

## Viruses and Simple Organisms

### Discovering the Virus Responsible for Hepatitis C

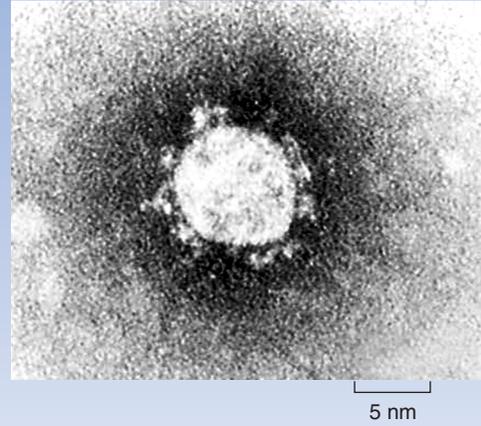
You may not be aware that our country is in the midst of an epidemic of a potentially fatal liver disease. Almost 4 million Americans are infected with the hepatitis C virus, most without knowing it. In the first years of this century, the number of annual United States deaths caused by hepatitis C is predicted to overtake deaths caused by AIDS.

Hepatitis is inflammation of the liver. There are three distinct forms. One, called infectious hepatitis or hepatitis A, is transmitted by contact with feces from infected individuals. A second form, called serum hepatitis or hepatitis B, is passed through blood and other body fluids. A third form, called hepatitis C virus (HCV), was only isolated in 1990 (see above photo). It too is passed through the blood.

HCV was difficult to isolate because it cannot be grown reliably in a laboratory culture of cells. Making the problem even more difficult, HCV is a strictly primate virus. It infects only humans and our close relatives, chimpanzees and tamarins. Because it is very expensive to maintain these animals in research laboratories, only small numbers of animals can be employed in any one study. Thus, the virus could not be isolated by the traditional means of purification from extracts of infected cells. What finally succeeded, after 15 years of failed attempts at isolation, was molecular technology. HCV was the first virus isolated entirely by cloning infectious nucleic acid.

The successful experiment was carried out by Michael Houghton and fellow researchers at Chiron, a California biotechnology company. What they did was shotgun clone the DNA of infected cells (that is, break the cell DNA into many pieces, and isolate each), and then screen each cloned piece of DNA for HCV.

The genetic material of HCV is RNA. So the first step was to convert HCV RNA to DNA, so that it could be cloned. There was no need to attempt to achieve entire faithful copies of the whole virus genome, a touchy and difficult task, because they did not wish to reproduce the HCV virus, only identify it. So the researchers took the far easier route of copying bits and pieces of the virus RNA, each piece carrying some part of the virus genome.



Electron micrograph of hepatitis C virus.

Next, they inserted these DNA copies of HCV genes into a bacteriophage, and allowed the bacteriophage to infect *Escherichia coli* bacteria. In a "shotgun" experiment like this, millions of bacterial cells are infected with bacteriophages. The researchers grew individual infected cells to form discrete colonies on plates of solid culture media. The colonies together constituted a "clone library." The problem then is to screen the library for colonies that had successfully received HCV.

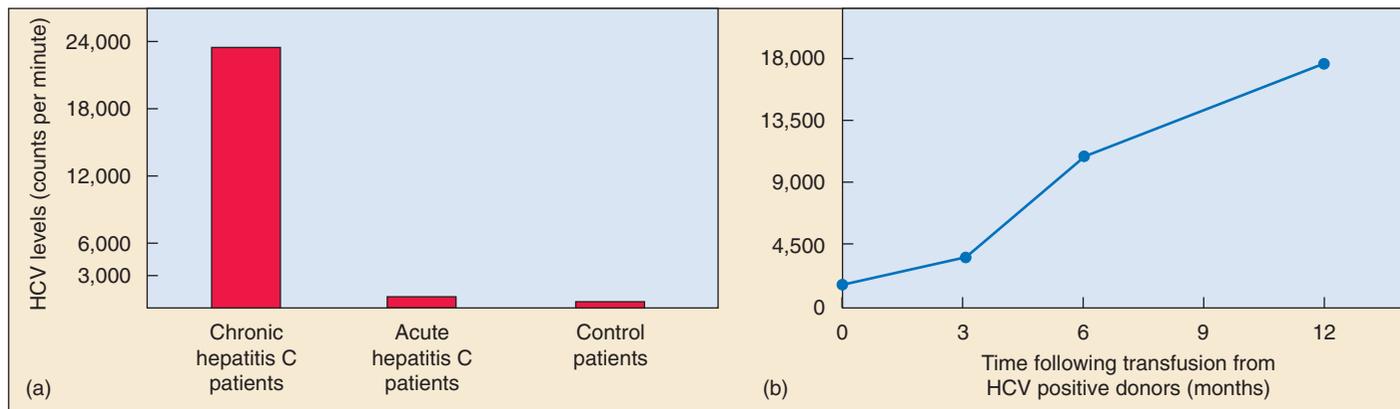
To understand how they did this, focus on the quarry, a cell infected with an HCV gene. Once inside a bacterial cell, an HCV gene fragment becomes just so much more DNA, not particularly different from all the rest. The cellular machinery of the bacteria reads it just like bacterial genes, manufacturing the virus protein that the inserted HCV gene encodes. The secret is to look for cells with HCV proteins.

