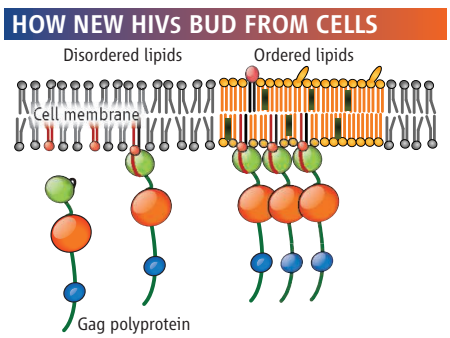
The Ins and Outs of HIV

Researchers first isolated HIV 27 years ago and arguably know more about how it behaves than they do any other virus. But presentations here challenged the most basic notions of how HIV enters and exits cells.

As landmark studies first revealed in 1996, HIV initiates an infection by binding to two receptors on the cell surface (*Science*, 10 May 1996, p. 809). But how that bound HIV then penetrates the cell membrane has remained murky. Many researchers believed that HIV sticks a protein into the cell membrane and then directly fuses with it. But HIV—and other viruses that have an outer coat made from a mishmash of its own proteins and cellular membrane—can enter via endocytosis.

In endocytosis, the membrane invaginates and pinches off to form an endosome—a bubble of membrane with the virus inside. The virus fuses with the endosome only after the bubble is floating in the cell. Biophysicist Gregory Melikian of the Institute of Human Virology at the University of Maryland School of Medicine in Baltimore presented provocative evidence that HIV primarily relies on the endocytic pathway.

In test tube studies with HIV and human cells, Melikian introduced a peptide that blocks direct fusion with the cell membrane. If endocytosis was a primary mechanism, the viruses already inside of endosomes could dodge the peptide. As a control, he used low temperature to block both direct fusion and fusion that occurs inside endosomes. Low temperature blocked fusion more potently than the peptide, indicating that many viruses were inside of endosomes and could proceed with the infection process. The block on direct fusion had no impact on the virus inside endosomes.



Self-coated. HIV selects specific lipids from membranes as it leaves the human cell.

This doesn't rule out the possibility that some virus enters by direct fusion, but in another experiment, Melikian made movies in which he labeled the virus with a fluorescent protein and recorded the infection process. HIV readily fused with the endosomes and initiated an infection, while the process of direct fusion aborted before the virus could unload its genetic cargo into the cell. In all, these data suggest that "the overwhelming majority of viruses are entering through endocytic pathways," Melikian concluded. "And with endocytosis, you hide the virus much sooner, narrowing the opportunities for antibodies."

Virologist Hans-Georg Kräusslich of the University of Heidelberg in Germany, whose lab first showed the importance of HIV endocytosis in 2004, said Melikian's work "certainly takes it further" but cautioned that it remains controversial whether that mechanism is an exclusive entry route.

Kräusslich presented data that tackled the other, often ignored end of the process: how HIV exits cells. "People thought the virus simply blebs out," said Kräusslich. "It doesn't. The virus controls the process." Over the past 6 years, Kräusslich's lab, along with several other groups—including Eric Freed of the U.S. National Cancer Institute in Frederick, Maryland; Michael Summers of the University of Maryland, Baltimore County; and Wes Sundquist of the University of Utah in Salt Lake City—have shown in exquisite detail

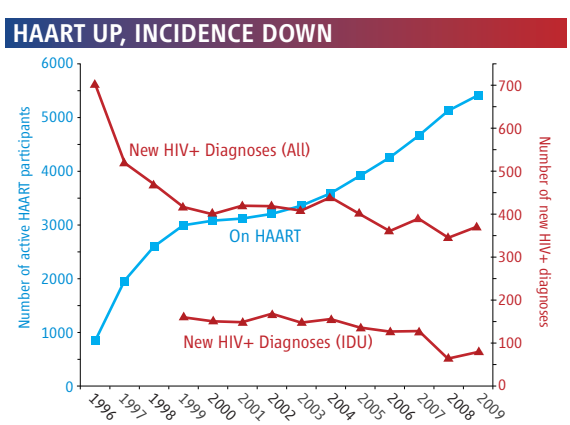
Treatment as Prevention

An ambitious idea to slow the HIV/AIDS epidemic is gaining traction: Test everyone for the virus and immediately start all HIV-infected people on treatment. But the test-and-treat scheme has epidemic modelers battling it out, with some insisting it's feasible, both financially and practically, and others denouncing it as a pipe dream and warning that it could increase drug resistance. At the meeting, two groups presented some of the firmest data yet to support the concept.

Although it's logical that if drugs reduce the amount of virus in individuals (the "viral load"), they then become less likely to transmit HIV, until now, the population data supporting the idea has remained sparse. Deborah Donnell of the Fred Hutchinson Cancer Research Center in Seattle, Washington, described a study of 3381 "discordant" heterosexual couples in seven sub-Saharan countries in which only one partner was infected with HIV at the outset. Over 2 years, Donnell and co-workers analyzed 103 new infections in which they could prove through genetic sequencing that the infecting virus came from the person's long-term partner. Of these, only one person who became infected had a partner who was receiving anti-HIV drugs, showing that treatment reduced the risk of transmission by 92%. "I think it's the single most important presentation here," said virologist Mark Wainberg of McGill University in Montreal, Canada.

