR

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NCI officials realized, of course, that in the long run a variety of HIV challenge stocks would be needed. Hence, after the initial IIIB stock had been prepared, they began wrestling with the question of which stock to make next. The first strain other than IIIB chosen was one called HIV-RF, also isolated by Gallo. But before RF could be put into animals, several labs reported that another Gallo isolate—MN—appeared to have infected more people in the United States than RF.

A vaccine based on a common isolate should theoretically protect more people than one based on a rare strain, so attention shifted to MN. NCI spent a few months last year making a clone of MN—a purified form of the virus based on a single viral particle—for use in challenge stock, but the virus didn't cooperate. So NCI decided to make the challenge stock from "wild type" (naturally occurring) MN, which some believed was the better choice anyway because it more accurately reflected the kind of viral challenge a vaccinated person would meet in the real world. Arthur had hoped to begin titrating the wild-type MN in chimpanzees as soon as this month, but unfortunately the wild-type virus has not proved to be as infectious as Arthur would like. He thinks the problem is surmountable, but he doesn't know exactly when the material will go into chimps.

One of those waiting for MN stock is Patricia Fultz. Fultz, who wants to do a demanding heterologous (cross-strain) challenge of a IIIB-based vaccine, says she's happy to see that Arthur is attempting to make MN stock. But, she notes that MN has been known to be a prevalent isolate for a year and a half. "I'm surprised that just now in 1991 they're [planning on] titrating MN," she says. And while Arthur works out the kinks in preparing MN stock, Fultz's vaccine challenge, which she plans to carry out with Marc Girard of the Pasteur Institute, must bide its time.

A heterologous challenge of Genentech's IIIB-based vaccine also is being held up for want of MN stock. And if that stock isn't available in a few months, its absence will begin to impose delays on a vaccine trial that is planned by Repligen and Merck & Co, in collaboration.

One strain that NCI has apparently never considered seriously as the basis for a challenge stock is the one called SF2, isolated in 1984 by Jay Levy at the Medical Center of the University of California at San Francisco. That choice has had frustrating consequences for Chiron—because its vaccine is based on the SF2 strain. The situation is doubly frustrating for Chiron because SF2 was one of the first isolates to be identified, meaning an SF2-based challenge stock could have been developed years ago. Nancy Haigwood, one of the lead researchers on Chiron's AIDS vaccine, expresses some tempered impatience with NCI's decision. "MN is a good choice," she says, "but SF2 is an equally good choice."

Although they are understandably impatient, Chiron researchers probably won't have to wait long for the challenge stock they need. The National Institute of Allergy and Infectious Diseases (NIAID), which helps evaluate AIDS vaccines, is edging into NCI territory by getting into the challenge stock business. On 20 December NIAID received permission from the necessary government committees to begin making SF2 challenge stock. NIAID's Division of AIDS hopes to have the SF2 challenge stock available by June, and Chiron's challenge experiments can then proceed.

Broad availability of one more challenge stock, however, won't lay the problems in the field to rest. One difficulty has been that the choices have never been put before a representative group of researchers in the field, enabling them to form a consensus on which challenge stocks are needed. Several key workers in the vaccine field think that the government should hold an open forum to decide which isolates should be titrated in chimpanzees. On that point Eichberg, former NCI administrator Fischinger, and Fultz all concur. NIH "should gather the experts in the field and get a consensus," says Eichberg, pointing out that in spite of the importance of the subject, no such forum has ever been held.

If a consensus conference on challenge stocks were held, it almost certainly would not settle on a particular strain as the only one to concentrate on. In the long run, chimpanzee stock made from MN, SF2, RF, IIIB, and many other isolates of the AIDS virus may well turn out to be useful to researchers. As AIDS vaccine research progresses, it is likely that heterologous challenges will become commonplace, and the more isolates a candidate vaccine protects against, the better its chances of one day sitting on a pharmacist's shelf.

Even that variety, however, isn't likely to be the final solution. A heterologous challenge stock of the type being prepared today may not be the most relevant test of an AIDS vaccine. Because sexual contact leads to transmission of the AIDS virus in cells as well as free virus in the seminal fluid or blood, a "real world" vaccine challenge must also include cell-associated virus particles. The current stock of IIIB—and NIH's planned MN and SF2—all include virus outside of cells only. Fultz says she and Girard hope to take the next step and titrate cell-associated MN stock in chimpanzees during the next year.

That project remains in the future. For the moment, researchers must grapple with all the complexities that surround the question of which stock to make and which stock to use right now. In that respect Larry Arthur compares chimpanzee challenge stock to computer software. "You know software's going to get better," Arthur says, "but you buy it because you need it now."

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