Hepatitis: A New Understanding Emerges

During the last 4 months, three different laboratories independently announced that they have successfully transmitted serum hepatitis to nonhuman primates. Although hepatitis has long been suspected to be caused by a virus, this demonstration is the first instance in which the suspected viral agent has been successfully transmitted to an animal model, and thus represents an important first step toward the eventual development of a hepatitis vaccine. Perhaps even more important, however, this successful transmission also represents a culmination of 5 years of near-explosive growth in knowledge about hepatitis, growth that has now led to a relatively broad understanding of at least one form of the disease.

Hepatitis has been a mysterious and intractable malady. Physicians have long recognized that its symptoms—inflammation of the liver accompanied by fever, weakness, loss of appetite, malaise, headache, and muscle pains—arise from two distinct types of infection. Hepatitis A, also called infectious hepatitis, is generally transmitted by fecal contamination of food and water, and is responsible for some 90 percent of the more than 74,000 cases reported in the United States every year. Hepatitis B, also called serum hepatitis, is most frequently transmitted by infusions of blood from infected individuals. Although hepatitis B is responsible for less than 10 percent of the reported cases, it is considered the more dangerous form because those exposed to it are generally already ill. Furthermore, the incidence of hepatitis B has been rising as a result of its spread by drug addicts (who do not sterilize their injection paraphernalia) and by increased use by blood banks of paid donors.

Until recently, little else was known about hepatitis, and even now there is neither a specific clinical test for either form nor a specific treatment other than rest and a nutritious diet. A major breakthrough occurred in 1964, however, when Baruch S. Blumberg of Philadelphia's Institute for Cancer Research observed a foreign substance, initially called Australia antigen, in the blood of an Australian aborigine. No clinical significance of this discovery was immediately apparent, but in 1968 Alfred M. Prince of the New York (City) Blood Center observed a similar substance in the blood of patients with hepatitis B. The two substances were quickly shown to be identical, and to be specifically linked to hepatitis B.

The isolation of this material, now called hepatitis B antigen (HBAg), provided a tremendous impetus to research about this disease. This antigen was the first serological marker for hepatitis B, and thus provided both a means for diagnosing the presence of hepatitis B and a tool with which to examine its nature. The availability of HBAg loosed an unprecedented flow of unexpected, and often conflicting, results that only within the last 6 months have begun to coalesce into a relatively clear picture.

Electron microscopy initially revealed that HBAg consists of viruslike particles approximately 20 nanometers in diameter, an indication that it might be the causative agent. This proposal was quickly discarded after chemical analysis showed that highly purified HBAg does not contain nucleic acids. Subsequent work, however, has led to the identification of three other viruslike particles associated with hepatitis B.

Three Other Particles Observed

The first, observed in blood serums by many investigators, has about the same diameter as HBAg, but is several times as long. It also is devoid of nucleic acids and is generally assumed to be another physical form of HBAg.

The second, identified in 1970 by D. S. Dane of London's Bland-Sutton Institute, is about 42 nm in diameter. It is composed of an outer coat that reacts with antibody specific for HBAg (hepatitis B antibody, or HBAb), and an inner core about 27 nm in diameter. The Dane particle has been found in blood serums and in the cytoplasm of infected hepatocytes (liver cells), but is usually present only in such small quantities that it has not yet been chemically characterized.

The third particle, discovered by several groups but examined most thoroughly by Shao-nan Huang of Montreal's McGill University, has a diameter between 23 and 27 nm. It was discovered in the nuclei of hepatocytes from liver transplant patients who contracted hepatitis, and has been characterized only by electron microscopy.

The emerging consensus is that the Huang particle is the DNA- or RNA-containing viral core that replicates in the hepatocyte nucleus and there (presumably) causes the tissue damage associated with hepatitis B. Through some as yet unknown mechanism, the viral core migrates to the cell cytoplasm, where it is sheathed in the HBAg coat to become the Dane particle—the presumed transmissible form of the virus. This mode of replication is similar to that of mouse leukemia and herpes viruses, whose protein coats are also synthesized in the cytoplasm.

For reasons that are also not well understood, the cytoplasmic messenger RNA directs the production of excess protein coat (HBAg). Excess protein production is also associated with other viral infections, notes Thomas S. Edgington of Scripps Clinic & Research Foundation, La Jolla, California, but the quantities involved with the putative hepatitis B virus are greater than those observed with other viruses. Adenoviruses and myxoviruses, for example, produce a ten- to thousandfold excess of coat protein, whereas it appears that the hepatitis B virus may produce more than a millionfold excess.

This replication scheme is thus amply supported by precedent, but the most convincing evidence in its favor, perhaps, was presented last winter by June D. Almeida of London's Royal Postgraduate Medical School. Almeida and her associates partially disrupted small quantities of Dane particles with a detergent to expose the core, which proved morphologically similar to picornaviruses—small (pico-) RNA viruses.

When the disrupted Dane particles are exposed to blood serums from patients convalescing from hepatitis, an antigen-antibody reaction occurs only with the core, and the resulting aggregates strongly resemble those observed by Almeida in liver homogenates from hepatitis patients. With serums obtained from the patients before they contracted hepatitis or with control serums from unexposed patients, no reaction occurs. With serums from patients multiply exposed to hepatitis (such as hemophiliacs, who have had many transfusions), antigen-antibody complexes are formed with both the coat HBAg and the core.

The results, Almeida suggests, indicate that antibody to HBAg is formed

during an attack of hepatitis B, but—unlike antibodies formed during other infections—disappears when the antigen is cleared from the system. Immune response to the core is more conventional: antibody to the core has been detected in serum as long as 2 years after a hepatitis infection.

