AIDS VACCINES

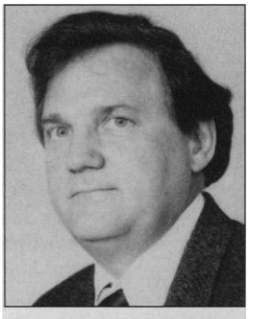
Errant HIV Strain Renders Test Virus Stock Useless

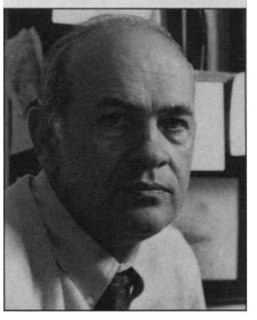
A federally funded AIDS vaccine development program has suffered an embarrassing and potentially costly setback that will add months to the time it will take to test candidate vaccines in chimpanzees. The problem could also delay the start of human trials slated to begin at the end of next year. A crucial stock of a single, well-characterized strain of HIV that researchers around the world planned to use as a standard in vaccine testing has been contaminated with another strain of HIV and is now useless. But, like many research "mistakes," this episode may have a hidden benefit, pointing the way to creating better test stocks in the future.

The contaminated stock, which had taken more than a year to develop, was to have been used to "challenge" chimpanzees inoculated with test vaccines to see whether the vaccines prevent infection. The new stock, based on an HIV strain known as MN, was being grown up as a replacement for a widely used strain called IIIB. Several researchers have already reported success in protecting immunized chimps from challenge with IIIB, but that strain is rare among people infected with HIV in the United States and vaccine researchers have wanted a challenge stock that was more representative of the virus infecting Americans (*Science*, 1 February 1991, p. 518). MN was supposed to fit the bill.

The task of making a challenge stock based on MN went to Larry Arthur, a virologist who works for Program Resources Inc., a contractor for the National Cancer Institute (NCI)-Frederick Cancer Research and Development Center in Frederick, Maryland. Arthur had successfully made a IIIB challenge stock that had been widely distributed for vaccine research and other basic work on the AIDS virus. From the start, however, Arthur ran into trouble with MN—it just did not infect chimp cells as readily as IIIB did, and it took him a long time to find a batch of MN that was infectious enough to infect chimp blood cells reliably. Finally, after 6 months of effort, Arthur, working with Dani Bolognesi and Al Langlois at Duke University in Durham, North Carolina, identified a good candidate. Using an immortalized human T cell line called H9, the collaborators grew sufficient quantities of MN virus to begin animal tests. On 22 October last year the NCI-Frederick Cancer Research and Development Center team injected three HIV-free, unvaccinated chimps with the MN stock. Each animal received a different dilution of the virus in order to get a rough idea of just how infectious the stock was.

But by February of this year, it was clear that something was amiss. "It's important to verify that the virus coming out [of the chimp] is the same as the one going in," says Bolognesi, so the Duke team conducted a series of experiments to characterize the virus that had taken hold in the three chimps. Duke molecular biologist Laurence Rimsky-Clarke was the first to identify the problem. Using the polymerase chain reaction to amplify the viral genetic sequences from infected cells, Rimsky-Clarke found that there was no trace of MN in the chimps: The only virus the researchers could recover was one that looked suspiciously like IIIB.





Taking stock. Larry Arthur *(top)* and Dani Bolognesi.

IIIB, or not IIIB. Arthur immediately notified researchers who had requested the MN challenge stock that there was a problem, and he and the Duke team began trying to figure out what had gone wrong. They now think they have the answer. At the time that the MN stock was being prepared at Duke, other researchers working in the same lab were doing studies with a variant of the IIIB virus that had been isolated from a chimpanzee previously infected with the IIIB challenge stock. Although all concerned insist the MN stock was tested for purity before it was used to infect those three chimps last October, Bolognesi speculates that a nearly undetectable amount of this chimp-adapted IIIB virus must have contaminated the MN stock. Arthur says that they have now gone back and analyzed the MN stock used to infect the chimps and found that it does appear to contain 1.0% of the IIIB strain the Duke researchers were studying.

