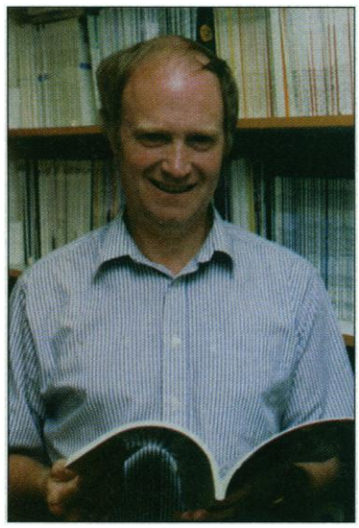
UNITED KINGDOM

Modest Briton Stirs Up Storm With Views on Role of CTLs

OXFORD, U.K.—Andrew McMichael, head of the Molecular Immunology Group at Oxford University, seems an unlikely candidate to kick off a scientific controversy. But to many AIDS researchers, this soft-spoken Briton is best known for his provocative and much-debated ideas about how HIV, the virus that causes the disease, escapes the wrath of the immune system.



OXFORD MEDICAL ILLUSTRATION

Escape clause. McMichael says HIV evolves to avoid immune response.

McMichael's theory stems from his 2 decades of solid work on immune cells called cytotoxic T lymphocytes (CTLs), among the most formidable weapons in the immune system's arsenal. CTLs home in on microbe-infected cells and destroy them before the microbe can reproduce and infect neighboring cells. In 1986, McMichael, along with Oxford University immunologist Alain Townsend and other U.K.-based co-workers, reported crucial evidence that CTLs move in for the kill when they recognize small fragments of microbial proteins that have been transported to the cell's surface. A decade later, McMichael, along with colleagues at Stanford University, unveiled a revolutionary new technique for identifying and quantifying CTLs that are primed to recognize specific foreign proteins—a tool that eager researchers are now using to dissect immune responses in previously unattainable detail (*Science*, 4 October 1996, p. 94).

"Andrew has made tremendous contributions to the field," says immunologist Bruce Walker of the Partners AIDS Research Center in Charlestown, Massachusetts. Immunologist Douglas Nixon at the Aaron Diamond AIDS Research Center in New York City, who did his graduate work with McMichael, adds that "Andrew was instrumental in educating immunologists on the role that CTLs play in viral infections and other diseases."

In the early 1990s, however, McMichael, together with Oxford University immunologists Rodney Phillips and Sarah Rowland-Jones and Oxford biochemist-mathematician Martin Nowak, set the AIDS community buzzing when they proposed that HIV avoids being destroyed by continually mutating until it is no longer recognized by CTLs. HIV has a very high mutation rate, largely because its replication mechanism, like that of many other viruses whose genomes are made of RNA, is inefficient. This mutability, McMichael and Nowak argued, eventually allows the virus to evolve through Darwinian natural selection and escape from even the most powerful CTL onslaught. Although the concept of "immune escape" had been proposed before to explain how HIV avoids being neutralized by antibodies, assigning such a central role to CTLs was controversial, largely because it was not clear how important these cells were in combating the virus.

Nevertheless, when McMichael's hypothesis was first put forward, some of his colleagues quickly latched onto it as an attractive explanation for why most HIV infections appear to be under control for many years before they eventually destroy the patient's immune system. Infected individuals produce varying levels of CTLs directed against the virus, and a growing number of researchers now believe that these cells may be key to keeping HIV at bay—and that boosting their numbers may be central to the development of a successful vaccine. But lingering doubts about the role of CTLs have made the model's key prediction—that the virus is evolving specifically in response to the natural selection "pressure" from these attacking cells—unconvincing to some researchers.

The recognition of invading microbes by CTLs is a complex business. An infected cell must first chemically chop up the microbe's proteins into small fragments, or peptides. These peptides are transported to the cell surface, where they become bound to specialized molecules called human leukocyte antigens (HLAs). Special receptors on the CTL recognize the HLA-peptide complex, and the CTL then kills the infected cell by unloading a cocktail of cytotoxic chemicals into it. But even a small mutation can change the peptide's structure enough so that it will no longer bind to HLA—or, alternatively, so that the peptide is no longer transported to the cell surface—and the infected cell then becomes "invisible" to the CTL.

