A few labs have been plugging away for years to develop cheap, malleable animal models for AIDS, say, a rat or a mouse. Recent findings have brought the goal closer, but some AIDS researchers remain skeptical

Building a Small-Animal Model For AIDS, Block by Block

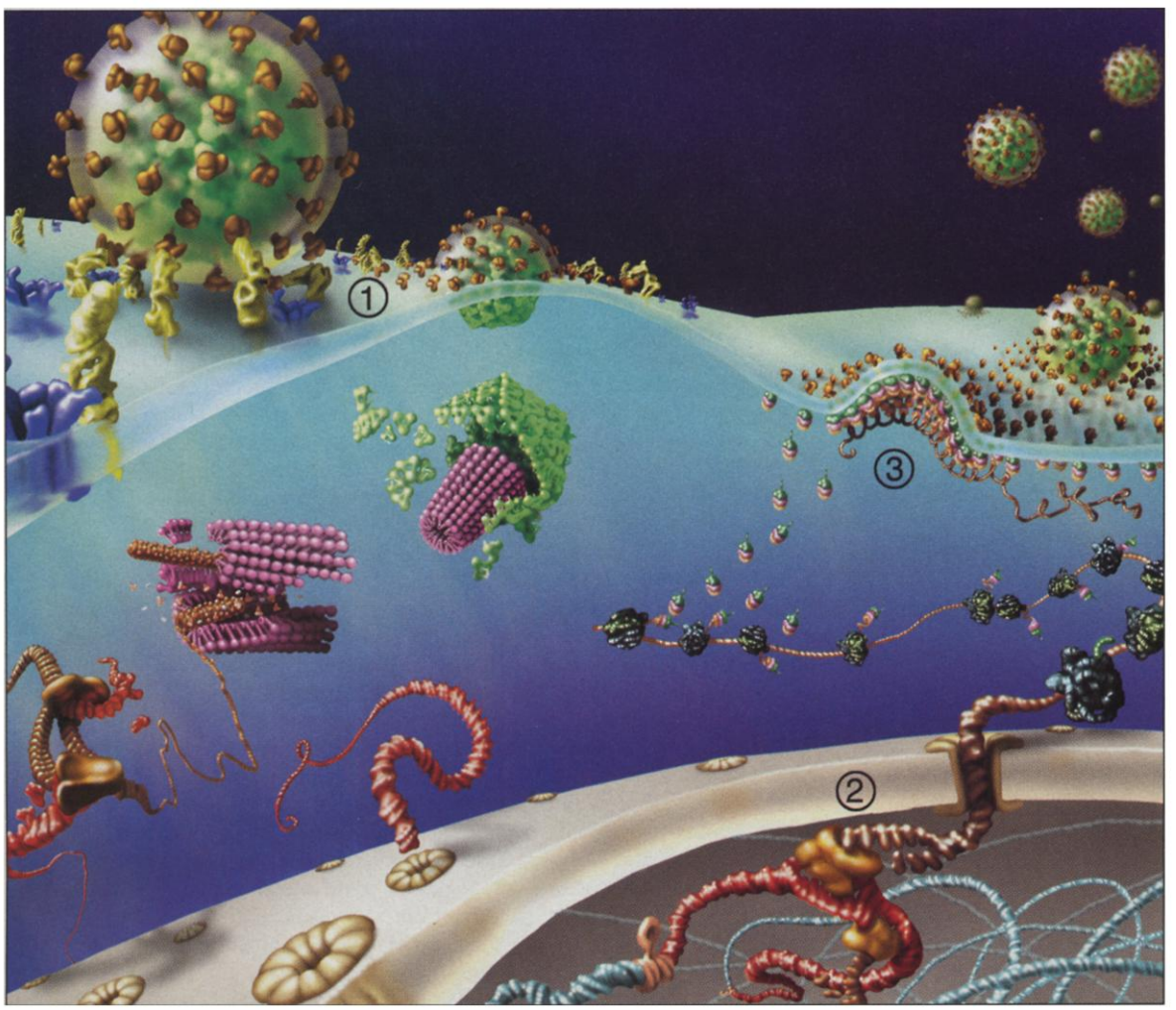
AIDS researchers typically describe HIV as a wily, stealthy, and clever killer. But researchers who have been struggling for the better part of 2 decades to get HIV to infect small animals have another adjective for the virus: impotent.