How to identify an HCV protein from among a background of thousands of bacterial proteins? Houghton and his colleagues tested each colony for its ability to cause a visible immune reaction with serum isolated from HCV-infected chimpanzees.

The test is a very simple and powerful one, because its success does not depend on knowing the identity of the genes you seek. The serum of HCV-infected animals should contain antibodies directed against a broad range of proteins, including HCV proteins encountered while combating the animal's HCV infection. Thus among the many proteins the serum can respond to in an antibody test will be some HCV proteins. The investigators can use the serum as a probe for the presence of HCV proteins in bacterial cells, which would not have any other animal proteins to confuse the meaning of a positive reaction.

Out of a million bacterial clones tested, just one was found that reacted with the chimp HCV serum, but not with serum from the same chimp before infection.

Using this clone as a toehold, the researchers were able to go back and fish out the rest of the virus genome from infected cells. From the virus genome, it was a straightforward matter to develop a diagnostic antibody test for the presence of the HCV virus.



**Detection of HCV antibodies.** (a) Levels of HCV were measured using radioactive antibodies in three general groups of patients. The counts-per-minute measure indicates the sensitivity of the antibody (samples >3549 were considered positive). (b) Levels of HCV antibody were measured in the blood of individuals who had received blood transfusions from donors infected with the hepatitis C virus.

## The Experiment

Using the diagnostic test, researchers found hepatitis C to be far more common than had been supposed. This is a problem of major proportions, because hepatitis C virus is unlike hepatitis A or B in a very important respect: it causes chronic disease. Most viruses cause a brief, intense infection and then are done. Hepatitis A, for example, typically lasts a few weeks. Ninety percent of people with hepatitis C have it for years, many of them for decades.

All during these long years of infection, damage is being done to the liver. Cells of the immune system called cytotoxic T cells recognize hepatitis C virus proteins on the surface of liver cells, and kill the infected cells. Over the years, many dead liver cells accumulate, and in response the cells around them begin to secrete collagen and other proteins to cover the mess. This eventually produces protein fibers interlacing the liver, fibers which disrupt the flow of materials through the liver's many internal passages. Imagine dropping bricks and rubble on a highway—it gets more and more difficult for traffic to move as the rubble accumulates.

If this fibrosis progresses far enough, it results in complete blockage, cirrhosis, a serious condition which may induce fatal liver failure, and which often induces primary liver cancer. About 20% of patients develop cirrhosis within 20 years of infection.

The development of a diagnostic antibody test was important for the screening of the blood supply for contamination. It was believed that contaminated blood through transfusions caused a large majority of new cases of hepatitis C. In order to develop a diagnostic antibody test an international research team headed by the Chiron group compared the DNA copies of several HCV clones and identified one common DNA sequence present in all clones. This section of DNA was reconstructed and then incorporated into the yeast genome along with another polypeptide gene, human superoxide dismutase (SOD), that assists in the incorporation of the HCV DNA copy into the yeast genome. Recombinant yeast cells express this SOD/HCV polypeptide, called C100-3, at high levels.

C100-3 was used to coat the wells of test plates so that HCV antibodies in blood samples could be captured and measured.

## The Results

To test the specificity and sensitivity of the antibody assay, HCV infected blood sera from patients with hepatitis C was tested (see graph *a* above). The researchers then assayed matched blood donors and recipients of both negative and positive donors and determined that recipients of negative donors did not develop hepatitis C. However, recipients of donors determined to be positive for HCV, based on reviewing stored samples, developed hepatitis C and showed positive HCV detection in the blood over a 12 month period (see graph *b* above).

These results indicated a specific association between the HCV antibody test and hepatitis C. Similar results obtained from patients in Italy and Japan confirmed the association of the HCV antibody and hepatitis C. These data supported the hypothesis that HCV is a primary cause of chronic hepatitis C and that use of this HCV antibody test would improve the safety of the world blood supply as well as become an important diagnostic tool.

Luckily, HCV is a very difficult virus to transmit. Unluckily, it is proving difficult to produce a vaccine directed against HCV. Antibodies directed against HCV are largely ineffective, and HCV mutates so frequently that, like AIDS, no vaccine seems likely to be effective. Attempts to combat hepatitis are focused on the virus itself. This virus carries just one gene, a very big one that is translated into a single immense “polyprotein” which enzymes cut into 10 functional pieces. Each of these 10 proteins is being investigated as a potential target for a drug to fight the virus, although no success is yet reported.