AIDS Vaccines: Is Older Better?

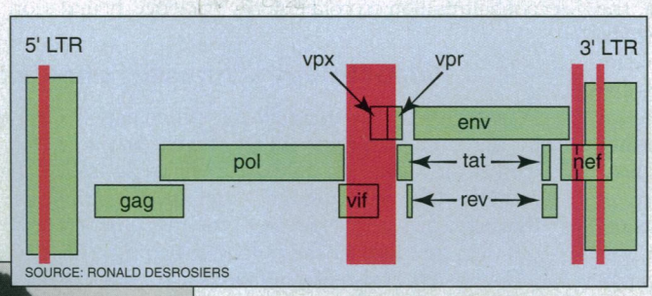
Researchers once wrote off vaccines based on live, weakened virus as far too dangerous, but recent data are causing some to rethink the old-fashioned approach

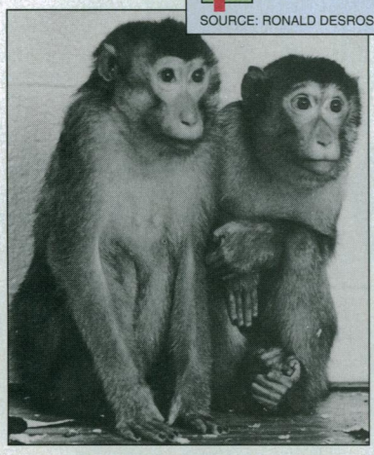
Scores of academic, government, and corporate labs around the world have entered the race to find an effective vaccine to prevent AIDS. But most are backing the same horse: genetically engineered pieces of the AIDS virus that they hope will be enough to trigger a protective immune response. In putting their bets on these engineered proteins, researchers have avoided the method used to develop human vaccines against most other viral diseases—a weakened version of the entire virus. AIDS vaccine developers recognize the power of attenuated virus vaccines, but they fear that even a weakened version of the stealthy and cunning HIV could lead to fatal infection.

A couple of recent developments have given researchers second thoughts, however, and some are now wondering whether they should hedge their bets. The first development is that the new-fangled, high-tech approach isn't working well: Human and animal experiments have yielded precious little data suggesting that vaccines made of HIV pieces will provide protection against the virus itself. The second is that an experiment involving attenuated virus has produced some startling results. On page 1938 of this issue of *Science* Ronald Desrosiers and his colleagues at Harvard's New England Regional Primate Research Center report the longest-lasting, strongest protection yet achieved in any AIDS vaccine experiment—using precisely that old-fashioned method.

More than 2 years after vaccinating four rhesus monkeys once with a weakened form of SIV (a close simian relative of HIV), Desrosiers "challenged" them with a low-dose injection of full-strength virus—and all the monkeys resisted infection. What is more, the monkeys later resisted another challenge with a much higher dose of SIV. "This is a significant advance," says Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases (NIAID). Alan Schultz, chief of the vaccine branch at NIAID's Division of AIDS, adds that "this is three orders of magnitude better than any protection we've seen." And Duke University's Dani Bolognesi calls the Desrosiers data "head and shoulders above everything else."

Desrosiers' findings don't mean that the AIDS vaccine community is about to switch horses and make attenuated HIV vaccines. Plenty of safety concerns remain, and it could turn out that the live, attenuated approach does no more than provide cues for a safer vaccine based on engineered proteins. But in a crucial field, where the outlook has been gloomy, Desrosiers' results could provide hope, especially if his vaccine works in more rigorous trials.





Delete key. Deleting part of the genome of viruses that cause AIDS *(deletions in red above)* forms the basis of a vaccine strategy that has protected monkeys from infection.

Like all vaccines, one made from an attenuated live virus is designed to fool the immune system into reacting as if it were meeting the actual, disease-causing organism. If the mock struggle works, the immune system will be primed to defeat the real enemy. What distinguishes the live viral vaccine from other approaches is that rather than simply presenting viral components, it actually causes infection. The virus replicates in the body, repeatedly providing a range of different proteins for the immune system to attack.

