EMERGING DISEASES

On the Trail of Ebola and Marburg Viruses

Hemorrhagic fever viruses have played starring roles in books and movies. Now researchers are making headway on understanding these real-life threats

MARBURG, GERMANY—This quiet town north of Frankfurt does not seem like the kind of place that would have a deadly virus named after it. On a typical afternoon, university students chatter by the fountain in the Marktplatz, where half-timbered Renaissance houses lean this way and that. Nearby, tourists crane their necks to admire the soaring spires of the 13th century St. Elizabeth's Church, the first Gothic church built in Germany.

But in August 1967, this peaceful scene was shattered when workers in a commercial lab fell ill with a series of alarming symptoms: fever, diarrhea, vomiting, massive bleeding, shock, and circulatory system collapse. Local virologists quickly traced the outbreak—which also occurred at labs in Frankfurt and Belgrade—to monkeys from Uganda that the three labs were using for polio vaccine preparation and other research. A total of 37 people, including lab workers, medical personnel, and relatives, caught the baffling disease, and a quarter of them died. Three months later, a team of German experts isolated the culprit: a dangerous new virus, shaped like a sinister, snaking rod, which had been transmitted to humans from infected monkeys.

The Marburg virus disappeared as mysteriously as it appeared, showing up next in 1975 when a single case was recorded in South Africa. But in 1976 a close cousin, the Ebola virus, made its first and fearsome appearance in the Democratic Republic of the Congo (DRC, formerly Zaire), killing 280 people. Since then, Ebola, Marburg, and other dreaded "hemorrhagic fever viruses" have achieved nearly mythical status. Last month, on the eve of the latest Ebola outbreak in Uganda, and in the midst of an ongoing Marburg outbreak in the DRC, about 100 Marburg and Ebola experts met in Marburg to share their latest results. Although many questions remain—including where these viruses hide between epidemics and how they cause such devastating symptoms—the new research raised hopes that treatments and vaccines might one day be a reality. The findings included the creation of a genetically engineered Ebola virus, which should provide a powerful molecular tool to analyze how these viruses cause disease, and promising, though preliminary, results in monkeys with an Ebola vaccine.

Sinister foe. The deadly Marburg virus *(above)* was first isolated in 1967 at the Institute for Hygiene and Microbiology *(left)* in a quiet German town.

CREDITS: (LEFT TO RIGHT) M. BALTER; CDC/SCIENCE SOURCE/PHOTO RESEARCHERS INC.

Mysterious reservoir

Although the deadly Ebola outbreak in Uganda has been grabbing headlines, the DRC is suffering a less publicized outbreak of Marburg virus, described in talks by Jean Muyembe-Tamfum of the National Institute for Biomedical Research in Kinshasa and Stuart Nichol of the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta. The epidemic began in November 1998 in the northern town of Durba. Workers at a gold mine just outside of Durba were the first to succumb. But the area's remoteness and ongoing local warfare prevented experts from CDC and the World Health Organization from arriving until the following May. Although the outbreak peaked in mid-1999, Nichol told those attending the meeting that new cases were still appearing as late as September 2000, at which time 99 people had been infected, with a mortality rate of more than 80%. Just over half of the victims were gold miners, which provided a possible clue to the virus's origin.

Working with virologist Robert Swanepoel of the National Institute of Virology in Johannesburg, South Africa, Nichol and his colleagues sequenced portions of the Marburg virus's genome. To the surprise of the researchers, the viruses showed an extraordinary genetic diversity—up to 16% difference in their nucleotide sequences—even though they came from what appeared to be a single outbreak. In contrast, the virus strain responsible for a dramatic 1995 Ebola epidemic in Kikwit, DRC, which infected 315 people, showed no genetic diversity at all. From this analysis at Durba, the team concluded that the Marburg virus must have been introduced into the populace at least seven separate times. This finding suggests that this once rare microbe is making new inroads into populated areas, Nichol and Muyembe-Tamfum said.

