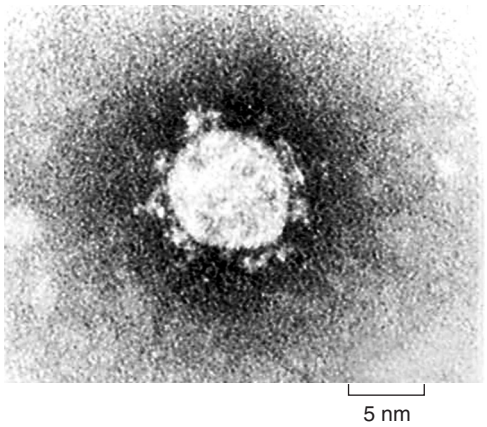
EXPLORE THE ISSUE BEING INVESTIGATED

Discovering the Virus Responsible for Hepatitis C



Electron micrograph of hepatitis C virus.

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.

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.