The Scientific Challenge of Hepatitis C

Jon Cohen

A virus that infects some 170 million people worldwide is causing rising rates of liver disease; like HIV, it is a wily foe for researchers developing drugs and vaccines. HIV may hold some lessons

Nobody would have mistaken the international conference on hepatitis C, held last month at the National Institutes of Health (NIH), for an international conference on AIDS. For the past decade, international AIDS meetings have attracted roughly 10,000 researchers; only 775 scientists attended the 4-day hepatitis C meeting. While drug companies working on anti-HIV drugs jam the exhibit halls at AIDS gatherings, not a single company set up a stand at the hepatitis C meeting. If activists infected with hepatitis C virus, or HCV, attended the gathering, none made their presence felt. Not a single press conference was held, reflecting the low media turnout (one). Yet HCV has infected an estimated 170 million people worldwide--more than four times as many as HIV--and, during the next few years, the number of annual U.S. deaths from HCV-caused liver damage and cancer may overtake deaths caused by AIDS.

Despite these disparities, on a scientific level, the similarities between the HCV field today and HIV research in the 1980s are striking. "There's so much you can learn from HIV," says David Thomas, a Johns Hopkins University clinician who studies and treats both viruses. Like their counterparts studying AIDS in the early 1980s, HCV researchers still can't grow the virus in laboratory cultures, and they don't know precisely how it infects a cell. They also have but foggy notions about the timeline between infection and illness, the so-called "natural history" of the disease. Currently available drugs, like early AIDS therapies, have serious toxicities and fail in most people--and no one knows for sure why some people respond to treatment and others do not. Nor have vaccines lived up to early hopes; just like HIV, HCV mutates rapidly, creating a swarm of different viruses in each infected person that can thwart antibodies easily. And, reminiscent of the struggles over patents on AIDS tests, lawyers from companies making diagnostics and drugs are firing salvos at each other over HCV patents (see sidebar, p. 28).

HCV is not, of course, HIV. The hepatitis virus does not splice itself into the genes of a host, which means it may be easier to eradicate from a person's body. Indeed, some people become infected for several weeks and then naturally clear HCV from their bloodstream. HCV also does not target and destroy the immune system, and it may not cause clinical symptoms for decades in most of the people who become chronically infected. And, unlike HIV, HCV is rarely transmitted sexually; it seems to require direct blood-to-blood contact. Still, differences aside, HIV holds up an interesting mirror to the young HCV field, where "I don't know" remains the most common answer to a question.

The NIH meeting came at an important juncture in the scientific battle against HCV. An improved therapy, approved just 6 months ago, has galvanized the field, and at least partial answers are now emerging to some of the most formidable unknowns about the virus. NIH also is increasing funding for HCV research (see table)--although the $33.6 million it plans to spend next year pales in comparison to the $1.8 billion to be spent on HIV. And as the barriers fall, more and more researchers--many of whom specialize in HIV--are being drawn to the field. Says Thomas: "It's like this smoldering fire that's finally starting to catch."
Elusive epidemic

For years, clinicians knew that something in the blood supply was causing a small fraction of transfusion recipients to suffer short-lived flu-like symptoms followed in some cases by liver disease years later. To distinguish the disease from better known forms of hepatitis, they referred to it by the ungainly but descriptive name of non-A, non-B hepatitis. Researchers from Chiron Corp. and the Centers for Disease Control and Prevention (CDC) finally unmasked the insidious agent in 1988. The next year, they jointly published papers in Science describing the new virus and a way to test for it in blood samples (21 April 1989, p. 359).

To the surprise of many nonhepatologists, hepatitis C has little in common with its more famous cousins, A and B, except that all inflame the liver. HCV hails from a family known as Flaviviridae, and its close cousins include viruses that cause bovine diarrhea, hog cholera, and yellow fever. Carrying a single strand of RNA, HCV contains just one gene, coding for a polyprotein that is subsequently spliced into at least 10 functional proteins (see figure). Scientists have identified more than 100 strains of the virus and grouped them into six major "genotypes," which tend to cluster in different regions of the world.

