Mycoplasmas in the AIDS Spotlight

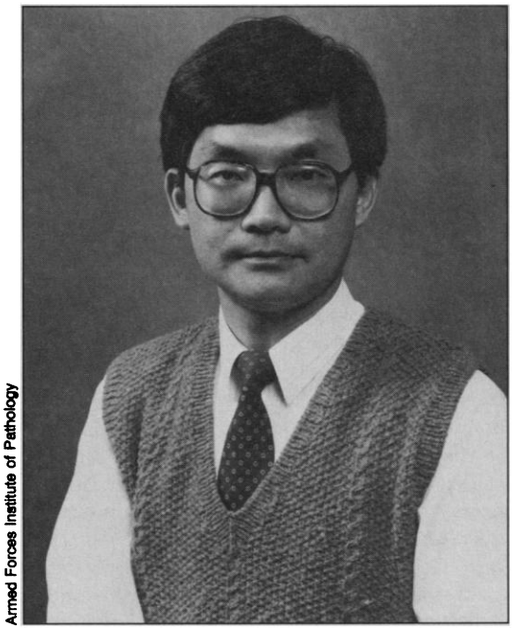
Luc Montagnier now thinks these microbes may have a role in AIDS—bringing a measure of delayed vindication to Shyh-Ching Lo, a tenacious young virologist

IN 1986, SHYH-CHING LO, a young virologist, brought a heap of abuse down on his head when he claimed to have isolated a "novel virus" in tumor cells taken from AIDS patients with Kaposi's sarcoma. In withering attacks, other virologists said Lo's results, based on work begun while he was at the National Cancer Institute (NCI), simply didn't justify his claims. And Lo, who is now at the Armed Forces Institute of Pathology, ruefully agrees. "The data were not very conclusive," he admits.

Many other researchers might have given up, but Lo persevered. Four years later, he is enjoying a measure of vindication. Last year he published a series of papers identifying the organism not as a virus but as a mycoplasma, a primitive microorganism that lacks a cell wall. Last December, his findings withstood intense scrutiny at a special workshop set up for the purpose by the National Institute of Allergy and Infectious Diseases (NIAID). And now Luc Montagnier, co-discoverer of the AIDS virus, has published the results of an independent study in which he names mycoplasmas as a possible cofactor in the cell death caused by HIV infection.*

There's still some uncertainty about the exact identity of the mycoplasma Lo has isolated, and several scientists are expressing reservations about the interpretation of Montagnier's findings. But there's no question that the possible role of mycoplasmas in AIDS is becoming a hot subject.

Montagnier's work, done in collaboration with the French pharmaceutical company Rhône-Poulenc Santé, shows that analogs of the antibiotic tetracycline can protect HIV-infected cells from being lysed by the virus, even though the cells continue to produce virus particles. "The most likely explanation for [our results] is that both HIV and a microorganism act synergistically to induce cell lysis," he concluded in the March issue of *Research in Virology* (formerly the *Annals of the Institute Pasteur*, where Montagnier works). Montagnier told *Science* that his group has since identified the microorganism. "We are completely sure it is a mycoplasma," he said.



Armed Forces Institute of Pathology

Battle scars from the science wars. *Shyh-Ching Lo of the Armed Forces Institute of Pathology.*

Montagnier said he did his experiments before he was aware of Lo's recent work and is now trying to determine if he and Lo are dealing with the same species of mycoplasma. Since lysis of T cells appears to be one way HIV cripples the immune system, the work may ultimately have significant clinical consequences. But most AIDS researchers are greeting Montagnier's report with measured caution.

"In vitro, many infectious agents interact with HIV," says Jerome Groopman of the New England Deaconess Hospital. He notes that cytomegalovirus, adenovirus, herpes simplex virus, and hepatitis B can all potentiate the cytopathic effect of HIV. But those interactions don't have any proven bearing on the clinical picture. Montagnier's findings may or may not, according to Groopman.

"My concern is that the mycoplasma is a contaminant of [Montagnier's] cell line," says Jay Levy of the University of California at San Francisco. Some species of mycoplasma are notorious and indiscriminate contaminants of tissue cultures, and they have been known to affect cell viability. Unless Montagnier can prove that his mycoplasma is not one of those common adulterants, Levy says, "I wouldn't universalize his results."