In contrast, the genetically engineered AIDS vaccines under commercial development typically consist of only a single HIV surface protein—most companies are focusing on a protein called gp120 or one called gp160. When mixed with enhancing chemicals, these single proteins can, in theory, prime the immune system without causing infection. To date, however, only one monkey experiment has had solid success with a recombinant protein SIV vaccine (*Science*, 24 January, p. 456), although a handful of vaccines containing recombinant HIV proteins have shown promise in chimpanzee tests.

Desrosiers himself tried—and failed—with the recombinant protein approach. He also had only marginal success with the so-called whole, killed virus method, in which a genetically inactivated version of the entire virus is used. Aside from the fact that the results from whole, killed SIV vaccine experiments have been difficult to sort out (see sidebar), many researchers dismiss this approach, too, for safety reasons, noting that if any genetic material remains intact, the vaccine could cause AIDS.

Desrosiers stumbled onto the live, attenuated approach while studying the mechanism by which the AIDS virus wreaks its damage. In that work, he had constructed a clone of a highly virulent SIV strain that did not include a viral gene called *nef*. Three years ago, he gave six rhesus monkeys an injection of this *nef*-deleted SIV to see how the deletion affected the course of the disease and simultaneously gave another group unaltered SIV. While the monkeys that got the natural virus began dying of AIDS, the six who received the *nef*-deleted strain remained healthy.

Apparently, the *nef* deletion had somehow rendered the virus harmless. If that was the case, Desrosiers wondered, could he have inadvertently created a vaccine? To find out, he challenged four of the "vaccinated" monkeys and four control animals with a small dose of infectious SIV. Within 36 weeks, the four controls were dead or sick, whereas the four primed with the *nef*-mutant strain remained healthy. The strain of virus used for the challenge couldn't be detected in the monkeys' blood, even with ultrasensitive tests, indicating that they had managed to resist infection.

Desrosiers had shown that the inoculated animals could resist a relatively low dose of SIV, but could they fight off a serious challenge? The answer, so far, appears to be yes. Desrosiers injected the monkeys with a huge dose of infectious SIV, and after 18 weeks these animals show no evidence of infection.

Virologist Muthiah Daniel, first author of

the *Science* paper, cautions that "we're many years away before this can be used in humans," but Daniel says researchers should think seriously about this approach—because there isn't much choice. "The recombinant vaccines," he predicts, "are not going to work." Murray Gardner, a prominent monkey researcher at the University of California, Davis, who calls the new data "striking," is also pessimistic about vaccines made from one genetically engineered viral protein. "To think that a simple, recombinant protein can do the job is foolish," he says, because single proteins can't induce the full immune response needed to fight off a virus. Gardner has also been swayed by encouraging results obtained by his colleague Marta Marthas, using her own attenuated SIV vaccine.

The companies pursuing recombinant or chemically synthesized candidate vaccines (including Chiron, Genentech, MicroGeneSys, Immuno, Viral Technologies, and Pasteur Mérieux) obviously disagree with Gardner and Daniel. Yet the companies do stand to gain from the attenuated experiments, because they could provide crucial indications of how the immune system can prevent HIV infection. In that regard, as Duke's Bolognesi says, "so far, we've been flying without a compass."

Phillip Berman of Genentech hopes to apply lessons learned from the attenuated vaccine. But Berman cautions that SIV is different from HIV and that monkey data may not apply to humans. "SIV grows fast and intense in rhesus, where HIV is slow and smoldering in man," says Berman. And he also thinks there's strong evidence that a specific portion of gp120 called the V3 loop can trigger production of antibodies that can stop HIV. "The data's very good that you can protect chimpanzees from infection with antibodies to the V3 loop," he says.

Desrosiers says he hopes a recombinant vaccine or some other obviously safe method will prove effective. But he says: "Our backs are getting to the wall in a dramatic and dangerous situation. We need to be ready to accept some radical approaches." Before the live, attenuated approach could be seriously considered for use in humans, however, at least three safety concerns would have to be dealt with. First, the AIDS virus in an attenuated vaccine could revert to a virulent state. Second, even if it didn't cause AIDS, the vaccine might cause cancer. And a virus that initially looks safe might, decades later, turn out to cause disease.