No compelling clues point to where or how HCV first infected humans, and no other species appears to serve as a natural host to the virus. Yet studies clearly have shown that the main routes of transmission are by tainted blood transfusions and dirty needles used by injecting drug users, practitioners of folk medicine, and even public health campaigns (see sidebar, p. 27).

Genetic blueprint. HCV’s one gene produces a polyprotein that splits into at least 10 proteins.

The development of a screening test in 1990 has virtually eliminated the spread of HCV through transfusions in industrial countries, and sharing contaminated needles is now by far the most common route of infection. As a result, CDC estimates that new U.S. HCV infections dropped from about 230,000 a year in the 1980s to fewer than 36,000 in 1996. But, because most of those infected in past decades are still alive, CDC estimates that perhaps 1.8% of the U.S. population harbors the virus. And as those patients age, HCV-related liver disease—which now accounts for 8,000 to 10,000 annual deaths in the United States and is the single most common reason for liver transplants—likely will increase.

Aside from direct blood contact, HCV is a very difficult agent to transmit. Even maternal-to-fetal transmission is low; no more than 6% of babies born to infected mothers will carry the virus. One question that is still being debated, however, is whether HCV can be transmitted sexually.

At the meeting, the CDC’s Miriam Alter reviewed several studies that "associate" HCV infection with having multiple sexual partners, leading to CDC's official conclusion that sex accounts for between 10% and 20% of the infection in the United States. "I think efficiency is very, very low, but it does happen," Alter says. "Given that sex is frequent and 80% of the population have had more than one partner in a lifetime and that there are a number of chronically infected people, it makes sense."

Yet several lines of evidence argue against sexual transmission. As HIV proves, homosexual men in the United States transmit blood-borne viruses more efficiently than do heterosexuals, yet gay men have no higher HCV infection rates than do heterosexuals. And in a long-term study of 116 "discordant" couples, in which only one partner was initially infected with HCV, Harvey Alter (not related to the CDC’s Miriam) and his co-workers at the NIH’s Clinical Center found just 16 cases of new infections. In every one of those cases, the person who became infected had a history of injecting drug use or a blood transfusion. "So we don’t have any direct evidence of sexual transmission,” concludes Alter. He now plans to compare the viruses in each of the 16 infected couples to confirm his suspicions that they did not infect each other.

Who will become ill?

Once scientists connect a bug to a disease, they can begin to unravel the natural history of a typical infection—how the disease progresses, and how long the process takes. Yet for HCV there appears to be no such thing as a typical infection. The severity of the disease varies greatly from person to person and—as the frustration of clinicians and patients—there are few reliable indicators to predict who will do well or badly.

Evidence accumulated over the past few years indicates that the immune systems of 15% to 25% of people infected with HCV will overcome the virus during the initial infection and clear it from the bloodstream. The remaining 75% to 85% will develop a chronic infection. HCV targets liver cells, called hepatocytes. As hepatocytes die off, fibrous tissue forms, which scars the liver, preventing blood from passing through it and leading to the life-threatening condition known as cirrhosis; this occurs in perhaps 10% to 20% of chronically infected people. Another 1% to 5% of the chronically infected also develop a liver cancer called hepatocellular carcinoma. Yet, as several studies presented at the international meeting show, the majority of patients have none of these symptoms even 20 years after infection.

NIH’s Alter and Jay Hoofnagle from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) reported results from a 7-year study of more than 400 would-be blood donors who had tested positive for HCV and whose infection could, in most cases, be traced to a transfusion or injection. Even though they had been infected for an average of nearly 20 years, only 13% had severe fibrosis and a mere 2%
had cirrhosis. These results closely match those from an Irish study published in the 22 April *New England Journal of Medicine* that charted disease progression over 17 years in 376 women who had received contaminated blood products in the 1970s. And a study by NIDDK's Leonard Seeff looked at 8568 blood samples stored by the U.S. Air Force between 1948 and 1954 and found that 17 tested positive for HCV antibodies; current records revealed that only one of those infected individuals (5.8%) died from liver disease.