Further supportive evidence for this scheme comes from immunofluorescence studies by Edgington and others, in which fluorescein-tagged HBAb is used as a probe for the presence and location of HBAg in hepatocytes. Such studies have produced conflicting results, Edgington notes: he demonstrated that HBAg is present only in the cytoplasm of hepatocytes, but some workers have found antigen only in the nucleus, and some have found it simultaneously in both sites.

After Almeida's discovery of the new antigen-antibody reaction of Dane particles, further investigation clarified these apparent conflicts. Using antibodies specific for the Dane particle core, Edgington has now demonstrated the presence of core-associated antigens in hepatocyte nuclei. He thus suggests that those laboratories that observed fluorescence only in cytoplasm used antibody specific for coat HBAg. Those laboratories that observed fluorescence only in nuclei, however, used antibody prepared with serums from convalescing hepatitis patients. And those that observed it in both locations used antibodies prepared with serums containing both antigens, thus confirming Almeida's findings.

Much is still unknown about the putative hepatitis B virus, however, and this lack of knowledge invites the search for animal models. The key to characterization of a virus, observes Robert H. Purcell of the National Institute of Allergy and Infectious Diseases, is to have an animal or tissue culture system in which the suspected virus can be grown, observed, characterized, and manipulated. Until this year, he adds, there was no such system for hepatitis other than man.

Using infectious human serum, Purcell and his associates at the National Institutes of Health have infected five rhesus monkeys with hepatitis. The agent responsible for this infection has subsequently been transmitted serially through four additional groups of monkeys by the use of serum from one group to infect the next. James E. Maynard's group at the Center for Disease Control, Phoenix, Arizona, working with Purcell, has also infected two chimpanzees in the same fashion.

Similar symptoms of infection were observed in both groups of animals. Transient antigenemia (circulation of antigen—in this case HBAg—in the blood stream) of 1 to 3 weeks duration occurred 8 to 13 weeks after inoculation, and was followed by development of antibody to HBAg. None of the animals developed clinical symptoms of hepatitis, Purcell says, but their responses are similar to subclinical infections previously observed in humans.

Alfred Prince, in cooperation with investigators from the Laboratory for Experimental Medicine and Surgery in Primates, at Sterling Forest, New York, infected five chimpanzees with serum from a chronic hepatitis carrier. He observed continuous long-term, rather than transient, antigenemia in the animals, beginning 3 to 4 months after inoculation. These infections were also subclinical, although a distinct rise in serum glutamic-pyruvic transaminase levels (a common indicator of liver function) was observed.

The third group, headed by Lewellys F. Barker of Food and Drug Administration's Division of Biologics Standards, infected two chimpanzees with hepatitis, then transmitted the suspected viral agent to two more. A fifth chimpanzee was also infected using partially purified blood plasma from one of the animals. Three of the chimpanzees showed transient antigenemia and development of antibody similar to that observed by Purcell, but the two others developed biochemical and clinical symtoms of infection. Electron microscopy of serum from one of these animals, moreover, revealed both intranuclear and Dane particles. There as yet appears to be no ready explanation for the disparities in the results from the different laboratories.

Sensitive Techniques Required

The absence of clinical symptoms in the majority of the animals, meanwhile, has necessitated the use of relatively new, highly sensitive techniques for confirmation of infection. Among the most important of these are radioimmunoprecipitation (RIP), which tests for HBAb, and radioimmunoassay (RIA), which tests for HBAg.

RIP, developed at NIH by Purcell and others is, he says, 2,000 to 500,000 times more sensitive for detecting HBAb than previous methods, such as complement fixation (which requires visual identification of a different antigen-antibody reaction in sheep blood cells). In RIP, radiolabeled HBAg is incubated with the serum being tested. Antigen-antibody complex, if present, is then precipitated and centrifuged. Radioactivity in the sedimented pellet then indicates the presence of HBAb in the serum.

The use of a radiolabel makes the technique expensive, Purcell concedes, but the expense is justified by the increased sensitivity. Using RIP, for example, Purcell and his associates last year demonstrated that the serums of more than 14 percent of a population of blood donors contain HBAb, even though most people in this group have not had hepatitis, blood transfusions, or other opportunities for parenteral transmission of the disease. These studies suggest not only that a significant proportion of the population has at some time had subclinical infections of hepatitis B, but also that the disease is (unexpectedly) readily transmissible via a nonparenteral route.

RIA is a "solid-phase" technique for detecting HBAg developed by Lacy R. Overby and Chung-mei Ling of Abbott Laboratories, Chicago, Illinois, and is of about the same sensitivity as RIP. In RIA, the serum to be tested for antigen is incubated in a plastic tube whose inner surface is coated with HBAb. The tube is then washed, radiolabeled HBAb is added, and the new mix incubated. After a final thorough washing, residual radioactivity in the tube then indicates the presence of HBAg in the original serum.

Without these techniques, Purcell argues, confirmation of infection in the primates would have been difficult, if not impossible. This also suggests, he adds, that previous investigators have also produced subclinical infections in various animals, but have been unable to detect it. It is a good bet, he suggests, that reinvestigation of their work will produce other animal systems—and perhaps tissue cultures—for further laboratory work on the characterization of the suspected virus.

It appears, however, that no group is in a position to produce a hepatitis vaccine within a short period. Much has been learned about hepatitis, but much remains unknown. Particularly discouraging, moreover, is the fact that no comparable breakthrough has been achieved with hepatitis A. Scientists have yet to obtain a "handle" for it similar to HBAg, and the growth of knowledge about hepatitis A has lagged far behind that of hepatitis B.

—THOMAS H. MAUGH II