Searching for the animal reservoir for the virus, the team also trapped more than 500 bats in the gold mine. Many scientists suspect that the natural reservoirs for both Marburg and Ebola are animals, such as rodents or monkeys, with which humans come into regular contact (*Science*, 22 October 1999, p. 654). Bats were a prime suspect because Swanepoel had earlier shown that they could be experimentally infected with Ebola. But so far, that hunch appears to have been wrong. By the time of the meeting, "the vast majority" of the bats had been examined, and none showed signs of infection with Marburg, Nichol said. Although there was "still a glimmer of hope" that some of the remaining bats might harbor the virus, other reservoirs—including possibly arthropods such as insects and spiders—now had to be considered, he concluded.

Leaky capillaries?

Equally perplexing is how Marburg and Ebola cause such devastating symptoms as shock and massive bleeding. Previous research has demonstrated that the virus targets many cell types, especially the macrophages of the immune system and liv-

* Symposium on Marburg and Ebola Viruses, Marburg, Germany, 1–4 October.

er cells. Less clear is whether the endothelial cells that make up the inner surfaces of blood vessels are directly attacked by Ebola and Marburg. Some—but by no means all—researchers believe that damage to these cells, resulting in an uncontrolled flow of blood from capillaries into surrounding tissues, is responsible for the circulatory system collapse that can lead to rapid death.

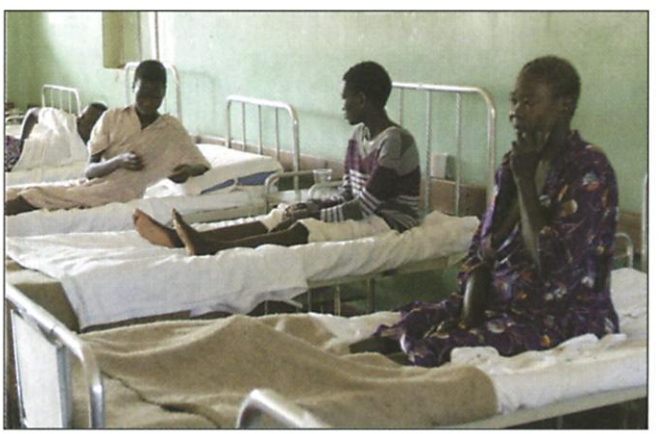
Both CDC pathologist Sherif Zaki and virologist Gary Nabel, director of the National Institutes of Health's (NIH's) Vaccine Research Center in Bethesda, Maryland, argued for a key role for endothelial cells. When Zaki examined autopsy tissues from victims of the Kikwit Ebola outbreak, he found that the capillary endothelium was severely damaged. To explore the cause of this damage, Nabel's team, in collaboration with others at NIH and CDC, genetically engineered cultured human endothelial cells to express the Ebola protein GP, which makes up the virus's outer coat. The results, published in the August issue of *Nature Medicine*, were dramatic. Within 24 hours, the cells could no longer adhere to each other; they died within a few days. And when the gene coding for GP was introduced directly into blood vessels that had been removed surgically from pigs or humans, the vessels suffered massive endothelial cell loss within 48 hours and became much more permeable to fluids. "Increased endothelial permeability and injury to the microvasculature appear to be central to the pathogenesis" of Ebola and Marburg infections, virologist Brian Mahy of CDC told *Science*.

Yet other researchers question the relevance of these findings during a real-life outbreak. Virologist Susan Fisher-Hoch of the Jean Mérieux Laboratory in Lyons, France, argued that Ebola and Marburg victims do not show characteristic signs of leaky capillaries, such as pulmonary edema and swelling of the head and neck. What's more, she added, survivors recover too quickly from even serious shocklike symptoms to have suffered extensive endothelial cell damage. Supporting that view, Thomas Geisbert of the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) at Fort Detrick, Maryland, reported preliminary results from a study of monkeys experimentally infected with Ebola. When the USAMRIID researchers examined the animals at various stages of infection, they saw little endothelial cell damage until the end stages of the disease, when severe symptoms had been present for several days.