Alter finds these results reassuring for the majority of patients. "What's needed in this field is some perspective," he says. "Hepatitis C is getting such big play, mostly with a sense of alarm. We spend a lot of time trying to calm people down. The real issue is, What proportion of people are going to reach the bad outcome?"

For individual patients, however, the real issue is, "Am I going to have a bad outcome?" So far, clinicians are hard put to provide an answer. They have found little correlation, for example, between the amount of virus in a patient's blood—the "viral load"—and disease progression. And several studies have found that none of the six HCV genotypes appears to be more pathogenic than the others. Alcohol consumption may increase the risk of disease progression--two of the seven patients with cirrhosis in the Irish study were heavy drinkers, for example--but, again, the risks appear to vary. Moreover, data from NIH's blood donor cohort presented at the meeting indicate that one commonly used test to assess liver damage may have little predictive value, too. This blood test measures an enzyme called alanine aminotransferase (ALT).

Hepatocytes release ALT when they die, so ALT levels should provide an indirect measure of how much damage HCV is doing to a liver. But in a study that began in 1993, Marc Ghany, a researcher in Hoofnagle's NIDDK lab, separated 60 HCV-infected people into three groups based on whether they had normal, mildly elevated, or moderately elevated ALT levels. Over the next 5 years, most participants' ALT levels remained in the same category. But liver biopsies showed that people with normal ALT levels had, on average, worsening of their fibrosis, while those in the mild and moderate categories had slight improvements. Hoofnagle cautions that these preliminary data come from a relatively healthy infected group, but he says they are "disturbing" nonetheless. "If you follow a person and ALTs are normal, we always thought that their [degree of fibrosis] would be near normal," says Hoofnagle. "This finding goes contrary to everything we believe."

Researchers say a better understanding of the natural history of HCV infections will require a major, long-term cohort study, such as the 15-year Multicenter AIDS Cohort Study (MACS) that has shed valuable light on the natural history of HIV. Leslye Johnson, who heads the enteric and hepatic disease section at the National Institute of Allergy and Infectious Diseases (NIAID), notes that a 1997 "strategic plan" put together by her institute called for such studies, but they have not been funded. "It's a money issue," says Johnson. Indeed, Johnson, whose institute this year spent about $7.7 million on HCV, says lack of funding is crimping other areas as well. "I put together an initiative list last year for internal consumption," she says. "I said I'm not going to ask for what I know I can get, I'm putting down what I need." Johnson's figure: $40 million. "Branch chiefs looked at it and rolled their eyes."

**A cultural barrier**

It's not money, however, that hepatitis C researchers mention when asked what the field needs most. As Frank Chisari, a leading hepatitis immunologist at The Scripps Research Institute in La Jolla, California, puts it, "We desperately need a culture system." To date, no one has been able to grow HCV reliably in a laboratory culture of cells, a lack that has slowed critical studies of everything from drugs to vaccines to basic understanding of the viral life cycle. Little wonder, then, that the showstopper at the NIH conference was a report of a new HCV culture system, which is described on page 110 of this issue.

Developed after 5 years of effort by Ralf Bartenschlager and colleagues at the Johannes-Gutenberg University in Mainz, Germany, the culture system does not actually grow HCV itself. Rather, Bartenschlager's group engineered a stretch of DNA that contains the mirror image of a portion of HCV's RNA. Bartenschlager injected this "replicon," which codes for HCV's nonstructural proteins but not its core or surface proteins, into immortalized human cell lines. The replicon then copied itself to high levels, which he showed both by polymerase chain reaction assays and by measuring viral proteins.

"It's a groundbreaking study," says NIDDK's Jake Liang, who with Hoofnagle co-organized the conference. "People have to be cautioned--this is not productive infection. It does not generate virus. Still, it's a major step in the right direction." Stanley Lemon of the University of Texas Medical Branch at Galveston adds that "if these results hold up, they'll be enormously useful for drug screens."

