

A painting of a person's arm with a tattoo of a skull and the words "Bad Blood". The tattoo is rendered in a dark, ink-like style with red highlights. The person's hand is visible, with red-painted fingernails. The background is a dark, textured green.

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# Secrets of a silent killer

*Emory collaborators are tracking the clues to hepatitis C*

By VALERIE GREGG • Illustrations by SHAWN BARBER

WHY THE IMMUNE SYSTEMS OF SOME PEOPLE CAN OUTWIT HEPATITIS C AND THOSE OF OTHERS CANNOT REMAINS A MYSTERY. But as more people than ever are experiencing liver failure due to the virus, the puzzle has become increasingly important to solve. Called the “silent killer” for its stealth, the hepatitis C virus (HCV) engages the human immune system in a dangerous dance for decades, hiding out in the liver and often identified only after the liver is irreversibly damaged with fibrosis, cirrhosis, or cancer. HCV is the No. 1 reason for liver transplants in the United States. And even transplanted livers can become reinfected, so transplant is not a sure cure. The other treatment—a tough regimen of the antiviral drug ribavirin and the chemotherapy agent interferon—clears the bloodstream of HCV only about 40% of the time. Time is of the essence, says immunologist Arash Grakoui, part of a diverse network of investigators in Atlanta working on some aspect of HCV. These physicians and scientists—from Emory, Grady Hospital, and Atlanta Veterans Affairs Medical Center (VA)—are adding different pieces to the HCV puzzle, working from its genetic and cellular interactions with the immune system to implications for patient care.



**Kim Workowski provides blood and liver samples from her HCV-infected patients.**

Growing numbers of people are learning that they have been living with HCV for years. Many who contracted the disease from blood transfusions before 1989 (when the U.S. blood supply began screening for the virus) are just now falling ill. And worldwide, 170 million people are infected with HCV, four times more than HIV.

“A crisis is on the horizon,” says Grakoui, whose lab at the Yerkes National Primate Research Center at Emory University examines blood samples and liver biopsies from patients referred from collaborating hospitals. “The demand for liver transplant is going to be enormous.”

Injection drug use, tattoos, or breaking of the blood barrier during sex represent the highest risk activities for contracting HCV via contaminated blood in the United States. While the blood supply is safe here, that is not the case in other countries, according to Grakoui. “In Egypt, for example, 20% of the population is infected,” he says, largely due to needle-stick exposures during the schistosomiasis eradication campaign.

Although some of those infected will never feel the disease coming, most eventually will suffer liver damage or failure. The body does offer some red flags, albeit subtle ones, early on.

Abnormal liver enzymes often show up in blood tests during the acute phase of HCV infection—the first three to five months. About 20% to 30% of people spontaneously resolve the infection, but for the rest, interferon/ribavirin therapy is the only option. It’s expensive and often carries severe side effects such as fatigue, depression, and low red and white blood cell counts.

### Uncovering the unknowns

Kim Workowski, an infectious disease specialist at Emory Crawford Long Hospital, collaborates with Grakoui’s lab and provides blood and liver biopsy samples from her HCV-infected patients. The infectious disease clinic where she works serves 1,200 patients with HIV, 15% of whom are co-infected with HCV. The standard

regimen for patients newly diagnosed with HCV is self-injection of interferon once a week and twice daily oral ribavirin (a broad-spectrum anti-viral drug) for 48 to 72 weeks. The clinic also provides social work and mental health support, counselors on site, psychiatry referrals, and nurses who teach patients how to perform weekly injections and assist in the management of medication side effects.

Many unknowns create challenges for studying HCV in human subjects, says Grakoui. “We don’t know when these patients were actually infected, how they were infected, or what dose of virus they were infected with.” The researchers hypothesize that chronically infected people may have an inadequate presentation of HCV antigens to cells of the immune system.

Grakoui studies HCV antigen presentation by using transgenic mice bred at Yerkes that have the same haplotype as some human immune cells from patients exposed to HCV. He hopes to find out exactly what happens to make HCV easy for immune cells to forget. His lab also is investigating changes in antigen-specific or HCV-specific T cells from patients starting therapy with interferon/ribavirin as well as during and after

therapy. Does the phenotype of these cells change? Does their function change?