Others cautioned that treatment might not have as powerful an effect with other transmission routes. But Julio Montaner, a key proponent of test-and-treat who directs the B.C. Centre for Excellence in HIV/AIDS at the University of British Columbia, Vancouver, in Canada, presented compelling data from one of the most susceptible groups, injecting drug users (IDUs). Test-and-treat is more feasible and possible to track in Canada because it has a national medical system with centralized, local control, he added.

Earlier, Montaner's group showed that as



All together now. As more people received highly active antiretroviral treatment (HAART) in British Columbia, new infections plummeted, even in IDUs.

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the structure and function of HIV's Gag polyprotein, which orchestrates the exit process with help from the cell.

Cell membranes consist of two sheets of many different types of lipids, including cholesterol. Kräusslich showed that new virions do not—as cartoons used by many in the field often suggest—bleb through the membrane and then randomly dress themselves in a coat made of these cellular lipids. Rather, HIV's Gag selects specific lipids to form the viral coat.

The lipid bilayer has two microdomains with different degrees of rigidity. The more rigid microdomain is "ordered" into "rafts" of regularly packed lipids that are straight and lined up like soccer players forming a wall to block a penalty kick. The other one is "disordered" with lipids that have kinks in them (see illustration). Gag selects lipids in the rafts—in particular, favoring one called PIP for short—or gathers other lipids to form its own rafts.

Using fluorescence microscopy, Kräusslich's lab showed the process of budding and release, detailing how the virus co-opts cellular proteins to pinch itself off from the cell. "What's remarkable about these new studies is what appeared to be such a simple process is so complex, and it shows how this virus interacts intimately with the machinery of the cell," said molecular virologist Nathaniel Landau of New York University School of Medicine in New York City. "It's one of the major advances in understanding HIV molecular biology."

–JON COHEN

2500 people started antiretroviral drugs (ARVs) between 1996 and 1999, new infections in their steadily expanding testing program dropped by 50%. The analysis presented here examined data from 2004 to 2009, when the number of treated people doubled to about 5000; many were IDUs. New infections fell, and although the drop wasn't as dramatic as before, there was a concomitant decline in viral load in the treated people. A subset analysis of IDUs also showed a particularly sharp reduction in new infections.

Several studies around the world will soon begin to evaluate the test-and-treat strategy. "It's a very challenging concept," said Anthony Fauci, head of the U.S. National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Maryland, who described obstacles, including the feasibility of conducting widespread, voluntary testing. But NIAID is funding a study of the concept at two U.S. sites that will start in June. "It's a bold, high-risk but high-return project that we are going to push the envelope on," said Fauci. **–J.C.**

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Upgrade time. Single-dose nevirapine, now deemed suboptimal, was celebrated when this makeshift clinic in Kampala, Uganda, proved it could protect many babies.

Limits of Success

Huge disparities in access to proven methods to thwart HIV still exist between rich and poor countries. Prevention of mother-to-child transmission (PMTCT) efforts are a case in point, explained pediatrician Elaine Abrams of Columbia University. In wealthy countries, where HIV-infected pregnant women receive cocktails of antiretroviral drugs (ARVs) and do not breastfeed, fewer than 2% transmit the virus to their babies. That's a drop from as high as 40% of the women who receive no treatment and breastfeed. "New pediatric infections have virtually been eliminated," said Abrams. "In contrast, the pediatric epidemic rages overseas." According to the best estimates, 480,000 babies worldwide became infected in 2008, with a mere 21% of pregnant women receiving an HIV test and only 45% of those who tested positive receiving drugs to prevent infection—and that treatment was often suboptimal.

The most commonly used intervention for PMTCT in developing countries is a single dose of the drug nevirapine given to the mother in labor and the baby at birth. This strategy, which first proved its worth in a Ugandan study that ended in 1999, is cheap and simple and cuts transmission rates in half—and, before the arrival of cheap ARVs, it was the only option for many poor women. But, said Abrams, abundant data now show the dangers of the "overreliance on single-dose nevirapine." Not only are cocktails of ARVs more effective at preventing transmission, but as many as 50% of pregnant, infected women have suffered severe immune destruction and need combination treatment for their own health. What's more, the single dose of nevirapine fuels the emergence of resistant strains, compromising the ability of mothers and their babies, if they do become infected, to benefit from that entire class of drugs—a key component of cocktails in developing countries.

Some countries now add another ARV or two for a short time before labor and during breastfeeding to reduce the risk of resistance emerging, and even when resistance does develop, it wanes over about a year's time. Still, the different standards of care for poor and rich are unacceptable, many researchers said at the meeting. "Our responsibility is to come up with consistent, across-the-board ARV recommendations," said pediatrician Arthur Ammann, who heads Global Strategies for HIV Prevention, a nonprofit based in San Rafael, California, that does PMTCT in the Democratic Republic of the Congo and Liberia.

Catherine Wilfert, a pediatrician emeritus at Duke University in Durham, North Carolina, stressed that the costs of ARVs are not the main roadblock. "The real funding challenge is the training," said Wilfert.

But if single-dose nevirapine is the only option available, it remains much better than doing nothing, said infectious disease specialist Nicholas Hellman, who heads medical and scientific affairs at the Elizabeth Glaser Pediatric AIDS Foundation: "We have to be careful not to throw out the baby with the bath water." **–J.C.**

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