A Surprise Animal Model for AIDS

Researchers have infected pigtail macaques with HIV, raising the possibility that the animals will be better—and cheaper—than chimpanzees for studying the disease

When microbiologist Michael Agy and his colleagues at the University of Washington's Regional Primate Center in Seattle decided to try infecting pigtail macaque monkeys with the AIDS virus a couple of years ago, few researchers thought they would be successful. Closely related monkeys had proved resistant to infection and "conventional wisdom said pigtails could not become infected," says Douglas Bowden, the center's director. Last week, however, Agy and a team of researchers from the primate center and the university's medical school announced that the conventional wisdom was wrong: They have not only successfully infected eight pigtail macaques (*Macaca nemestrina*), but the animals have also shown symptoms of acute disease.

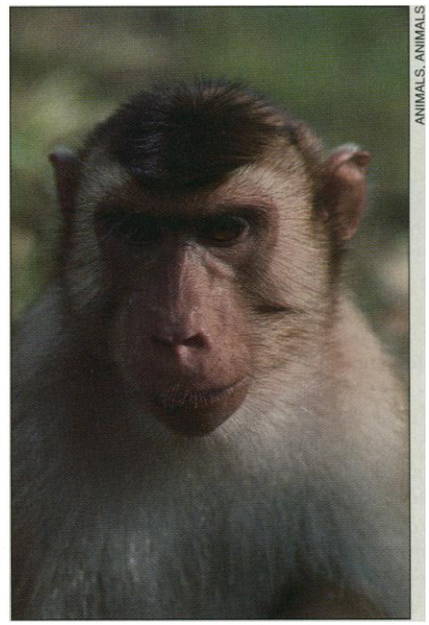
The achievement has sent a ripple of excitement through the AIDS research community, for it may help remove one of the biggest obstacles to studying the disease: the lack of a good animal model for monitoring the course of infection and for testing candidate drugs and vaccines. This is "a major logistic advance for pathogenesis and vaccine and therapy studies," says Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases.

Until now, the only animals researchers have been able to infect with HIV-1 are chimpanzees, but these animals have major drawbacks: They are relatively scarce, expensive, and show no symptoms of disease after being infected. Researchers have also been trying to glean clues to HIV-1's properties by infecting some species of monkeys with a closely related virus known as simian immunodeficiency virus, or SIV. Mostly, however, they have been forced to assume that the AIDS virus behaves in the body the same way it does in the test tube—an assumption that recent studies suggest is not always accurate.

The fact that pigtail macaques can be infected with HIV-1 is therefore potentially a major advance, but further work will be required to determine just how good a model the animal will be. For one thing, none of the infected macaques has developed full-blown AIDS, and none has been reported to show a decline in CD4 cells, the white blood cell depleted in human AIDS. Moreover, while pigtail macaques are not an endangered species, there is not yet an adequate supply of the animals from breeding colonies—and importing wild animals is becoming increasingly difficult (see box). Even if it's not perfect, however, the model appears to be better than anything researchers now have, and the fact that the animals develop symptoms soon after being infected could be especially important in studying the early course of infection. (A scientific paper describing the macaque model will appear in the 3 July issue of *Science*. The article was accepted for publication on 4 June, and *Science* lifted its normal embargo on 11 June after the National Institutes of Health [NIH] issued a media alert announcing the new model.)

When Agy, along with cell biologist Michael Katze and veterinarian William Morton of the primate center and Lyn Frumkin, Lawrence Corey, Robert Coombs, and James Koehler from the University of Washington medical school, started out, they knew they were embarking on a long shot. In the early days of AIDS research, several teams tried without success to infect two more commonly used macaques, the smaller rhesus (*Macaca mulatta*) and the longtail macaque (*Macaca fascicularis*). "You assume that if it doesn't work in [other macaques], it's not going to work in pigtails," says Robert Whitney, director of the National Center for Research Resources at NIH, which supports the Washington primate center. But with a large population of pigtails close at hand, they decided it was worth a try.