HIV causes disease only in humans and chimpanzees. If it could be coaxed to infect mice and rats—and, better yet, make them sick—the payoff could be enormous. "Instead of five animals in an experiment, we'd have 500," says Robert Gallo, head of the Institute of Human Virology in Baltimore, Maryland. "Instead of waiting 2 years to get results, you'd wait 2 months. It would greatly catapult the field forward." But until recently, attempts to develop a rodent model for AIDS have been frustrating. HIV, it seemed, is just too picky.

Over the past couple of years, however, researchers have identified several critical steps in the delicate pas de deux between HIV and the cells it infects, and those insights are breathing new life into efforts to engineer a rodent susceptible to HIV's depredations. A few groups have succeeded in infecting genetically engineered mice and rats, and a couple of groups, including Gallo's, have even managed to cause disease in both animals with one novel approach. "We're seeing incremental improvements in the field," says Janet Young, a program officer at the National Institutes of Health (NIH) who oversees extramural research efforts to develop these animal models.

The driving force behind these efforts is the lack of any good animal model to study HIV's wily ways. For the first decade of the AIDS epidemic, researchers conducted experiments in chimps held in primate colonies. But the animals are scarce and expensive, costing up to $50,000 each. More recently, researchers have used much more plentiful and cheaper rhesus macaque monkeys, originally from India. These monkeys develop an AIDS-like disease when infected with either SIV, a simian cousin of HIV, or a laboratory-made SIV/HIV hybrid called SHIV. The monkey model is a big improvement, but it has serious drawbacks of its own: SIV and SHIV are not HIV, one animal costs up to $5000, breeding takes years, and now Indian rhesus macaques are in short supply (*Science*, 11 February 2000, p. 959).

The development of a good rodent model for AIDS is still undeniably a long shot, however. "This is the most difficult project I have in my lab right now," says Paul Jolicoeur of the Clinical Research Institute of



Duplication demands. HIV requires human factors to: (1) enter a cell, (2) transcribe viral DNA into mRNA, and then (3) properly assemble the newly minted core proteins of the virus.

ILLUSTRATION: C. SLAYDEN

Montreal, who is attempting to make a transgenic, infectable mouse that develops disease. Some researchers even argue that the whole effort is an exercise in futility. "They're wasting their time," says Malcolm Martin of NIH's National Institute of Allergy and Infectious Diseases (NIAID), who once worked on the mouse model. By the time researchers engineer both the mouse and the virus to produce a model, he says, "you're going to wind up with an animal that's no longer a mouse or a virus that's no longer HIV."

But the prospect of using rodents to study AIDS is so intriguing that about half a dozen groups around the world are persevering. "They say, 'It'll never work,' " says Ned Landau, who is attempting to make an HIV mouse model at the Salk Institute for Biological Studies in La Jolla, California. "It *is* a difficult problem. But you'll never know if you don't try."

Block by block

Landau and his colleagues are trying to identify and remove the "blocks" that prevent HIV from copying itself in species other than humans and chimps. In 1996, Landau helped unravel one of the most confounding blocks facing the field: cell entry.

Shortly after Gallo's lab proved in 1984 that HIV causes AIDS, researchers discovered that the virus infects T cells by first binding to a receptor on their surfaces called CD4. Several groups quickly stitched human CD4 receptors into mouse T cells, but HIV still couldn't get into the cells. The implication: Factors in addition to CD4 are required to establish an infection. In 1996, again building on a finding from Gallo's lab, Landau and others discovered that the mystery cofactors were a family of receptors for chemokines, immune system messengers.

Again, several labs quickly engineered mice to express human CD4 and a human chemokine receptor on their T cells. These transgenic rodents were more promising. In 1997, Harris Goldstein of the Albert Einstein College of Medicine in the Bronx, New York, published evidence that he and his co-workers had infected one with HIV. But that success came with a big qualifier: Once the virus entered mouse cells, it did not copy itself.

The next year, the Salk's Katherine Jones reported a finding that knocked down another major block involving HIV replication. After HIV enters a cell and weaves its genes into the host's DNA, the virus copies itself first by transcribing its DNA into messenger RNA (mRNA). To make the mRNA, HIV relies on a protein it produces, called tat (transactivator of transcription). Jones and colleagues reported in the 20 February 1998 issue of *Cell* that they had identified a human protein, cyclin T1, that tat needs to do its job. Moreover, when the Jones group added human cyclin T1 to HIV-infected mouse cells, the cells churned out the proper viral mRNAs. "That really set things in motion," says Paul Bieniasz, an investigator at the Aaron Diamond AIDS Research Center in New York City who studies the HIV mouse model.

