HIV/AIDS VACCINES

Monkey Puzzles

As a growing number of vaccines move through the pipeline toward clinical trials, experiments with monkeys are producing puzzling data—and doubts

The first full-scale trial of an AIDS vaccine is scheduled to end in November, and the world soon will learn whether it works. A second product will move into a large efficacy trial this fall. Earlier in the pipeline, the array of AIDS vaccines entering human studies is more diverse than ever before (see table, p. 2326). "The pipeline is dramatically improved in many ways from 5 to 6 years ago," says Peggy Johnston, who heads the AIDS vaccine program at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland. But, Johnston cautions, "significant scientific and operational challenges remain."

Foremost among those scientific challenges: Just what does it take for a vaccine to work? Almost 2 decades after HIV was identified as the cause of AIDS, researchers are still debating which immune responses are likely to provide the best protection against the virus. The answer is proving elusive in part because experiments with monkeys are coming up with puzzling, even contradictory, data. "We're so much in the dark about what we would need for a protective vaccine," laments Ronald Desrosiers, head of Harvard University's New England Regional Primate Research Center in Southborough, Massachusetts.

Monkeys are the favored model for testing vaccine strategies. (Researchers no longer use chimps for ethical and cost reasons.) Although HIV does not infect monkeys, a cousin simian virus, SIV, does: Some two dozen African species of monkeys are now known to harbor SIV in the wild, and it causes them no harm. But when SIV infects Asian monkeys, it causes an AIDS-like disease. In the most common type of experiment, researchers vaccinate Asian rhesus macaques and then "challenge" them with either SIV or a laboratory-made hybrid of the simian and human viruses, called SHIV.

Although some researchers question whether the monkey model truly mimics HIV in humans, the field at large has embraced it as the best way to determine which vaccine strategies hold the most promise. But a slew of recent monkey experiments has raised questions about most of the vaccine approaches now being pursued.

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Half a loaf

When HIV was first discovered, the way to a vaccine seemed clear: Find a part of the virus that triggers an antibody response capable of "neutralizing" HIV before it can establish an infection. The AIDS vaccine that has moved furthest in human trials—a genetically engineered version of HIV's surface protein made by VaxGen of Brisbane, California—banks on this concept.

But monkey studies with AIDS vaccines have completely failed to elicit antibodies that can neutralize the virus. "I think the Holy Grail in the field of AIDS vaccine development is how to generate a broad, neutralizing antibody response," says Norman Letvin, a primate researcher based at Harvard's Beth Israel Deaconess Medical Center in Boston, "but we don't know how to do it."

As a result, many have shifted their attention to the arm of the immune system that dispatches killer cells, tiny missiles that seek infected cells and obliterate them. Because killer cells, by definition, can do their thing only if an infection has already occurred, the goal now is not prevention of infection but of disease.

Immune conundrum. Sooty mangabeys have high viral loads of SIV, yet the virus does not make them sick.

Some monkey experiments have given heart to those taking this approach. Researchers at Merck Research Laboratories in West Point, Pennsylvania, working with Letvin, reported in the 17 January issue of *Nature* that two AIDS vaccines they used back to back in monkeys constrained SHIV and protected the animals from disease by triggering killer cell responses. The Merck data have buoyed spirits among vaccine researchers, and the vaccines now are in early human trials. But other monkey studies have raised doubts.

In the same issue of *Nature*, Letvin, Dan Barouch of Massachusetts General Hospital in Boston, and co-workers reported that similar vaccines initially protected eight monkeys against the identical SHIV strain Merck used, whereas unvaccinated control monkeys had high levels of virus and subsequent disease. But 6 months later, one vaccinated monkey no longer could control the virus, and by 1 year it had developed AIDS-like symptoms and died. The researchers discovered that the SHIV had mutated its way around the killer-cell response. It's an ominous development, Letvin thinks, because it threatens in time to undermine every vaccine that relies on killer cells alone.

Then again, Letvin points out, seven of the vaccinated animals are still controlling their SHIV infections, and even the animal that died fared better than the unvaccinated controls. "I'm willing to take half a loaf if that's all we have," says Letvin.

Experiments conducted by immunologist David Watkins of the Wisconsin Primate Research Center in Madison suggest that even half a loaf might be optimistic, however. Like the Merck team, Watkins and his colleagues used two vaccines back to back that triggered strong killer cell responses. Yet, as Watkins's team reported in the April issue of the *Journal of Virology*, when they challenged the animals with a famously nasty strain of SIV rather than SHIV, the virus was blunted for a time but ultimately ran wild. A growing number of researchers contend that this more vigorous challenge accurately reflects the way that HIV behaves in humans. "Watkins gets better cellular immunity than I've seen before, better than we can hope to get in humans, and it had a modest effect," stresses Jeffrey Lifson, a virologist at SAIC-Frederick, a company that runs the National Cancer Institute's AIDS Vaccine Program in Frederick, Maryland. "I'm very depressed by these studies," says Watkins. Killer cells by themselves, he suggests, "are not going to be protective."

Triple surprise

African monkeys' impressive ability to withstand infection by SIV might provide some answers to how the immune system can keep the virus at bay. A few recent discoveries, however, have only made the puzzle seem more complex.