Agy and his colleagues began by trying to infect pigtail white blood cells in the test tube with four different HIV-1 strains—three from well-characterized, laboratory grown isolates and one from a recently infected AIDS patient. All four infected the pigtail white blood cells, and all showed a particular affinity for CD4 cells, the so-called helper T cells that are devastated in AIDS. The researchers also showed that the infected cells produced new virus particles.



ANIMALS, ANIMALS

Big macaque. Pigtails are larger than their more common laboratory cousins.

Buoyed by this success, the Washington researchers decided to try their luck in infecting two animals. They removed some white blood cells from each animal, mixed them with a strain of HIV-1 known as LAI, and then reinjected the cell and viral suspension back into the animals. Beginning 3 days after infection, the researchers periodically removed white blood cells from the pigtails, mixed them with a cell line that is readily infected with the AIDS virus, and tested to see if the macaque cells still contained infective virus. Bingo. The researchers could detect virus as long as 52 weeks after infection. Both animals also developed antibodies to the virus 3 to 4 weeks after infection as measured by a standard enzyme-linked immunosorbance assay, and both had enlarged lymph nodes and bouts of fever.

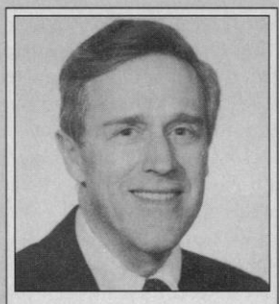
One question remained to be cleared up: Could the monkeys have been infected with some related virus, rather than the HIV-1 the researchers had injected into them? They turned to Steven Wolinsky at Northwestern University medical school in Chicago for the answer. Wolinsky has been studying changes in the virus after it infects a living organism. Using the polymerase chain reaction, Wolinsky showed that the viral DNA from infected pigtail cells was indeed from HIV-1, and not SIV or the related HIV-2 human virus, both of which have been previously shown to infect macaques.

More convinced than ever that they were on the right track, the Washington team injected six more pigtails with HIV-1. All six not only became infected but also showed the swollen lymph glands typical of acute infection. In addition, two animals developed a rash around the groin and abdomen, and several had bouts of diarrhea.

If these results hold up, researchers can at last contemplate an alternative to the

The Indonesia, Russia Connections

If the pigtail macaque (*Macaca nemestrina*) does become the animal model of choice for AIDS researchers, demand for the animals will quickly outstrip the current supply, according to Douglas Bowden, director of the Regional Primate Research Center at the University of Washington. Breeding colonies in the United States are limited, and the wild population is dwindling, Bowden told the advisory council of the National Center for Research Resources at the National Institutes of Health last week. But Bowden said that, thanks to some novel arrangements with the government of Indonesia and a research institute in Russia, he's hopeful that supplies can be stepped up.



Douglas Bowden

Right now, the Washington primate center has the largest captive population of pigtail macaques in the world, with approximately 450 animals, 400 of which are females suitable for breeding. There are smaller colonies at the Yerkes Regional Primate Center in Georgia and at the University of Puerto Rico. In addition, a few animals are available from commercial breeders.

Pigtail macaques come primarily from the Indonesian islands of Sumatra and Kalimantan (formerly Borneo). While the animal is not on the endangered list, the indigenous population has been under severe pressure since 1981, when the Indonesian government began a massive migration program to these islands to reduce overcrowding on neighboring Java. Not only do human settlers decimate the monkeys by clearing the vegetation that forms the animals' habitat, but, says Bowden, the macaques face a more immediate threat: "They taste good." Indonesian officials have therefore restricted export of wild pigtail macaques, and this year's quota of 500 animals has already been spoken for, Bowden says.

There is, in any case, a potential problem in using wild animals—infections with other retroviruses that cause immune deficiency. Type D retroviruses in particular are known to cause such symptoms, and to be quite common in macaques. "If you are going to use these animals for HIV-1, you need to be darn sure they don't have these other viruses," says Murray Gardner of the University of California, Davis.

