INFECTIOUS DISEASES

Hard-Won Advances Spark Excitement About Hepatitis C

Years of research are paying off with a new mouse model and insights into natural immunity, raising hopes about drugs or vaccines to fight the epidemic

PARIS—In the mid-1970s, Harvey Alter of the U.S. National Institutes of Health sent out an SOS. A new form of hepatitis was attacking the livers of some blood-transfusion recipients, and Alter wanted help tracking down the culprit. Not until 1989 did Michael Houghton and his team at Chiron Corp. in Emeryville, California, identify the elusive pathogen—hepatitis C virus (HCV). Blood centers began screening for the virus the following year, stanching the flow of HCV-contaminated blood into the blood supply.

But scientists soon realized they were dealing with a viral time bomb that they had few tools with which to disarm. Spread primarily by direct contact with human blood, HCV had already infected millions of people through transfusions or unsafe medical practices. HCV continues to infiltrate developed countries, mainly by needle-sharing drug users. And in developing countries, medical practitioners reusing inadequately sterilized needles inadvertently inoculate millions of patients with the pathogen.

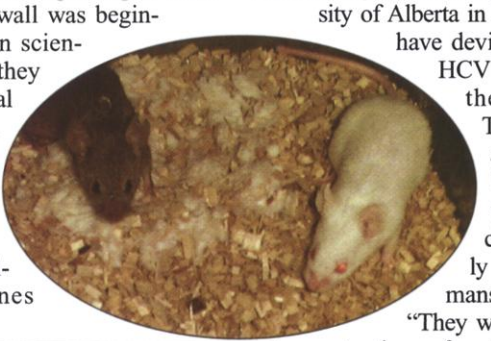
At least 3 million to 4 million people worldwide are infected each year, according to the World Health Organization, compared to an estimated 5.6 million new infections of the virus that causes AIDS. Once infected, about 20% of people clear the virus from their bloodstream, but the rest will harbor HCV the rest of their lives. Of those chronic cases, many may never have symptoms, but 10% to 20% eventually develop liver-destroying cirrhosis or cancer.

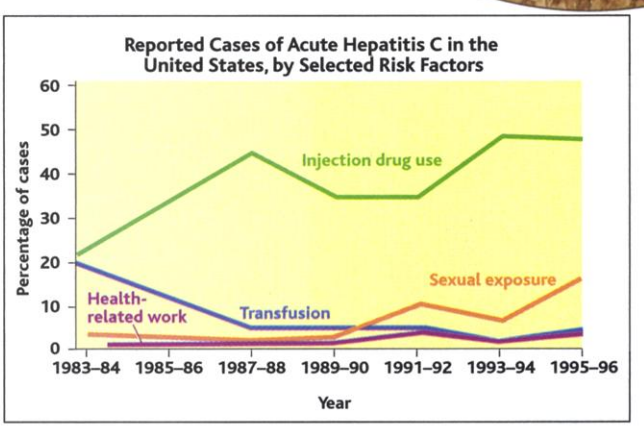
As the extent of the HCV epidemic hit home, dozens of research teams began trying to find ways to prevent infection or more effectively treat it. But the scientists immediately ran into a brick wall: For reasons still baffling, the virus stubbornly refuses to grow in the lab, greatly handicapping research efforts. Now, a dozen years after unmasking the pathogen, there is still no vaccine or specific antiviral drug. The best available treatment combines interferon, which modulates the immune system, with ribavirin, a nonspecific antiviral drug. But the regimen succeeds in only 30% to 50% of cases, and side effects are significant.

Last month, some 1000 hepatitis C researchers met here at an annual conference to discuss the headway they have made recently in understanding and taming the virus—including a new animal model—as well as some letdowns.

At a meeting 2 years ago, hopes were raised that the brick wall was beginning to crumble when scientists announced that they had devised a potential way to grow HCV in tissue cultures (*Science*, 2 July 1999, p. 110). Ralf Bartenschlager and his team at the Institute for Virology at Johannes Gutenberg University in Mainz, Germany, built truncated versions of the virus's genetic material, called replicons. Inserted into human cells grown in tissue culture, the replicons began to churn out proteins the real HCV uses to make copies of itself. About a year later, the Mainz team as well as researchers led by Charles Rice, then at Washington University School of Medicine in St. Louis, Missouri, announced separately that they had each improved on the replicon by identifying mutations in HCV genes that increased replication (*Science*, 8 December 2000, p. 1870). Since then, several scientists have been adding the complete complement of HCV genes to the truncated replicons, hoping to produce intact virus.