Because the replicon does not produce whole viruses and their attendant envelope proteins, however, researchers cannot use it to determine how HCV infects cells--a critical question that has been frustratingly difficult to answer. As a team led by Sergio Abrignani of Chiron's Siena, University School of Medicine in St. Louis, Missouri.

**Treatment: Limited success**

Until last year, people infected with HCV had only one choice of treatment: three injections a week of interferon--a chemical messenger naturally produced by the immune system--for up to a year. For most, it wasn't much of a choice; the drug eliminated the virus in less than 20% of patients. But last fall, the field was jolted by the results of two major trials that showed that combining interferon with an antiviral drug called ribavirin at least doubles the chances of success in people who had never been treated.
The first of the two studies, published in the 31 October 1998 *Lancet*, compared 48 weeks of treatment with either interferon-α-2b alone or the two-drug combination in 832 HCV-infected people. Forty-three percent of the patients on the combination therapy had no detectable HCV RNA in their blood 24 weeks after treatment, while only 19% of those taking interferon alone had a similar “sustained” virological response. One promising sign: Unlike HIV, HCV does not appear to lurk in hard-to-treat reservoirs from which it will reestablish an infection.

The second study, published in the 19 November 1998 *New England Journal of Medicine*, involved 912 patients. Again, 24 weeks after treatment stopped, 38% of those who took both drugs had undetectable HCV RNA, compared to 13% of those on monotherapy. Just 1 month after the study was published, the Food and Drug Administration approved the combination therapy for previously untreated patients.

Promising as these results are, the drugs are expensive—a 48-week treatment costs nearly $20,000—and cause debilitating flulike symptoms in most people, leading about 20% of the patients in both trials to stop treatment early. "Interferon is a difficult drug to take, and ribavirin makes it worse," says NIH’s Alter. More sobering still, the drugs are less effective against the most common strain in the United States, known as genotype 1, which is responsible for about 70% of U.S. infections. The *New England Journal* study, for example, found that only 28% of people with genotype 1 had a sustained response, compared to 66% of those infected with other genotypes. (Other factors also affect the likelihood of success: Women, patients under 40, those with low viral loads, and those without severe liver damage all tend to do better.)

These results have sparked a sharp debate over whether to treat asymptomatic patients. Alter says the high failure rate and the toxicity make him cautious. "Drug companies are pushing everyone to treat everyone, but often it's a mild disease," he says. And he notes that the treatment seriously disrupts most infected people's lives. "These people all feel well and all of a sudden they feel badly," he says, echoing a refrain that is often heard when asymptomatic HIV-infected people begin taking AIDS drugs.

Why some people fail therapy and others succeed is a mystery, in part because nobody knows precisely how interferon and ribavirin work. But a study published on page 107 of this issue offers one possible explanation for why some HCV strains are more resistant to interferon than others. Michael Lai of the University of Southern California in Los Angeles and his co-workers focus on an enzyme called protein kinase PKR that derails viruses by inhibiting their protein synthesis. Interferon turns up the rate at which cells make PKR. But the researchers' data suggest that HCV has a weapon against the enzyme: one of its two surface proteins, dubbed E2, which inhibits the activity of PKR. Based on sequence analyses of E2, they further contend that the protein made by HCV genotype 1 is especially good at binding to PKR and blocking its function. "This is an interesting observation that has to be further explored," says Michael Katze of the University of Washington, Seattle, whose lab focuses on a nonstructural HCV protein called NS5A that inhibits PKR. "There's no precedent for an envelope protein being a PKR-like molecule.

Clinicians now are tweaking treatment regimens and testing improved versions of both interferon and ribavirin. But, as with anti-HIV drugs, bigger gains are expected from compounds tailored specifically to attack key HCV proteins. Several companies are searching for inhibitors to enzymes that HCV uses to copy itself: protease, helicase, polymerase, and replicase.