But again, hopes were dashed. Landau and, separately, Dan Littman of New York University (working with Jones) showed that murine cells engineered to express human cyclin T1, human CD4, and a human chemokine receptor still failed to produce high levels of new HIVs. "There's good evidence that something else is missing," says Littman.

Gag order. Salk's Ned Landau found that mice mangle HIV's gag protein.

In the April 2000 *Journal of Virology*, Landau identified one of those missing players. HIV mRNA codes for a protein, gag, that travels from the cytoplasm to the cell's membrane, where it is processed into smaller proteins. One of those proteins, p24, assembles into a capsid, a key internal structure that forms a shell around HIV's genetic material. Landau and colleagues showed with electron microscopy that in mouse cells, gag becomes trapped in the cytoplasm and never gets chopped into p24. Without a capsid, the new virus can't put all of its pieces together. "In our opinion the remaining obstacle to overcome is the ability of the virus to assemble," says Landau.

Did the mouse cells somehow inhibit the assembly of the capsid, or do human cells provide a critical factor? The human contribution is the key, conclude Bieniasz and his former postdoctoral adviser, Bryan Cullen of Duke University, who found evidence pointing to a factor in human cells. Last November, they reported in the *Journal of Virology* that they could produce infectious HIVs by fusing HIV-infected mouse cells with human cells. "We saw a substantial increase in viral production," says Bieniasz. Another group may have fingered the mystery factor: In April, Jaisri Lingappa of the University of Washington, Seattle, reported at an AIDS meeting in Keystone, Colorado, that her lab had identified a protein, HP68, that appears to chaperone p24 to form a tighter capsid.

After her Keystone presentation, Lingappa says, "we got barraged" by researchers working on developing an animal model who want to collaborate. But Lingappa, who has submitted the work for publication and does not want to discuss it in detail, is circumspect about the impact her results will have on those efforts. "My worry is this might be one of several factors," she says. "I don't think it's the whole story."

Indeed, at an AIDS vaccine meeting held in Puerto Rico this May, Goldstein of Albert Einstein presented evidence for yet another block. Most labs in this field, including Goldstein's, have focused on stitching human genes into mouse T cells. But Goldstein decided to try another tack: engineering mice to express human CD4 and a human chemokine receptor on a different cell in the immune system that's infected by HIV, the dendritic cell. Several labs have shown that dendritic cells in humans play a starring role in establishing an HIV infection by presenting the virus to T cells and other HIV targets.

Goldstein's group then crossed mice carrying his modified dendritic cells with others that had the human CD4 and chemokine receptors on their T cells. (Neither animal had cyclin T1.) The researchers then injected HIV into the animals' spleens. In Puerto Rico, Goldstein reported that these mice "developed sustained in vivo infection."

Others in the field are not convinced that Goldstein's mice truly had a sustained infection. "There might be some low-level replication, but it's not sufficient to make

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an animal model," says Landau. Littman agrees: "There was just so little virus there. It could be virus just sticking around." Goldstein says he, too, looks at his data cautiously: "When you delve into these stories, it's not as clear-cut as it first seems." But he notes that he found evidence of HIV in mouse plasma, which suggests that the virus infected cells in the spleen, copied itself, and traveled to the blood. "It's not as robust as infection in humans," acknowledges Goldstein. Still, he says his lab is now gearing up to do a "major experiment" to confirm these results. "If the results of that experiment duplicate our original results," he says, "I think we're in good shape."

Handy model? HIV-transgenic mice develop an AIDS-like disease.

Rat race

While mouse engineers have been plugging away at the blocks in their favorite species, Mark Goldsmith and his postdoc, Oliver Keppler, of the Gladstone Institute of Virology and Immunology in San Francisco, have been making steady progress with the rat. "It seems like they're very close in getting something with the rat model," says Salk's Landau.