But at last month's meeting, Bartenschlager's team announced that although they successfully made an entire HCV genome that replicates efficiently in tissue culture, the proteins it produces fail to knit themselves together into virus particles. "It is disappointing," says Bartenschlager. "What's more disappointing is we really don't know why. Maybe there is a host cell factor required for proper interaction of the proteins, and this factor is missing in the cell culture. But that's just one possibility."





Blood-borne danger. Hepatitis C is spread by direct contact with blood, although scientists suspect other routes may exist. A new mouse model (*above*) may accelerate research.

Supermice

While researchers continue trying to grow an intact virus in cells, Norman Kneteman and a team of surgeons and scientists at the University of Alberta in Edmonton, Canada, have devised a way to grow HCV in mice: by giving them human livers. The chimeric mice are a handier and less expensive in vivo model than chimpanzees, the only animal besides humans that HCV infects. "They will revolutionize the testing of antivirals," predicts Christopher Richardson of the Ontario Cancer Institute in Toronto, who is already using the mice to test a potential gene therapy for HCV infection.

These are no ordinary mice, even before they receive grafts of human liver. Kneteman's group spent 3 years selectively breeding them from two types of genetically engineered mice: mice with severe combined immunodeficiency (SCID) and mice carrying a sequence of genes, called *Alb-uPA*, that disrupts normal liver cell function. The Alb-uPA mice have sickly, white livers. But, thanks to the unique ability of mammalian livers to regenerate, faulty livers are constantly being stimulated to grow new cells. So, the offspring of the Alb-uPA and SCID mice have livers that work in overdrive to grow new cells and immune systems that are unable to recognize foreign tissue.

And that is just what the researchers needed for successful transplants of human tissue, as they reported in the 1 August issue of *Nature Medicine*. With a technique honed to a 5-minute procedure, the surgeons transfer about a million human hepatocytes, taken from either fresh or frozen liver tissue, into the spleen of a 10- to 14-day-old SCID/

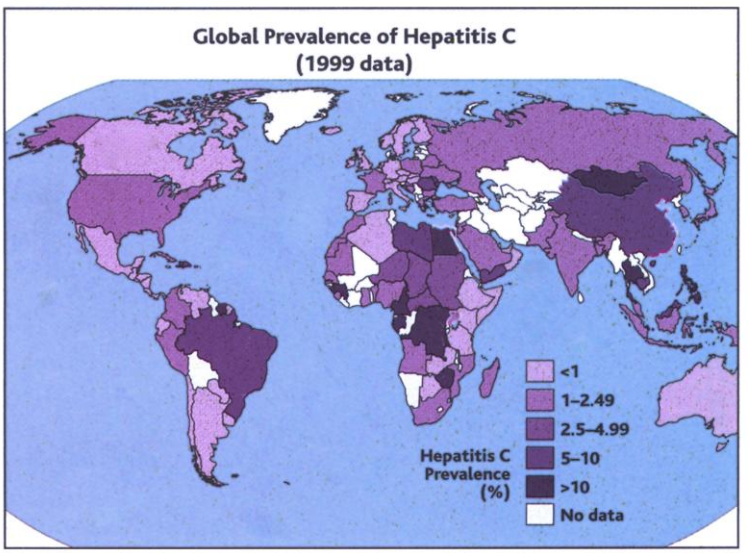
CREDITS: (LEFT TO RIGHT) SOURCE: CDC; BELINDA HSI/UNIVERSITY OF ALBERTA AND N.M. KNETEMAN

Alb-uPA hybrid. After 6 weeks, the rodent liver boasts red nodules of functioning human cells. Mice that receive a copy of the *Alb-uPA* gene sequence from each parent grow enough human liver to develop a chronic hepatitis C infection when inoculated with the virus.

Kneteman's group is already using the homozygous SCID/Alb-uPA hybrids in a number of experiments. At the meeting, the team reported that HCV-infected mice treated with interferon for 2 weeks had lower amounts of HCV in their blood than untreated mice. Scientists think interferon works by both revving up the immune system and directly disarming HCV. Because the mice lack key immune system defenses, having fewer viral particles indicates that interferon does indeed exert an antiviral effect. Next, the team plans to expand the study to the widely used combination of interferon and ribavirin, the newer pegylated interferon, and small antiviral molecules.

