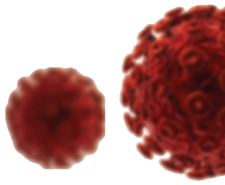
Gut warfare



Far from the unhurried killer it seemed to be, HIV is a swift assassin, gutting the body's immune system within days of infection. Erika Check finds out how this new paradigm is transforming AIDS research.

HIV is supposed to be a slow and stealthy killer. For years, scientists have thought the virus begins its assault in the blood, destroying just a few of its favorite targets—specialized immune cells called CD4 T-helper cells, which anchor the body's defenses against infections.

After that initial attack, HIV appears to move in slow motion, taking years to deplete enough CD4 cells to fatally decimate the immune system.

But in the 20 years that scientists have been studying AIDS, they haven't been able to explain this seemingly massive leap: how do so few infected cells drive the inexorable progression to death?

The answer may lie not in the blood, but in the gut.

Doctors have known for more than a decade that HIV affects the digestive system, causing intractable diarrhea and severe weight loss. But until recently, few had grasped the importance of those symptoms. Exactly how big a role the gut plays in the disease progression is still controversial, but a series of papers have redefined our understanding of how HIV kills and how to prevent it.

"We have ended up with a completely new view of HIV infection," says Daniel Douek, chief of human immunology at the US National Institute of Allergy and Infectious Diseases' Vaccine Research Center, who published some of those studies.

Far from being slow, all evidence now indicates that HIV's initial attack is swift and deadly, destroying CD4 cells in the gut where, in fact, most of the body's CD4 cells reside. In a monkey model, about 80% of CD4 cells in the gut are wiped out within the first four days of infection.

"There's been a complete turnaround," Douek says. "This has changed people's attitudes, from thinking that this disease is slow and indolent to thinking that it is extremely aggressive."

The fact that HIV's devastation happens so quickly suggests that the infection should be treated as early as possible and that vaccines should focus on generating immunity at mucosal surfaces, such as those that line the gut. It has also boosted interest in prevention strategies such as microbicides, which aim to block infections at the mucosal lining of the vagina.

"The work on the acute infection has led to a massive reappraisal of the mechanisms of disease pathogenesis, has helped refocus our views on where and when therapies should be instituted, and has led to the search for vaccines that elicit robust mucosal immunity," Douek says.

Digestive troubles

Why it took scientists so long to understand the gut's importance is a mystery in itself—particularly given that HIV researchers work more closely with clinicians than do researchers in most fields.

The reason may partly be that it is easier to study T cells in the blood than in the gut, and finding newly infected individuals is an impossible task. Still, there have been glaring signs from both monkey and human studies for more than a decade.

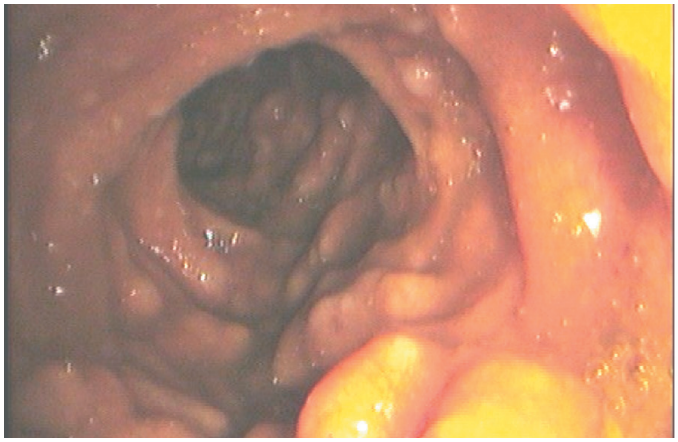
Digestive problems are a hallmark of AIDS—so much so that veterinarians always interpreted diarrhea in infected monkeys as a sign of full progression to the disease.