Most researchers agree that HIV's escape from CTL surveillance could play a role in the immune system's collapse. But just how great a role is a matter of debate, and the experimental evidence brought to bear on the issue has been contradictory. "Immune escape probably has some role in a complex process that involves a large number of variables," says Walker. "[But] a question remains as to the magnitude of its contribution to overall progression" of the disease.

A dimmer view is taken by Simon Wain-Hobson, an AIDS researcher at the Pasteur Institute in Paris. Wain-Hobson, together with other European colleagues, looked for HIV "escape mutants," but found no evidence that mutations were occurring at a faster rate in the protein segments specifically recognized by anti-HIV CTLs. Some other researchers who have looked for evidence that immune escape actually occurs have also drawn a blank. Wain-Hobson argues that the model is an "unsatisfactory" explanation for why the immune system loses control of HIV, because other fast-mutating RNA viruses, such as those that cause measles and yellow fever, can be controlled by the immune system either unaided or after vaccination. "If RNA viruses were [inherently] able to escape, we wouldn't be here talking about it," he says.

But over the past several years, McMichael and other colleagues at Oxford, including Phillips and Rowland-Jones, have continued to accumulate evidence that they believe supports the model. The group has identified a number of HIV-infected patients who had strong CTL responses against certain viral peptide sequences early in their infections. When these peptides were later altered by mutations, the patients were left with apparently weaker CTL responses directed against other sequences—a key prediction of the model. Similar evidence that viruses can escape from CTLs was reported last year in *Nature Medicine* by Persephone Borrow at The Scripps Research Institute in La Jolla, California, George Shaw at the University of Alabama, Birmingham, and other colleagues.

Given this evidence, McMichael says, "it is hard to believe that [immune escape] doesn't have an effect" on the course of the disease. "This virus makes some of the strongest CTL responses we've ever seen; ... you can't have strong CTLs without escape mutants. Invariably, there must be selection."

Now, a powerful new technique McMichael helped develop may finally resolve the debate. McMichael and other Oxford coworkers, together with Mark Davis, John Altman, and their colleagues at Stanford, have developed a highly sensitive assay to

identify and quantify CTLs that recognize specific microbial peptides. The method, called tetramer staining, uses genetically engineered complexes consisting of four HLA-peptide subunits—each of which contains the specific peptide of interest—to detect CTLs taken directly from patients. The complexes bind so tightly to the CTLs' receptors that even small numbers of cells are readily detectable.

Using this technique, a team led by McMichael, including Oxford researchers Graham Ogg, Rowland-Jones, and Nowak, along with Nixon and David Ho at Aaron Diamond, published in *Science* earlier this year the first clear evidence that higher levels of HIV-specific CTLs are correlated with lower concentrations of HIV in the blood of infected patients (*Science*, 27 March, p. 2103)—a finding consistent with the view that CTLs play a major role in controlling the virus. "This technology has had a tremendous impact on our understanding of the relationship between CTLs and [viral burden]," says Walker. As for how important immune escape will turn out to be in the development of AIDS, Walker says, "I am sure Andrew will be the one to sort this out."

–Michael Balter

FRANCE

Duo Brings Hope of Immune Restoration

PARIS—When Brigitte Autran and Christine Katlama attended medical school in Paris together in the 1970s, they had much in common: Both were specializing in infectious diseases, a field long dominated by men; both were avid and competitive skiers; and a few years later, as newly graduated doctors, they were among the first French physicians to take care of AIDS patients. Yet their paths diverged soon afterward. While Katlama continued caring for patients, Autran left clinical medicine for a career in basic immunological research.

