The Hepatitis G Enigma

Researchers corner new viruses associated with hepatitis

By KATHLEEN FACKELMANN

The surgeon first noticed an easily dismissed symptom, fatigue. Then his skin turned mustard yellow. The color was a dead giveaway—he had contracted hepatitis B. This virus attacks the liver and can cause not only the skin-yellowing, flulike illness called jaundice, but sometimes more severe inflammation or even death.

He recovered from that initial bout with the virus and continued to operate. As always, he followed standard infection control procedures. Before entering the operating suite, he scrubbed his hands and forearms with a germ-killing soap. He wore a sterile surgical gown and gloves.

Despite all these precautions, a 47-year-old female patient became ill and was diagnosed as having the hepatitis B virus. Moreover, a squad of federal investigators determined that the surgeon had passed the virus to 19 patients in all. The investigators and their coauthors detail the inquiry in the Feb. 29 NEW ENGLAND JOURNAL OF MEDICINE.

That report on hepatitis B attracted a lot of media attention. Yet another hepatitis story has been unfolding, without fanfare, for more than a year. Researchers have identified a cluster of viruses associated with hepatitis, or inflammation of the liver. They are known in scientific circles as the G viruses.

The alphabet of well-established hepatitis viruses starts with hepatitis A and ends with hepatitis E. These viruses belong to several different genuses. An international team reported finding a hepatitis F virus recently, but others have yet to confirm that discovery.

Researchers worry that, like the other hepatitis viruses, the G viruses will set up shop in the liver, causing persistent infection, damage, and sometimes cancer. In severe cases of hepatitis, the liver may fail, requiring a risky and expensive transplant operation.

Virologist Bernard Roizman of the University of Chicago calls the discovery of the hepatitis G viruses "very important." Researchers may use such knowledge to prevent hepatitis or ward off the harsh consequences of the disease.

As it turns out, the hepatitis G story also starts with a surgeon. This physician, whose initials are G.B., developed a mysterious liver inflammation in 1964. In the years that followed, researchers tried, but failed, to pin his illness on any of the known hepatitis viruses.

That puzzling case would have remained in the archives of medical history had it not been for Isa K. Mushahwar and his viral discovery group at Abbott Laboratories in North Chicago. For 30 years, researchers there kept in storage a frozen sample of the agent that caused illness in the surgeon.

A variety of researchers over the years introduced the infectious agent from G.B. into different primates. It produced noticeable illness only in monkeys called tamarins. Despite many studies of infected tamarins, the identity of the agent continued to elude the scientists.

The Abbott researchers recently decided to try again to pin down the agent—this time using the tools of modern molecular genetics. They began by collecting blood from healthy tamarins and then injecting the animals with serum from animals infected with the G.B. agent.

These tamarins developed hepatitis, and the researchers again drew blood. Next, they turned to a technique called representational difference analysis (RDA) to compare the pre- and post-illness blood of each tamarin. With RDA, researchers could identify DNA sequences present only in the blood of the sick animals.

"To our surprise, we found the existence of not only one virus but two," Mushahwar told SCIENCE NEWS. The Abbott team's evidence suggested that the viruses belong to the *Flavivirus* genus, which includes the viruses that cause hepatitis C, yellow fever, and dengue. Mushahwar and his team described their work in the April 11, 1995 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES.

The group then began to study blood collected from people living in West Africa, a region where hepatitis is endemic. That effort flushed out yet another hepatitis-associated *Flavivirus*, the team reported in the June 1995 NATURE MEDICINE.

The researchers named the trio GBV-A, GBV-B, and GBV-C, after the surgeon whose blood left a legacy of hepatitis. SCIENCE NEWS refers to them as hepatitis G-1, G-2, and G-3, respectively, to avoid confusion with the other hepatitis viruses.

The similarity of the new virus to hepatitis C is worrisome, notes James Koziarz, vice president of research and development at Abbott Labs. The various hepatitis viruses differ in their likelihood of producing chronic infection. Almost everyone infected with hepatitis C becomes a carrier of the virus, Koziarz says. In about 20 percent of those cases, hepatitis C infection eventually destroys the liver.