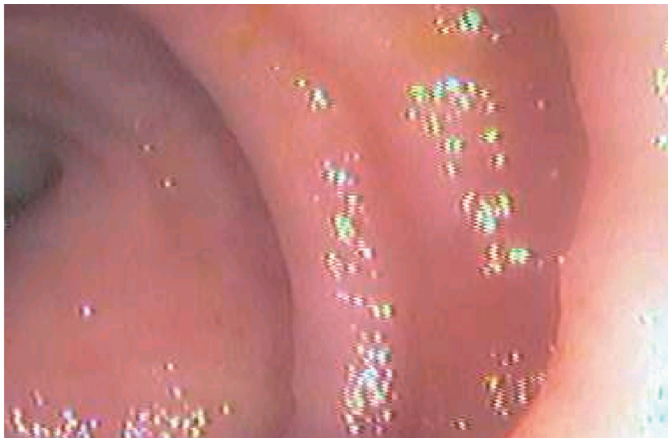
But in people, doctors assumed that gut problems were a result of opportunistic infections caused by the weakened immune system. "From the beginning, gut complications were very clearly linked to the disease, but most studies of gut tissue were focused on advanced infection," says Satya Dandekar, chair of medical microbiology and immunology at the University of California in Davis.

Most doctors never thought to order invasive tests, such as biopsies, to assess the virus's effect in the gut in early infection.

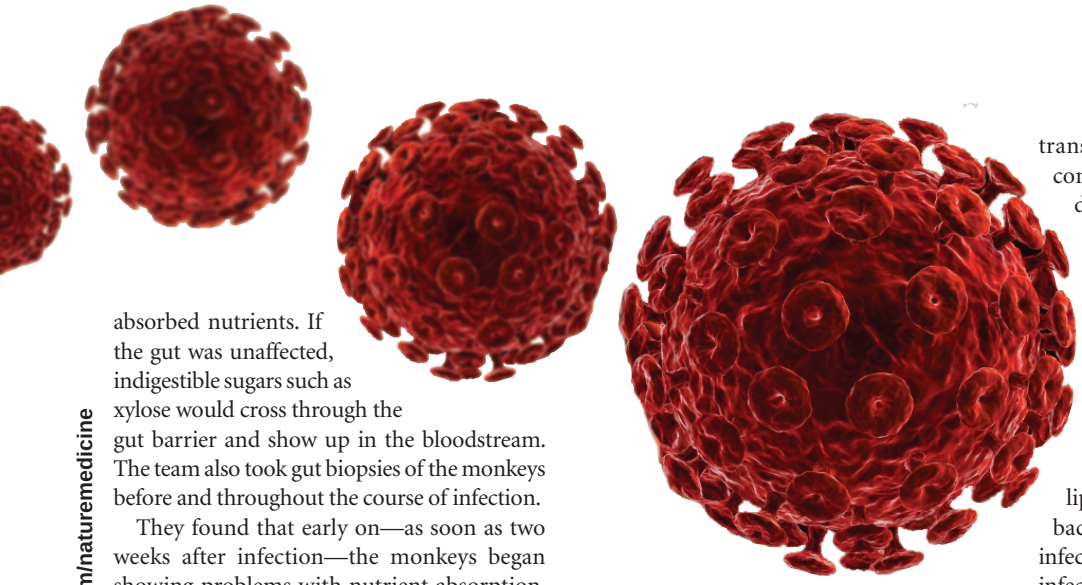
For people with HIV, the digestive problems were of serious concern. Dandekar noticed, for instance, that patients waiting to be seen by doctors would often have to run to the loo. To understand how HIV causes these gastrointestinal problems, Dandekar turned to rhesus macaques infected with the simian immunodeficiency virus, a relative of HIV that has been used to create monkey models of the disease.

As early as 1994, Dandekar's team began testing the gut lining by assessing how well it





Clean sweep: The gut harbors most of the body's CD4 immune cells (left), but HIV infection wipes them out (right).



absorbed nutrients. If the gut was unaffected, indigestible sugars such as xylose would cross through the gut barrier and show up in the bloodstream. The team also took gut biopsies of the monkeys before and throughout the course of infection.

They found that early on—as soon as two weeks after infection—the monkeys began showing problems with nutrient absorption. Gut biopsies revealed that large numbers of T cells and macrophages—which play a key role in fighting pathogens—are infected with SIV as soon as one week after infection (*J. Infect. Dis.* **169**, 1116–1120; 1994). These changes were apparent before complications such as diarrhea.

Four years later, Andrew Lackner and colleagues at the New England Regional Primate Center also showed that SIV has a quick, extensive and devastating impact in the monkey gut. In the rhesus macaque model, they found, SIV infection in the first two to three weeks of infection wipes out large numbers of CD4 T cells from the intestine—but not from the blood, spleen and other peripheral lymph organs (*Science* **280**, 427–431; 1998). The virus seemed to selectively destroy the protective mucosal lining of the gut.