In 1998, Lisa Chakrabarti, then at the Pasteur Institute, and her colleagues surprised many investigators when they report-

AIDS VACCINE PIPELINE

Vaccine	Developers	Location/Comment
Efficacy trials:		
Recombinant gp120	VaxGen	Thailand, North America, Europe/ Results expected in 2003
Canarypox (multigenes)/rgp120	Aventis Pasteur and VaxGen	Thailand/Late 2002 launch, U.S. and Thai govt. collaboration
Safety and Immunogenicity trials:		
Canarypox (multigenes)/rgp120	Aventis Pasteur and VaxGen	Caribbean, South America/Similar to Thai study but smaller
Lipopeptides	ANRS	France/Better as boost than prime?
DNA/modified vaccinia Ankara (MVA) (gag + CTL epitopes)	Oxford and Nairobi U., International AIDS Vaccine Initiative (IAVI)	U.K., Kenya/IAVI's most developed project
DNA/adenovirus (gag)	Merck & Co. Inc.	U.S./Worked well in monkeys, international trial sites soon?
Nef-tat fusion protein +/– rgp120	GlaxoSmithKline	U.S./NIAID-sponsored
DNA (gag/pol)	Vaccine Research Center (VRC), NIAID	U.S./VRC's first effort
Preclinical testing:		
DNA/adeno (multigenes)	VRC, NIAID	U.S./Made from multiclades
DNA-IL2/IG (multigenes)	VRC, NIAID	U.S./Builds off monkey study
Adenovirus (multigenes)	National Cancer Institute	U.S./Differs from other adeno vaccines because it replicates
DNA/fowlpox (multigenes) +/– cytokines	U. New South Wales, Australian National U., Virax	Australia/Contains "immunity-enhancing" genes. NIAID-sponsored
MVA/fowlpox (multigenes)	Therion	U.S./NIAID-sponsored
DNA/MVA (multigenes)	Emory U., NIAID	U.S./Worked well in monkeys
DNA/MVA (multigenes)	Aaron Diamond AIDS Research Ctr.	U.S./Plan to make version for China, IAVI support
DNA (epitopes)	Epimmune	U.S., South Africa/Trying to make one vaccine for world
Salmonella delivering DNA vaccine	Institute of Human Virology, IAVI	U.S./Given orally
Venezuelan equine encephalitis (gag)	AlphaVax, IAVI, NIAID	South Africa, U.S./Unusual vector

A variety of approaches. When the search for an AIDS vaccine began, the field trained its sights on preparations that contained genetically engineered versions of HIV's surface protein. Only one of those early concepts survives, a gp120 made by VaxGen. The next vaccine in line, made by Aventis Pasteur, "primes" the immune system with a live-virus vector, canarypox, that carries HIV genes, and then "boosts" it with gp120. Although the prime-boost concept still holds center stage, naked DNA—at least for now—is the priming vector of choice, followed by a boost with a viral vector. As time moves on, expect more exotic vectors, such as Venezuelan equine encephalitis, and unusual carrying systems, such as *Salmonella* shuttling in a DNA vaccine. And if history repeats itself, you can bet that most of these vaccines will disappear before they ever make it to the finish line.

ed that SIV-infected sooty mangabeys maintain terrifically high levels of the virus in their blood. If their immune system is protecting them, it's not by traditional means. Last year, Jonathan Allan of the Southwest Foundation for Biomedical Research in San Antonio, Texas, and co-workers reported that the same holds true for African green monkeys. Mark Feinberg of Emory University in Atlanta, Georgia, who collaborated with Allan and also has confirmed the French findings with sooty mangabeys, suggests that these monkeys might benefit from a sluggish immune response that keeps many immune cells—SIV's target—out of the line of fire. "More isn't necessarily better," says Feinberg. "We don't know enough to point to what are the really good immune responses and which ones aren't so good."

Two experiments that have protected Asian monkeys also raise intriguing questions. A decade ago, Desrosiers's lab reported that a vaccine made from a live, weakened version of SIV offered the best protection seen to this day—and he still cannot completely explain why (*Science*, 18 December 1992, p. 1938).

Another surprise has come from an experiment that resulted in substantial protection, but doesn't even involve an AIDS vaccine. Lifson of SAIC-Frederick and his co-workers, including Desrosiers, infected five monkeys with a highly lethal strain of SIV and began treating the animals the next day with tenofovir, an anti-HIV drug that also works against the monkey virus. After 28 days, they stopped all treatment. As they reported in last November's *Journal of Virology*, the researchers could not detect SIV in the monkeys' blood, and the animals continued to control the virus once the treatment stopped. Moreover, when the researchers challenged the monkeys with the same SIV strain more than a year later, they all beat back the challenge.

To further test the monkeys' immunity, the researchers injected the animals with antibodies that temporarily deplete CD8 cells, the family from which killer cells originate. SIV spiked, but they quickly reestablished control. The investigators also upped the ante, using a different SIV strain that should be much more difficult for the animals to recognize and contain. All five monkeys substantially controlled the new virus.

Although CD8 cells appeared to play a role in the protection of some animals, the results perplexed many researchers because in other vaccine experiments, monkeys developed more impressive immune responses but still failed to contain the challenge virus. The animals in Lifson's study, Desrosiers says, "are as well protected as any vaccinated monkeys on Earth."

One lesson, says Lifson, is that blunting the initial burst of virus—whether by drugs or a vaccine-fortified immune system—is crucial, perhaps because it protects critical immune responses that otherwise would be lost for good. And he thinks this might help explain why Watkins and others could not defeat SIV with their vaccines. "None of these vaccines seem to give us enough blunting of the early viremia to allow development of an immune response that can give a good chance of solid, prolonged protection against SIV," he says.

Given the contradictory evidence from monkey studies, Desrosiers, the field's resident skeptic, says he has little hope that any of the vaccines now in human trials will work. "The breakthrough discovery that's going to lead to an AIDS vaccine hasn't been made yet," he says. "And if it happens at all, it's going to be serendipitous." Then again, many vaccines—including the one that eradicated smallpox from the world—went into widespread use long before humans had a clue how they actually worked.

–JON COHEN

SOURCES: IAVI, NIAID, UNIVERSITIES, AND COMPANIES