"Everyone who works with tissue cultures works with the fear of having mycoplasmal contamination," agrees Barbara Laughon, a senior scientist in the developmental therapeutics branch of NIAID. She calls Montagnier's report "an observation that raises more questions than it answers." For example, she notes, the effect of tetracycline could be explained without postulating the presence of a cofactor. Tetracycline antibiotics might alter membrane integrity by binding calcium ions and would thereby interfere with the packaging of virus particles as well. Yet Laughon also says: "We're following this with a great deal of interest."

For Lo, the fact that a co-discoverer of HIV would mention mycoplasmas in the same breath as the AIDS virus is in itself a sort of triumph.

After shooting himself in the foot with his 1986 paper, Lo labored long and hard to gather better data on the agent he had isolated from AIDS patients. To begin with, he detected ribosomal genes in the isolate, indicating that it was not a virus at all, but a prokaryote. He abandoned the term "virus" in favor of the more ambiguous "virus-like infectious agent."

He then marshaled the strengths of gene amplification, electron microscopy, in situ hybridization, and immunohistochemistry to identify the putative agent in tissues. And he found it—in the spleen, liver, brain, lymph nodes, and blood of AIDS patients as well as in the sarcoma tissues.

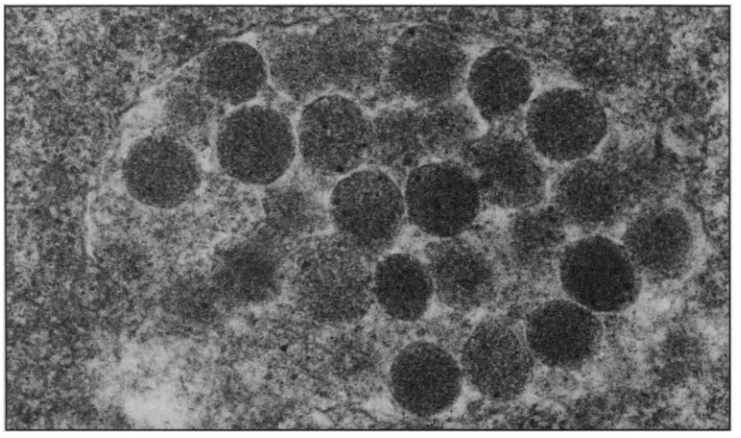
Even more dramatic was his discovery that the agent could cause death on its own. Lo injected four silverleaf monkeys with the isolate; they all died within 9 months. And Lo found the agent in damaged tissues from six HIV-negative patients who had died from unspecified causes 1 to 7 weeks after presenting symptoms suspiciously like those of AIDS.

Predictably enough, Lo had a tough time getting his findings published. "I forget how

* M. Lemaître *et al.*, "Protective activity of tetracycline analogs against the cytopathic effect of the human immunodeficiency virus in CEM cells," *Res. Virol.* **141**, 5 (1990).

many journals turned us down," he says. One colleague put the figure at more than half a dozen. But the delay wasn't entirely unproductive. By the time he had persuaded the *American Journal of Tropical Medicine and Hygiene* to publish the first of five papers in 1989, Lo knew the "VLIA" was a mycoplasma.

That identification prompted a new rash of skepticism similar to the one now being leveled at Montagnier's report. "A lot of people suspected that the agent was a contaminant," says Joel B. Baseman, a microbiologist at the University of Texas Health Science Center at San Antonio, who has studied human mycoplasmal infections for 20 years. But after chairing the December NIAID workshop, Baseman says, he is convinced Lo's results cannot be dismissed. "This is no minor-league effort."



Secret agent. *Lo's mycoplasma shown in the cytoplasm of liver cells of silverleaf monkeys. This may be the first mycoplasma known to kill human beings.*

"Lo's stuff deserves serious review," concurs Jay Levy, who has followed the drama with renewed interest since Lo began publishing again last year.

"Once people become familiar with this data, they generally agree that it's significant," says Robert L. Quackenbush, a bacteriologist at NIAID who pushed for the workshop. "Until last year, most people just thought this stuff was a crock."