"That really set the stage," says Douek. "But even that paper was overlooked."

Mucosal immunity

Over the following five years, Lackner and his colleagues showed that SIV preferentially targets T cells not only in the intestine, but also at other mucosal surfaces such as the lung and vagina, depleting the body's defenses at its primary barrier sites.

Finally, in 2004, Douek's group, in collaboration with researchers at New York's Aaron Diamond AIDS Research Center, published results that made it impossible to overlook the gut's importance.

Scanning intestinal biopsies of individuals recently infected with HIV, they found that all the observations in the SIV model hold true in people as well: the intestine is the first site of CD4 cell depletion, and memory T cells in the gut are wiped out early in infection (*J Exp. Med.* **200**, 761–770; 2004, *J. Exp. Med.* **200**, 749–759; 2004).

"[This] supports a simple hypothesis to explain much of the pathogenesis of HIV infection… that disease progression may correlate with turnover of specific cell subsets in mucosal tissues," Veazey and Lackner wrote in a commentary on the two papers (*J. Exp. Med.* **200**, 697–700; 2004).

It's still not entirely clear how the HIV's invasion of the gut leads to an overall immune failure.

In 2005, Dandekar's group reported a study on people who are infected for years with HIV, but who seem to keep the virus at bay—so-called long-term non-progressors. The group found that these non-progressors have normal levels of CD4 T cells in the blood and the gut. They also found that genes associated with inflammation and immune activation—the state in which the immune system is turned on to respond to an infection—are expressed at lower levels in non-progressors than in infected individuals who were losing the fight against the virus. This constant state of immune activation is thought to eventually exhaust the immune system of HIV-infected individuals.

But in both the non-progressors and susceptible individuals, genes associated with digestive functions are expressed at lower levels, indicating that the gut of non-progressors is also affected by the virus (*Proc. Natl. Acad. Sci.* **102** 9860–9865; 2005).

There is some evidence that this effect doesn't improve even with treatment. Even after years of retroviral therapy, as many as 70% of those infected still have depleted T-cell populations in the colon, even though T cells in the blood bounce back—suggesting that a blood T-cell count may not always accurately reflect the severity of the disease.

Despite all these pieces of information, however, it's not clear how the gut depletion affects the course of the disease.

Leaky gut

One theory, proposed in November 2006 by Douek, has to do with 'microbial translocation'—a concept well known in conditions such as inflammatory bowel disease, but foreign to most HIV researchers.

Microbial translocation is, as Douek puts it, "a leaky gut"—a condition in which the gut barrier ceases to function normally, and the enormous amount of bacteria normally found in the gut swarms out of the intestines and into the blood.

Douek measured levels of lipopolysaccharides, a component of the bacterial cell wall, in the blood of HIV-infected individuals. Those with chronic HIV infection—and monkeys infected with SIV—have elevated levels of blood lipopolysaccharides. Higher amounts of these substances correlate with higher levels of immune activation. The levels of lipopolysaccharides also drop after antiretroviral treatment (*Nat. Med.* **12**, 1365–1371; 2006).

"We think microbial translocation causes systemic immune activation and therefore progression in HIV disease," Douek says.

Douek's idea has its share of supporters and skeptics. But in the meantime, there is a stronger emphasis on mucosal immunity within the field. The National Institutes of Health and the Bill and Melinda Gates Foundation are both, for instance, funding attempts to make a vaccine that generates mucosal immunity and microbicides, which work at mucosal surfaces.

If Douek is right, antibacterial drugs could be given along with antiretroviral therapy to combat the leaky gut.

But it is too early to start advising doctors to change the way they treat people with HIV, says Marty Markowitz, researcher at the Aaron Diamond AIDS Research Center.

"At this point, there's no indication at all that treatment approaches should be changed," Markowitz says. "But I do think the field is remiss in not identifying enough patients in acute and early infection."

Still, the fact that researchers have finally begun paying attention to the gut might elicit some answers—and changes in the way HIV is treated. And that change is long overdue, says Dandekar.

When she first decided to look at HIV in the gut, senior faculty members advised her against it. "They said, 'Why are you working in the gastrointestinal tract?'" Dandekar recalls. "'Work on the lymph nodes instead.'"

Had she and others heeded that advice, Dandekar says, it may have taken even longer to realize the gut's importance. "I'm glad I didn't listen," she says. "I listened to my gut."

Erika Check writes for Nature Medicine from San Francisco.