The Indonesian government has, however, been receptive to the idea of treating the monkeys as a renewable resource, and is willing to use money generated from the sale of the monkeys to develop infrastructure in the country for sustaining the population. The Washington primate center, along with the Oregon Regional Primate Center and Bowman Gray Medical School in Winston-Salem, North Carolina, recently formed an alliance with the Indonesian agricultural institute in Bogor to develop a breeding colony in Indonesia. The three institutions are also planning to release some pigtail macaques on Tinjil Island, which has no human population but is home to about 1000 longtail macaques (*Macaca fascicularis*) that were introduced there as part of another captive breeding program.

Bowden says it will be at least 2 years before these proposed colonies are producing animals in any reasonable numbers, however, so researchers will have to scramble for additional supplies in the short run. That's where Russia comes in. The Russian Academy of Medical Sciences maintains a colony of about 120 pigtail macaques at the Institute for Biomedical Primatology in the Black Sea town of Adler, just across the border from Georgia. The Washington center is exploring a cost-sharing arrangement that would provide U.S. researchers access to the monkeys in exchange for hard currency and supplies to maintain the colony.

–J.P.

chimp—and on logistical grounds alone that would be a huge bonus. Chimps are an endangered species, and they can be obtained only through captive breeding programs. NIH's Whitney reckons that only about 25 animals are being produced domestically per year through NIH-supported programs, and each costs between $60,000 and $100,000. By contrast, the pigtail costs about $1000 to $1500. The University of Washington primate center's Morton argues that researchers will now be able to consider experiments involving 20 animals, something that is virtually unthinkable with chimpanzees. The pigtail macaque "should eliminate the chimp from most considerations," says Morton.

But others are more cautious. "It's too early to say if it would wipe chimpanzees off the map," says Murray Gardner, a vaccine researcher at the University of California, Davis. Gardner says other researchers will have to replicate the Washington results and show that animals can be infected by HIV-1 strains other than LAI and NL4-3, the two the Washington team used. Alan Schultz, the acting chief of the vaccine research and development branch at NIAID, also says the pigtail model will need more study before it is fully accepted by AIDS researchers. Schultz's big fear is that HIV-1 may never replicate inside the pigtail monkeys: It may simply get inside cells and stay there until the cells die naturally, even though it replicates in the cultured cells. Although Wolinsky's data show that the viral sequences can still be found 1 year post infection, Schultz says the infection appears to be weakening over time. Schultz would also like to see data from more animals indicating how large a dose of virus is needed to cause infection.

Washington's Corey says that's just what they intend to do next. He is planning to infect several animals with a standard vaccine challenge stock developed by Larry Arthur for the National Cancer Institute. The goal will be to determine the minimum infectious dose for pigtails, which will be a crucial requirement for designing vaccine trials. He is also collaborating with other AIDS researchers to see if he can find a way to induce the infected pigtail CD4 cells to start producing massive quantities of virus. If this causes disease, Corey says, then they will have a terrific model for studying the pathogenesis of AIDS.

But even if the pigtails never develop full-blown disease, Wolinsky says the macaque model will still produce extremely valuable information about the course of infection. "It will allow us to understand pathogenesis especially following acute infection," he says. Like other retroviruses, HIV-1 makes a lot of "mistakes" as it replicates its genetic material inside infected cells. These mistakes can lead to changes in the viral proteins, which make it difficult for the host's immune system to recognize the virus. Researchers are now anxious to know how the virus changes from the early days after infection—when it causes only mild disease—to some later time when it triggers severe immune deficiency. Using the pigtail model, researchers could also look for drugs and vaccines that could prevent the acute infection.

Although the University of Washington results have yet to be formally published, Morton says the primate center is already inundated with requests for animals and reagents. But Corey is also aware that there have been a lot of false alarms in the AIDS world. "I hope to see our work duplicated quickly, and that people will pick up on it," he says. That's just what people are beginning to do.

–Joseph Palca