The million-dollar question for Abbott and other scientists is whether the G viruses account for the substantial number of viral hepatitis cases not linked to hepatitis A through E. The puzzle of what's behind such unexplained viral hepatitis drives a number of research labs, including one led by Jungsuh P. Kim, a molecular virologist at Genelabs Technologies in Redwood City, Calif.

Kim and her colleagues at the Centers for Disease Control and Prevention (CDC) in Atlanta focused on a vial dubbed PNF2161. That vial contained serum taken from a patient in the United States who had contracted a mysterious case of viral hepatitis.

Using a different molecular technique from the Abbott group's, Kim and her colleagues isolated a hepatitis virus from the serum. Kim first described that virus at the 16th U.S.-Japan Joint Hepatitis Panel Meeting held in Tokyo in January 1995. The team published a full description of its work in the Jan. 26, 1996 SCIENCE.

Kim and her colleagues compared their virus to those isolated by the Abbott group. They reported that their virus appears to be "very closely related" to the hepatitis G-3 virus, which was isolated from a West African patient.

In a commentary in the March 2 LANCET, Arie J. Zuckerman of the Royal Free Hospital School of Medicine in London points out that the sequence of chemical constituents, or nucleotides, of each of the two viruses match up closely. They are

"independent isolates of the same virus," Zuckerman writes. SCIENCE NEWS refers to both isolates as hepatitis G-3.

Researchers would expect slight differences in the nucleotide sequence that makes up each isolate's genetic material, Mushahwar says, adding that viruses tend to mutate as they travel around the globe.

Further testing by the Abbott group revealed five subtypes of hepatitis G-3. Moreover, certain ones appear to flourish in discrete regions of the world. For example, one subtype is found primarily in the United States and Europe. Another makes its home in Southeast Asia. Abbott scientists plan to describe the five types of hepatitis G-3 at an international symposium on viral hepatitis and liver disease later this month in Rome.

The Abbott group began to focus on hepatitis G-3 when research showed that G-1 appears to be primarily a tamarin virus that was transferred from one monkey to another along with the G.B. infectious agent. They also failed to find hepatitis G-2 in most of the human blood they have screened—at least so far. In contrast, the Abbott team and others have found a high rate of human infection with hepatitis G-3.

"We started testing, and we found it everywhere," Koziarz says. The team has documented evidence of hepatitis G-3 infection in people in the United States, Canada, Peru, Egypt, West Africa, and Europe.

In unpublished research, the Abbott group notes that about 1.5 percent of Japanese hepatitis patients who do not have the A through E varieties are infected with the G-3 virus. Moreover, 18 percent of similar West African patients have hepatitis G-3 RNA in their blood.

The data from Kim's group also show infection with the virus. Six of 48 patients with unexplained hepatitis in South America had hepatitis G-3 infections, as did 9 of 110 such patients in Europe.

Healthy people may also carry the hepatitis G-3 virus. The Abbott team's research indicates that the virus was present in the U.S. blood supply 25 years ago. Kim's group now finds that between 1 and 2 percent of today's volunteer blood donors, at least in the United States, have a previously undetected hepatitis G-3 infection. That's higher than the rate of infection with hepatitis B or C, notes Harold Margolis, chief of the hepatitis branch at the CDC. Margolis is a coauthor of the SCIENCE article.

Blood banks currently screen for the hepatitis B and C viruses. (The hepatitis A virus typically isn't transmitted through exposure to infected blood.) They do not yet have a way to screen for hepatitis G and the other newly discovered viruses, points out another SCIENCE coauthor, Harvey Alter of the National Institutes of Health in Bethesda, Md. The Abbott team and Genelabs are working on a way to screen blood for hepatitis G-3.

If up to 2 percent of blood donors carry this virus, and if it triggers disease, one would expect to see a spiraling incidence of transfusion-associated hepatitis, Alter says. However, blood banks have seen just the opposite. "The rates of transfusion-transmitted hepatitis are coming way, way down and are now approaching zero."