How could such a tiny amount of IIIB in the challenge stock overwhelm the MN after it was injected into the chimps? The answer to that, says Bolognesi, may be the silver lining to the dark cloud the contamination has cast over vaccine research. Clearly the IIIB variant, which had been growing for some time in the infected chimp before it contaminated the MN stock, is better adapted to growing in chimpanzees. If researchers can find why, they may gain important clues about what makes an HIV strain particularly infectious in a live animal. Moreover, the contamination has suggested a better way to create a challenge stock: First find a variant of MN that will infect chimp white blood cells, next infect a chimp with that strain of the virus, let it grow for a while, and then use the chimp-adapted virus to create a standardized challenge for future animal vaccination studies.

Although nearly every virology lab has suffered a contamination at one time or another, there is an acute irony in this case: The IIIB strain itself is a notorious contaminant. IIIB is the strain on which National Cancer Institute researcher Robert Gallo based his 1985 patent for an AIDS blood test. It is virtually identical to a virus dubbed LAV that was first isolated by researchers at the Pasteur Institute in Paris and sent to Gallo's lab in 1983. Last year Gallo conceded that his IIIB cultures must have been contaminated by LAV. Soon after that, Pasteur researchers discovered that LAV is in fact another virus called LAI (which they isolated around the same time as LAV) that had apparently contaminated their LAV cultures.

For vaccine researchers, the delays caused by the contamination of the MN challenge stock are already causing problems. National Institutes of Health officials were originally expecting that the stock would be available by last summer, but when that proved impossible, the goal was pushed back to the start of this year. The current snafu means that it will be several more months before a new stock will be ready.

"If we had [the MN stock], we'd be using it," says vaccine developer Phillip Berman of Genentech Inc. of South San Francisco. Genentech has already shown that their vaccine, based on the genetically engineered form of the HIV envelope protein, can protect animals from IIIB challenge. Now the company wants to try a different virus to see how broadly effective its vaccine is. On the other side of San Francisco Bay, Chiron Corp. in Emeryville is in a similar position. Chiron researchers have been hoping to use a different challenge stock based on a viral isolate called SF2. "But we don't know if SF2 is going to be infectious enough," says Kathelyn Steimer, who heads Chiron's vaccine development project. And since SF2 belongs to the more generic family of MN-like viruses, she too is anxiously awaiting the MN challenge stock.

Liability fears. But Alan Schultz, the acting chief of the vaccine research and development branch at the National Institute of Allergy and Infectious Diseases (NIAID), is less troubled by the delay in producing the MN

stock. "I don't see it as an overwhelming problem," he says, "but it may become one if companies withhold their products because they don't have a chimpanzee protection trial." Schultz argues that results from a chimpanzee protection trial will be just one of the factors federal health officials will consider when choosing which candidate vaccines to use in human efficacy trials. NIAID does not insist that companies interested in participating in future human trials conduct chimp challenge experiments, but Schultz admits that since such trials have traditionally been required in vaccine development, companies may feel easier about liability issues if they have done them.

Schultz says it is not clear at this point whether NIAID will be forced to delay the human efficacy trials it was hoping would kick off by the end of next year. Laboratory results, trials in other animal models, and experience from phase I trials already under way in humans will all go into deciding when to start efficacy trials, and which vaccine to use. For now, Schultz says, the idea is to prepare for all eventualities: "We want to have the [vaccine] syringe ready to go, and then see if we're going to fill it." Certainly the delay in creating the MN challenge stock is not going to make that decision any easier.

–Joseph Palca

PHYSICS FACILITIES

NASA Researchers Protest DOE Turnoff

The Bush Administration has long championed an ambitious manned space program, including such projects as a lunar base and a manned mission to Mars by 2029. But researchers at the National Aeronautics and Space Administration (NASA) are now complaining that the Administration appears to be undercutting its commitment to multibillion dollar long-term space voyages—and some less splashy space research as well—in order to save a few million dollars in an apparently unrelated area: the nuclear physics program at the Department of Energy (DOE).