But the new SCID/Alb-uPA mice have drawbacks. "They are tricky to propagate," explains Rice, now at Rockefeller University in New York City. "And anything that involves getting very fresh and, hopefully, normal liver tissue is challenging."

Scientists won't have to build their own mice, however. Kneteman and colleagues have applied for a patent and formed a company, KMT Hepatech, to expand their breeding colony of homozygous mice and make them available to researchers. For now, to ensure quality control, all studies with the mice will be done in the Edmonton lab, Kneteman says. Outside scientists doing basic research on HCV can use the mice in exchange for co-authorship of published papers resulting from the work; studies of potentially commercial ideas or products would be covered by an upfront agreement to share research costs and resulting revenue. "Our goal is to make the biggest impact on the disease," says Kneteman. "We don't want to limit the availability of these animals."



Silent spread. Because symptoms often don't appear until years after infection, the virus has spread across the globe; figures are rough, but an estimated 3% of the world's population is now infected.

CREDIT: WHO

Vaccine hopes

Although the lack of a small-animal model has been a big handicap for efforts to develop a vaccine against HCV, researchers have also been discouraged by hints that the immune system doesn't seem to remember earlier encounters with the virus. This could be a huge drawback, because vaccines rely on immunologic memory: the ability to recognize an earlier invader. At the meeting, however, several groups reported that human and chimpanzee immune systems do indeed remember HCV and can thus mount quick and robust counterattacks.

In the early 1990s, scientists noted that chimps that had overcome an initial acute HCV infection would develop acute infections again whenever they were reinoculated. The reinfections suggested that T cells and other components of the immune system failed to recognize HCV even though they had battled it before. Those studies "really boded poorly for HCV vaccine development," says Christopher Walker of the Children's Research Institute at Ohio State University, Columbus.

Walker is one of several researchers who recently repeated the "rechallenge" experiments on chimps, using more sensitive techniques for measuring the level of cellular immune response. Examining the liver and blood of chimps that had cleared an HCV infection 4 to 5 years earlier, Walker's group found that large numbers of CD4 and CD8 T cells were still primed to attack the virus. When reinoculated with HCV, the chimps developed less severe infections compared with their first bout with the virus. The number of viral particles in the blood at the peak of infection was 100 times lower, and the virus disappeared from the blood in an average of 14 days rather than 4 months.

Human immune systems are similarly primed, other scientists reported. Georg Lauer of Massachusetts General Hospital in Boston described two patients who had shrugged off HCV infections at least 2 years earlier but still had a "huge" number of CD8 T cells—at least 5%—preprogrammed to recognize HCV. The cases are "rare extremes," says Lauer, but they could provide insights into mechanisms for controlling HCV or developing long-term immune responses to it.

Another team, led by David Thomas of Johns Hopkins University School of Medicine in Baltimore, reported that humans have shorter and milder acute infections on subsequent exposure to the virus, much as chimpanzees do. In a 2-year study, Thomas and colleagues tracked HCV infections in more than 250 injection drug users who came in for checkups every 6 months. During the study, some individuals became infected with HCV for the first time, and others became reinfected. When the researchers compared the two groups, they found that those with first-time infections, as opposed to reinfections, were twice as likely to have viral particles in their blood for at least two consecutive visits and the number of particles was 100 times more at peak infection.

Finding natural immunity, say Thomas and others, suggests that a therapeutic vaccine may be possible. Such a vaccine may not prevent an acute infection, but it might prime the immune system to clear out the virus quickly and prevent a life-threatening chronic infection. A vaccine might also benefit people with chronic infections by eliminating the virus or keeping the viral load in check so that cirrhosis or cancer would be less likely to develop.

Pharmaceutical companies are already working on such vaccines. Innogenetics NV in Ghent, Belgium, for instance, is now conducting a phase II clinical trial for a therapeutic vaccine based on recombinant E1, a viral glycoprotein found on the surface of HCV. Chronically infected patients produce few or no E1 antibodies, whereas patients who shrug off the virus make large quantities of E1 antibodies, often in combination with a strong T cell response. Similarly, Chiron is gearing up for phase I trials of two potential vaccines. One is based on E1 and E2, another viral surface glycoprotein. Chiron's second candidate is based on the HCV core protein, which surrounds the genetic material of the virus. Chiron's Houghton envisions using the vaccines "alone or in combination."

"The mood among researchers has really changed," says Houghton. "There's now more optimism for vaccine development. More and more of us think we may be able to protect the majority of individuals from chronic HCV infection." **–CHARLENE CRABB**

Charlene Crabb writes about science from Paris.