Supported by a five-year, \$1.25 million grant from NIH, Grakoui's lab is examining samples from Workowski's patients as well as patient samples from the VA, Grady, and Emory in a similar approach. The patients—including those with HCV mono-infection as well as those with HIV co-infection (more than 200 so far)—are analyzed according to whether their HCV infections resolve with therapy or remain chronic. Grakoui hypothesizes that in those who become chronically infected, antigen presentation is inadequate, preventing the right immune cells from being activated. He hopes to find what goes wrong in this process by comparing ongoing samples from patients in each cohort—those who resolve and those who do not.

Macrophages and B cells in the immune system also play a role. "They need to pick up the vital antigens from the infected cell and process them in such a way that they can present them on their cellular surface to T cells," says Grakoui. "This alerts the entire immune system that 'Hey, we have something important here, and we need to work in tandem to take care of it.'"

For example, a subset of killer T cells known as CD8s are needed to prevent an infection from becoming chronic. Chronic infection is the result of T cell depletion. When first confronted by an acute viral infection, CD8 killer T cells proliferate rapidly, produce cytokines to kill infected cells, and lower the viral load in the bloodstream. When things go well, a group of these cells remain to "remember" the viral threat after the pathogen is eliminated, ready to mobilize quickly if the virus reappears or if a person is exposed again. Sometimes this process works with HCV. Much of the time it does not.



Arash Grakoui uses the samples to see why immune cells forget HCV.

### Fixing a faulty memory

Other scientists at Emory are working in concert with Grakoui and Workowski to solve the mystery of HCV. Immunologist Rafi Ahmed, director of the Emory Vaccine Center, studies the ability of the immune system to remember a particular antigen and kill it when necessary. His research has delved into how HCV and HIV immune memory cells are created and how long they survive. He also has developed a new strategy to re-energize T cells that used to have this immune memory but have become "exhausted" in research published in *Nature* (February 2006).

With a \$12.5 million grant from the Grand Challenges in Global Health Initiative, funded by the Bill & Melinda Gates Foundation, Ahmed's team is studying HCV immunology. The aim: to understand why immune cells become exhausted in one type of battle and not in another. They already have found that exhausted T cells express high levels of an inhibitory receptor, whereas highly functional memory T cells had no detectable level of this response.

Likewise, Grakoui's team has shown that an inhibitory response is highly expressed on HCV-specific T cells from

chronically infected people, and he hopes to build on these findings. Because special helper T cells known as CD4s are important in HCV biology, he hypothesizes that people who become chronically infected with HCV have an inadequate antigen presentation to the CD4s.

The Emory collaboration has yielded other findings showing T cell exhaustion in HCV. For example, Henry Radziewicz, a scientist in Grakoui's lab, wrote a landmark article in the March 2007 *Journal of Virology*, showing stark differences in phenotype between the signatures of HCV in the peripheral blood versus blood in the liver.

"Since the business end of this virus is in the liver, you may be missing a great deal of important information if you don't look in the right place—the liver," says Grakoui.

Workowski says that a better understanding of the immune mechanisms of persistent infection will help her treat patients more effectively. "Volunteers provide samples throughout treatment so we can assess if changes in immune markers can help predict treatment

responsiveness. Early predictors of success would be extremely useful because hepatitis C treatment is difficult for many patients to tolerate, and we would like to provide a higher success rate for our patients.

"It's an exciting time for hepatitis C research because several promising new drugs are being investigated," she adds. "We haven't had anything new to offer our patients for a long time. This research will help us study not only the clinical effects of these novel medications and combinations of these agents but also their immunologic effects, both in the blood and the liver."

Grakoui feels lucky to be in a place with investigators from multiple disciplines interested in HCV. "I'm surrounded by a new breed of researcher—the physician/scientist who speaks both languages, basic science and clinical," he says. "They are the people who can take this work from our labs to directly benefit patients." ●

*Valerie Gregg is a freelance science writer in Atlanta.*

## Glossary

**Cytokines**—a group of proteins or peptides that are used in organisms as signaling compounds, allowing one cell to communicate with another

**Haplotype**—the genetic constitution of a person at a set of closely linked genes on a given chromosome

**Macrophage**—cells within tissues that originate from specific white blood cells called monocytes

**Phenotype**—the observed physical, biochemical, and physiologic makeup of a person as determined both genetically and environmentally, as opposed to genotype, the inherited instructions of an organism

**Transgenic**—genetically modified using recombinant DNA technology