The most popular target to date has been the "serine" protease, one of two viral enzymes that helps clip HCV's polyprotein into functional...
proteins. "Everybody picked the protease target first simply because of the success with HIV [protease inhibitors]," says Lewis "Rusty" Williams, Chiron's chief scientific officer. Like other companies, Williams says Chiron--which has teamed up with Pharmacia & Upjohn to develop anti-HCV drugs--is pursuing other targets, too. "We have progress in several of those targets, and it's hard to say which is furthest," says Williams. "I'm sure a number of companies are at work on the same targets. It's a race." Neither Chiron nor its competitors, however, have indicated that they are far enough along to set a timeline for human trials. And the dearth of presentations on these efforts at the meeting indicates that the companies are holding their cards closely to their chests.

Vaccine vacuum

While companies are elbow-to-elbow in the HCV drug development race, Chiron--in part because of its patent position (see sidebar, p. 28)--has had the vaccine field largely to itself. It is facing a tough challenge. Like HIV vaccine developers, it must contend with the fact that antibodies directed against rapidly changing viral proteins are unlikely by themselves to offer protection. In one test, for example, Chiron found that chimps vaccinated with genetically engineered versions of the virus's two surface proteins, E1 and E2, failed to fend off infection when they were inoculated with a strain of HCV that differed from the strain used to produce the vaccine. This has led the company to put increasing emphasis on the cell-mediated arm of the immune system, which uses killer cells and other strategies to clear the body of already infected cells.

In the April issue of Immunity, Stanford University immunologist Stewart Cooper, in collaboration with Chiron researchers, published the results of a study with chimpanzees that hints at the power of this approach. The researchers inoculated one chimp with E1/E2 antibodies derived from humans and another with the Chiron E1/E2 vaccine. The study also included two other animals (LouLou and Todd) that had previously been injected with HCV but had cleared the virus, and two naive animals as controls. When the animals were "challenged" with HCV, the first two and the controls readily became infected. LouLou and Todd, however, resisted HCV. Subsequent analyses revealed that while neither LouLou nor Todd had antibodies from their previous exposure to HCV, both had robust killer cell responses. This implies that cell-mediated immune responses may have wiped out their earlier infections and could now protect them from subsequent infections.

To trigger a strong cell-mediated immune response, Chiron is investigating vaccine strategies that produce viral proteins inside the body's cells. One such approach involves a so-called DNA vaccine, in which the injected preparation contains viral DNA by itself. Chiron also is exploring the possibility of stitching viral genes into safe viruses that then can infect cells and cause a mock infection.

Chiron may soon have some competition in these efforts. Scripp's Chisari and Chris Walker--a co-author on the Immunity paper who recently left Chiron for Ohio State University in Columbus--are working with a San Diego biotech, Epimmune, to develop an HCV vaccine that exploits cell-mediated immunity. And on the academic front, Fred Prince at New York Blood Bank is combining an HCV DNA vaccine with one that stitches HCV genetic material into a fowlpox virus; and NIAID's Robert Purcell is also exploring the DNA vaccine approach.

Walker, a former AIDS researcher, cautions that even if these efforts produce a promising vaccine, testing it in humans will be difficult. In the United States, efficacy tests probably could be done only in injecting drug users, a difficult group to follow for the length of a trial. Tests in poor countries would run into the same logistical and ethical problems now facing trials of anti-HIV vaccines. "I think we have a lot to learn from HIV," says Walker. "The efforts going on there are going to blaze the trail for HCV."

For most of the HCV-infected world, the development of vaccines will be crucial. Although hepatitis C could become a curable disease in a decade or so, if the next generation of drug therapies live up to their promise, the treatments will only help people in those countries that can afford the drugs. And that is perhaps the most sobering lesson from AIDS.

* Sixth International Symposium on Hepatitis C and Related Viruses, 6-9 June, National Institutes of Health, Bethesda, Maryland.