They may have an easier task: As Goldsmith, Keppler, and co-workers explain in a paper in the September issue of the *Journal of Virology*, HIV appears to replicate much more readily in rat cells than in mouse cells. In particular, they found that HIV-infected immune scavengers called macrophages and microglia ("brain macrophages") produce "substantial" levels of p24. "We're totally ecstatic about that," says Goldsmith.

Goldsmith's team now plans to test the ability of HIV to replicate in a rat that they've engineered to express human CD4, chemokine receptors, and cyclin T1. "If we ever get to the point where we have a predictive model," says Goldsmith, "people will go wild with it and begin producing their own versions."

Goldsmith recognizes, however, that researchers could do more with a mouse model than a rat model. Not only do scientists have a much better understanding of the mouse immune system, they also have developed many transgenic mice that have specific genes added or "knocked out," which theoretically could easily be crossed with an HIV-infectable mouse. "You can test all these different components of the immune system to see what's important," explains Landau. Still, Goldsmith contends that the rat has much to offer: "The tools in the rat are not quite so advanced as in the mouse. But they're pretty darn good."

Alternative approaches

Instead of dismantling the blocks that prevent HIV from establishing an infection in rat or mouse cells, a few investigators are trying to bypass them. As far back as 1988, NIAID's Martin and co-workers showed that they could create an AIDS-like disease in mice by stitching the genes for HIV itself into an animal (*Science*, 23 December 1988, p. 1665). But these mice died because of a lab accident—someone left the air conditioning off too long—and several other groups subsequently had trouble creating a similar mouse.

Canada's Paul Jolicoeur in 1998 reported that after a long effort he had engineered a transgenic, HIV-infected mouse that within 1 month developed muscle wasting, lymph damage, kidney disease—and died. This closely mirrored Martin's HIV transgenic mouse. "I just couldn't believe it the first time we had a diseased mouse," says Jolicoeur.

This brute-force approach avoids all the barriers that prevent HIV from getting into the cell and copying itself. But that very asset is also a handicap. "You get a toxic effect that's not related to any spreading [of HIV]," says Martin. "That's what turned me off about the model."

Although many researchers question the utility of such a model, Jolicoeur and coworkers have used the animals to investigate several aspects of HIV's modus operandi. In the 16 October 1998 issue of *Cell*, for example, they showed that the progression of disease in the mice appeared to depend entirely on levels of a little-understood HIV protein called Nef, and they spelled out possible mechanisms. Jolicoeur now has papers in press at *Immunology* and the *Journal of Virology* that further use the model to explore how HIV causes disease.

Joseph Bryant, Gallo, and colleagues at the Institute of Human Virology are following a similar approach with the rat. In the 31 July *Proceedings of the National Academy of Sciences*, Bryant and colleagues reported the creation of a transgenic rat carrying seven of HIV's nine genes. The animals suffer from immune damage and some AIDS-like diseases within 9 months. Bryant says the rat has a few potential advantages over HIV-transgenic mice. The larger rat has nearly 20 times as much blood as the mouse, making it easier to study its immune system, Bryant notes. His rats also provide a better model to study HIV-related damage to the central nervous system, he says, because they produce higher levels of the viral surface protein, which others have tied to the disease process. "There may be clues that you can get from this model, even if you can't get definitive answers," says Goldsmith.

Practical problems

In spite of these promising developments, efforts to create a rodent model for AIDS still face many obstacles, not the least of which is the lackluster support this avenue of research receives. Currently, no more than a half-dozen labs have serious efforts under way to develop a transgenic small-animal model. This high-risk endeavor not only has trouble winning funds from granting agencies—the NIH spent a mere $1.7 million on work last year that explicitly develops these models—graduate students and postdocs shy away from devoting themselves to projects that may not lead to publications. "I don't put graduate students on it," says Jolicoeur. "I do it only with my senior people who don't [need to build up their publication records]."

And even if a model works in the eyes of some researchers, others may be reluctant to embrace it. "If we had such a mouse today, what would I do with it?" asks Gary Nabel, who heads the NIH's Vaccine Research Center. "I'd work with the primate as much as possible still, because there are so many different aspects of the biology of the mouse that we don't understand, and we wouldn't want to make critical decisions about vaccine trials where it's so poorly understood."

But Goldsmith remains undeterred. "The bottom line is we don't yet have a robust model, but we have things that argue to us that we should keep going forward," he says. "We'll keep doing it until we have success or run out of money." —JON COHEN

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