Nevertheless, a hepatitis G-3 threat, if it exists, may take years to become apparent. After a brief attack, the virus may remain in the body for years. Margolis and his group at the CDC studied 38 cases of unexplained viral hepatitis reported to U.S. county health departments. Of the five people with hepatitis G-3 infection, four carried the virus for 2 to 9 years after the acute illness subsided. The researchers have not yet examined whether these people have liver damage.

Some researchers speculate that it takes years for hepatitis G-3 to do its dirty work. They hypothesize that at first, the virus replicates quietly in the liver. Only after many years does the damage show up.

Evidence that the G-3 virus is linked to long-term disease was published in the Oct. 28, 1995 LANCET. Japanese researchers led by Shunji Mishiro at the Toshiba General Hospital in Tokyo looked at six cases of fulminant hepatitis, a progressive form of the disease in which the liver fails. The cases were not linked to any of the known hepatitis viruses. Mishiro's team found that three of the six people were infected with hepatitis G-3.

"The clinical course of the disease in these patients was characterized by slow progression," they write in the journal. "The results suggest the importance of [hepatitis G-3] in the etiology of fulminant hepatitis." But the Japanese authors point out that their sample is too small to draw any firm conclusions.

Much of the basic biology of hepatitis G-3 remains an enigma. What researchers can say for certain is that it can be spread by contact with infected blood.

Mushahwar and his colleagues wonder if the virus, like the one that causes yellow fever, can be spread through the bite of an infected mosquito. Their study of infected families in West Africa has yet to turn up a route of transmission for hepatitis G-3. In one case, a teenage boy and several members of his family all had the virus.

"There was no history of blood transfusion, no tattooing, no intravenous drug use," Mushahwar says. "How did those people get it [the virus]?"

The clinical picture of hepatitis G-3 infection also remains murky.

"We do get the sense that people remain persistently infected, " Margolis says. However, infection doesn't necessarily translate into disease. People with hepatitis G-3 infection seem to remain healthy for years.

Over decades, however, some people infected with the hepatitis C virus do develop serious problems, including liver damage. Will hepatitis G-3 show the same pattern? "We don't yet have the data," Alter says.

He goes on to caution that researchers have yet to close the link between hepatitis G-3 and liver disease. To clarify its relationship to hepatitis, researchers still need to determine whether hepatitis G-3 lives in and destroys liver tissue.

As for its origin, researchers won't even hazard a guess. "It's not a new virus," Alter says. Hepatitis G-3 may simply be an old virus that is new to the scientific community. □

Viral transmission

The U.S. surgeon who gave hepatitis B to 19 patients is not alone. A Spanish surgeon who passed hepatitis C to five of his patients while performing open-heart surgery is also described in the Feb. 29 NEW ENGLAND JOURNAL OF MEDICINE.

In 1991, the Centers for Disease Control and Prevention (CDC) in Atlanta issued guidelines designed to minimize the risk of patients' catching a virus from an infected doctor or other health care worker. The guidelines require hospitals to inform patients of the risk of viral transmission during an invasive procedure such as surgery.

The safety rules sometimes fall short, says Julie Louise Gerberding of the University of California, San Francisco in an editorial accompanying the articles.

The U.S. surgeon told CDC investigators he had often noticed a pain in his index finger while tying sutures. The researchers discovered that the force of tying knots in heavy-grade suture material created tiny nicks in the surgeon's skin. Still, the virus had to pass through breaks in the surgeon's glove to infect the patient, notes CDC epidemiologist Rafael Harpaz. The suture theory has yet to be proven, he told SCIENCE NEWS.

In the case of the Spanish heart surgeon, Jaime Guardia of the Universitat Autònoma in Barcelona and his colleagues determined that the surgeon probably passed hepatitis C along when he was closing the sternum with wire sutures. That strenuous part of open-heart surgery often results in cuts on the doctor's hands, they note.