For more than 20 years, NASA researchers have conducted a series of important experiments on the Bevalac, an aging heavy ion accelerator that DOE supports at the Lawrence Berkeley Laboratory (LBL). Concerned by predictions of flat or declining research budgets over the next 5 years, however, DOE announced earlier this year that it will close the Bevalac by mid-1993. The move has prompted strong opposition from NASA scientists and officials, who warn that many agency programs, including one that calibrates the instruments for space probes, may grind to a halt without the Bevalac. Negotiations between NASA and DOE have so far failed to produce a plan either for keeping the Bevalac open or building a replacement, suggesting that without congressional intervention, U.S. scientists soon may have to do without the Bevalac's capabilities altogether.

While about three-quarters of the accelerator's operating time is devoted to basic nuclear physics research and cancer radiotherapy, NASA has a special interest in the facility because it is the only accelerator in the country able to simulate the galactic cosmic radiation background with energetic beams of heavy ions such as iron and uranium. Using the Bevalac, NASA life scientists have begun to explore the biological effects of heavy ion cosmic radiation, work of particular importance to planners of long-term manned space missions. "Our whole radiation program is dependent on high-energy ion beams at the Bevalac," says Walter Schimmerling, senior scientist for NASA's space radiation health program. "There's just no way to do our program without a U.S. capability in this area."

More than NASA's life science program is threatened by a Bevalac shutdown. Since the late 1960s, NASA has used the facility to calibrate the particle detectors aboard probes flown in space and aboard high-altitude balloons. "It's safe to say that every satellite program has been calibrated here," says Jose Alonzo, a deputy director at LBL with responsibility for Bevalac operations. "We are the only cosmic ray factory on this side of the Atlantic." Vernon Jones, NASA's chief scientist for cosmic and heliospheric physics, notes that while some facilities in Japan, Europe, and Russia might be able to carry on some of the same work now done at the Bevalac, all would require some upgrading—and all are currently oversubscribed. "[The Bevalac] is the only facility in existence that can provide the beams we now need," he says.

LAWRENCE BERKELEY LABORATORY

Under the shadow of the ax. Berkeley's Bevalac.

DOE has long had the Bevalac on the chopping block, but it only recently moved up the facility's execution to 1993. Last summer, DOE's Nuclear Science Advisory Committee recommended closing the accelerator by 1995 in order to keep a newer facility—the Relativistic Heavy Ion Collider (RHIC) at Brookhaven National Laboratory—on schedule for a 1997 completion. According to Schimmerling, that original timetable might have allowed NASA time to figure out some way to come up with at least a portion of the Bevalac's $18 million annual operating budget. Just last fall, however, another advisory panel on research priorities recommended shutting the facility in mid-1993, and DOE agreed. Negotiations have been under way since then, but no one seems optimistic about their progress. One reason for pessimism is a 5 May letter from DOE nuclear physics program director David Hendrie to a NASA official effectively stating that the Bevalac will be closed to outside users beginning this October.

As a result, Congress appears to be the researchers' only hope to keep the facility going in the short term. The House recently passed a bill authorizing NASA to spend $3 million on Bevalac operations in the next fiscal year, essentially as a stopgap aimed at keeping the program going until DOE can fully fund the facility. But the real decision for this year rests with the congressional appropriations committees, which are under the same fiscal pressures DOE now faces. "There are a lot of things the committee would like to do, but the availability of money is a real problem," says one House appropriations staffer.

As a long-term solution, researchers have begun planning an extension to an existing Brookhaven accelerator that will allow them to tap an ion beam equivalent to that of the Bevalac by late 1995 or early 1996. But DOE has given no indication that it would be willing to pay the estimated $30 million such a facility would cost. In the meantime, "We're sitting on the sidelines wringing our hands, waiting for the giants in Washington to settle our fate," says Alonzo.

–David P. Hamilton