Desrosiers has thought about all three concerns. Because the virus requires certain genetic elements to cause disease, he says, he is confident that if you remove enough of the viral genome, it would be practically impossible for an attenuated virus to revert to virulence; he is now testing mutants with up to five genetic deletions. As for a vaccine causing cancer, Robert Gallo of the National Cancer Institute argues that HIV is implicated in B cell lymphomas and Kaposi's sarcoma. But Desrosiers counters that the causal link isn't clear, and that, in any case, these cancers seem to be dependent on HIV replicating, and his attenuated virus doesn't replicate much.

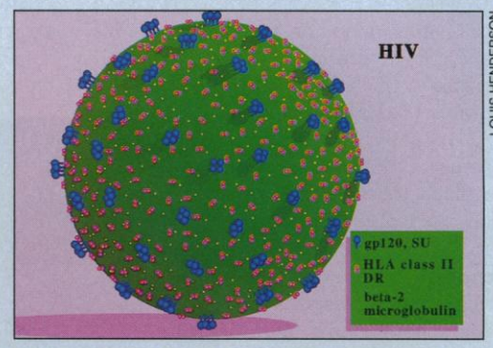
Long-term safety concerns, however, are worrisome, concedes Desrosiers. "It will take 10 or 15 years of safety testing before we can be comfortable putting this into thousands of people." Which is why he thinks the process ought to begin soon. He's about to begin chimp trials of triple and quadruple deletion mutants. If those preparations prove safe and give hints of efficacy, he believes tests should begin in a small number of human volunteers at high risk of HIV infection.

That's not a thrilling prospect, all involved concede. Yet some researchers believe it's time to rethink things. Patricia Fultz of the University of Alabama, Birmingham, who has tested AIDS vaccines in chimps and monkeys, says that 5 years ago she ruled out live vaccines. Now, she says, "we may be forced to use this as the only method that appears to have an impact." Which highlights the fact that, as in all AIDS research, the choices are between rocks and hard places.

–Jon Cohen

Explaining Puzzling Vaccine Results

A letter published in *Nature* last year by James Stott of England's National Institute for Biological Standards and Control didn't fill even one page—but it stood the AIDS vaccine world on its head. Stott described how his group had, as a control to a monkey vaccine experiment, injected four animals with human white blood cells. When the animals were "challenged" with virulent SIV (a monkey version of the AIDS virus) two failed to become infected, suggesting that something in the blood cells protected the monkeys. That worried researchers because human white blood cells are used to culture SIV and they have been used in many monkey vaccine trials—possibly skewing the results. In this issue of *Science* (page 1935), a team led by Larry Arthur of Program Resources Inc., a contractor with the National Cancer Institute, helps explain Stott's findings. But the new results also show just how wily the AIDS virus is in evading the immune system.



Studley. HIV studded with immune proteins.

The vaccine that has worked best in monkey experiments to date has been made from SIV whose genetic material is chemically or physically inactivated. Though researchers have serious safety concerns about this method (if any virus accidentally was not inactivated, the vaccine could cause AIDS), the failure-riddled AIDS vaccine field was heartened when this "whole, killed" virus approach began succeeding. Then Stott's letter threw into question the validity of every experiment involving whole, killed SIV vaccines. Were the immune responses stimulated by viral proteins in the vaccine or by an artifact?

Stott and his co-workers quickly came up with a theory. Like all retroviruses, SIV grows in cells and buds through their membranes, picking up cellular proteins in the process. Stott's study suggested that antibodies made to these cellular proteins—not to the SIV proteins—were the key to protection. Now Arthur spells out just what those cellular proteins are and how they could have provided the protection.

Arthur took purified HIV and SIV and separated out the cellular proteins, which include immune-system molecules called β2 microglobulin (β2m) and HLA DR. Strikingly, they found more β2m and HLA DR than HIV's own surface protein, gp120. Arthur also showed that concoctions of concentrated antibodies to β2m and HLA DR can block HIV and SIV infection in the test tube, supporting Stott's hypothesis. Arthur now intends to test an SIV vaccine made from cellular proteins alone.

Michael Murphey-Corb of the Tulane Regional Primate Research Center says that while cellular proteins explain some of the protection seen with whole, killed SIV vaccines, researchers should not write off the approach. "This whole inactivated vaccine worked too well not to be real," says Murphey-Corb, who is investigating other explanations for the Stott experiment.

–J.C.