"The question is still open at the moment," says virologist Heinz Feldmann of the Canadian Science Center for Human and Animal Health in Winnipeg, Canada.

Designer Ebola

Sorting out the mechanisms of infection may soon be easier, thanks to an advance described by molecular virologist Viktor Volchkov of Claude Bernard University in Lyons, France: a genetically engineered Ebola that will enable researchers to mutate the virus at will to see which of its genes and proteins are most responsible for its deadly effects. "This is a great tool," says Fisher-Hoch. USAMRIID virologist Mike Bray agrees: Volchkov's work was at the "top of the list" of important talks at the meeting, he told *Science*. Last year, Volchkov, in collaboration with colleagues at the Institute for Virology in Marburg, determined the complete nucleotide sequence of the Ebola genome: an 18,959-base-long single strand of RNA.



The current plague. As of 29 October, the Ebola outbreak had sickened 211 people and killed 72. Above, people suspected of being infected are isolated in Lacor Hospital in Gulu, northern Uganda.

CREDIT: S. AZIM/AP

The researchers have now engineered copies of the virus by constructing a DNA molecule with a nucleotide sequence complementary to that of the Ebola genome. When they introduced this complementary DNA into cultured cell lines, along with genes coding for four key Ebola proteins, including the structural protein GP, the cells proceeded to make new Ebola RNA. The result was a lab-created virus that is fully infectious when transferred to other cell lines.

"We can now answer a lot of questions about virulence and pathogenesis," says Bray. By altering the sequence of the complementary DNA, Volchkov's team has already created a mutant Ebola with which to explore how the virus modulates its deadly effects. The mutation in the gene coding for GP—which is highly toxic to target cells—causes the virus to make many more copies of the protein. Volchkov identified a mechanism that gives the virus some "self-control" over how much GP it produces, so as not to kill off infected cells before it can spread efficiently to noninfected cells. Feldmann sees possible applications in vaccine strategy as well: "If we knew how to attenuate the virus, we could make a genetically engineered vaccine."

For years, various teams have been struggling to make vaccines against Marburg and Ebola, with some success in animal models such as guinea pigs and monkeys. At the meeting, Nabel reported that he had made some headway with a DNA vaccine. Using the so-called "prime-boost" vaccine technique, Nabel and his collaborators—including postdoc Nancy Sullivan of NIH and Anthony Sanchez of CDC—injected four monkeys with a prototype vaccine consisting of "naked DNA" complementary to the Ebola GP gene, followed by later injections of the same gene using an adenovirus vector. The vaccinated monkeys, as well as four unvaccinated controls, were then infected with Ebola. Within 7 days, the controls were either dead or dying, while the vaccinated monkeys were still alive and healthy many months later.

These findings, in press at *Nature*, drew mixed reviews from the assembled scientists. "I think [the vaccine] worked, because [the challenge virus] killed the controls," says Bray. Even so, Bray and others cautioned that further experiments are needed before concluding that Nabel's team has a working vaccine. They were especially concerned that Nabel did not specify in his talk the "challenge" dose of Ebola virus used to infect the monkeys—the higher the challenge an animal can withstand, the greater the protection. In earlier work with an experimental vaccine against Marburg, USAMRIID virologists Alan and Connie Schmaljohn had protected monkeys challenged with high doses of that virus. A number of researchers at the meeting told *Science* privately that they believed Nabel's challenge dose was much lower than that used by the Schmaljohns.

Nevertheless, "I am cautiously optimistic that [Nabel's vaccine] is a significant step forward," says Alan Schmaljohn, while also stressing the need for further studies to verify the results. "I will be more comfortable once it is repeated with a higher challenge dose."

Although the meeting revealed considerable progress in understanding these deadly viruses, it may be many more years before this research pays off in terms of help for their future victims. Says Mahy: "Ebola and Marburg will continue to cause severe illness and deaths in Africa … until we have an effective drug treatment or vaccine. This should be a high priority." **–MICHAEL BALTER**