Several scientists who attended the December workshop said no effort was spared to try and punch holes in Lo's findings. But the work, on which Lo collaborated with colleagues at the National Institute of Health's Warren Grant Magnuson Clinical Center and the Centers for Disease Control, held up under the gaze of a dozen mycoplasmologists and infectious disease specialists.

And the 40 or so AIDS researchers who saw Lo's presentation at a meeting of the NIAID-sponsored AIDS Clinical Trials Groups in March also gave his work good reviews. "Those people were quite impressed," says John Bartlett, chief of infectious diseases at the Johns Hopkins Hospital. "They agreed that it has to be pursued."

Bartlett and NIAID's Joseph G. Tully, whom some scientists regard as the country's leading mycoplasmologist, have decided to collaborate with Lo and his colleagues to explore the implications of Lo's findings. Tully wants to ascertain whether the agent, which Lo has tentatively called *Mycoplasma incognitus*, is in fact a new mycoplasma species. So far, he says, it appears to be a strain of *Mycoplasma fermentans*, a microbe previously isolated from the urogenital tracts of healthy men and women.

At the very least, Tully says, Lo may have identified the first mycoplasma that is a significant human pathogen. "No other mycoplasma has been found to be clearly associated with fatal infections," he says. Until now, mycoplasmas were known to cause walking pneumonia, arthritis, and occasional spontaneous abortions.

Yet Lo says that since the NIAID workshop at least ten more cases of acute flu-like disease, apparently caused by the mycoplasma, have been documented in HIV-negative individuals. Most resulted in death. But one patient in Fargo, North Dakota, first seen by doctors in December, seems to have recovered after "aggressive" treatment with the tetracycline analog doxicycline.

The question of treating this infection may turn out to be a crucial one for AIDS patients. Lo claims to have found the agent in DNA from 22 of 32 AIDS patients. "If this organism is as prevalent as Lo's data indicate, treatments directed against it could radically improve the quality of life for most AIDS patients," says Quackenbush. But Laughon cautions, "It's premature to think about clinical trials until we know more about the agent."

Montagnier says he is trying to find out how the mycoplasma he identified could potentiate the cytopathic effect of HIV. "This is the beginning of a very interesting story," he hints.

Workshop participants agreed it is crucial to find a quick way to test for the presence of the agent. Standard serological tests for antibodies may not work, because mycoplasmas lack the cell wall of bacteria and therefore often elude immune surveillance. "Without the cell wall, the host just doesn't recognize them as being very bad," says Tully, who adds that the new agent may also actively suppress the immune system, as HIV does.

The search for a diagnostic test won't be aided by the fact that Lo's mycoplasma has proven difficult to culture. In fact, Lo's group has the only apparently pathogenic isolate. That exclusivity caused some rancor last year when Lo delayed sending samples of his agent to several researchers who requested them. He says he wanted to wait until his last paper was published to distribute samples and that he has now sent them to 30 labs.

One of the loudest critics of Lo's failure to give out his materials at the time was Robert Gallo of NCI, whose voice also joined the chorus of disapproval over Lo's 1986 "virus" paper. But for the moment Gallo doesn't have anything more to say about Shyh-Ching Lo. Through an NCI spokesman he refused to be interviewed for this article.

In spite of the changing tone with which scientists are receiving Lo's work, some puzzles linger. The most controversial issue, according to Baseman, has to do with how he obtained his isolate. Lo says he got the isolate by transfecting mouse cells with DNA from an AIDS patient's sarcoma. But only viruses are known to be able to reproduce by transfection, which is one of the reasons Lo initially assumed he had identified a virus. Even simple prokaryotes such as mycoplasmas are thought to be too complex to be assembled by other cells.

"I know plenty of scientists who would say it can't happen," says Baseman. "Unlikely," agrees Montagnier. But the researchers at the NIAID workshop agreed that it was worth trying to replicate the results. Lo's claim that he procured the agent through transfection is at least consistent with another surprising (but less controversial) finding that the mycoplasma appears to be residing inside mammalian cells as well in the extracellular space.

In spite of these remaining scientific questions, one thing seems certain: the claims Lo has made are significant—and problematic—enough to assure that the spotlight will be trained on mycoplasmas for some time to come. Indeed, for these low-profile parasites and veteran contaminants what is coming may be the end of a long, sleepy era.

■ KAREN WRIGHT

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