But in the early 1990s, the pair teamed up again, to lead one of France's most successful AIDS research collaborations. Over the past year, Autran and Katlama—who now both work at the Pitié-Salpêtrière Hospital in Paris—have published a series of encouraging reports showing that the battered immune systems of HIV-infected patients may recover, at least partially, if powerful combinations of drugs are used to reduce their viral burdens. Although AIDS researchers are still debating how much recovery actually takes place, most agree that the French team's findings have helped open the door to this important possibility. "[This] research has had a significant impact on the understanding of the immunopathogenesis of HIV disease," says Mario Roederer, an immunologist at Stanford University.

Immunologist Quentin Sattentau, of the Center for Immunology in Marseilles, says that Autran and Katlama have gained a place among France's leading AIDS researchers because "they are tough when they need to be and are not worried about stating their opinions in a forceful way." The two began laying the groundwork for their recent discoveries more than a decade ago, while they were still working independently. After leaving the clinic for the laboratory, Autran began working with Pitié-Salpêtrière immunologist Patrice Debré to elucidate the role of the immune cells known as T lymphocytes in fighting HIV infection. In 1987, Autran and Debré, along with other French co-workers, published a landmark paper in *Nature* demonstrating that HIV-infected patients produce large numbers of killer cells, called cytotoxic T lymphocytes (CTLs), directed specifically against the virus. This key finding, simultaneously reported by Bruce Walker and his colleagues at Massachusetts General Hospital in Boston, effectively countered the views of some researchers at the time that CTLs were of little importance in the immune system's response to HIV.

Meanwhile, Katlama was making her own mark on HIV research. As one of the small number of French physicians willing to devote themselves to studying the disease in the 1980s, she quickly developed a reputation as an expert on the opportunistic diseases, such as cytomegalovirus (CMV) infection, that ravage AIDS patients. And in 1985, Katlama's insistence that a patient from the Cape Verde islands off the west coast of Africa had AIDS—a diagnosis many of her colleagues doubted—led to the discovery of HIV-2, a West African variant of the virus that usually causes a milder form of the disease. Later, Katlama would emerge as an international leader in clinical trials of anti-HIV therapies. For example, her team was the first to demonstrate the efficacy of the anti-HIV drug 3TC and among the first to show that combining drugs could lead to better control of the virus.

FREDERIC REGLAIN/GAMMA LIAISON

Reunited. Brigitte Autran *(left)* and Christine Katlama believe the immune system can recover if viral load is reduced.

Thus when Autran and Katlama reunited several years ago, their experiences in basic and clinical research meant they were well placed to study how the immune systems of HIV-infected patients were responding to combination therapy. Their biggest breakthrough came in 1997, when, in collaboration with other French colleagues including Debré and immunologist Jacques Leibowitch at Raymond Poincaré Hospital outside Paris, the pair reported in *Science* that patients in advanced stages of HIV infection who were treated with combination therapy could recover some of their ability to mount immune responses against CMV and the tuberculosis bacterium—two of the most important opportunistic infections afflicting AIDS patients (*Science*, 4 July 1997, p. 112).

In addition, after a year or so of therapy, these patients apparently begin regenerating so-called "naive" T lymphocytes, immune cells that have not yet been exposed to foreign antigens—a key criterion for immune system reconstitution. And more recently, Autran and Katlama, along with Guy Gorochov of Pitié-Salpêtrière, have demonstrated that antiviral treatments can help the crippled immune systems of HIV-infected patients, which are able to respond to fewer and fewer invaders as the disease progresses, to partially recover their capacity to respond to a wider range of invading organisms—another crucial indicator of immune system health.

Autran cautions that she and Katlama have yet to demonstrate that the immune system of HIV-infected patients can be completely restored to normal, although she adds that there may be "no major limitations" to this restoration if the virus can be adequately controlled over the long term. But many researchers credit Autran and Katlama with providing new hope that this welcome possibility might become a reality. The pair's work, says Roederer, "demonstrates that there may be a slow, sustained reconstitution of the immune system ... [which is] the ultimate goal of AIDS therapy."

–Michael Balter