Virus-like Particles in Sera of Patients With Infectious and Serum Hepatitis

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In a prospective study of patients with serum hepatitis, 46 (74%) of 62 had Australia antigen in their serum. This high frequency of detectable antigen was probably due to sampling at weekly intervals during the acute phase of the disease. Antigen became detectable 35 to 120 days after exposure to contaminated blood products and persisted for one week to three months in 42 patients, and for more than ten months in 4 patients. Two of 128 healthy chimpanzees and one of 14 healthy gibbons also had the antigen. Antigens found in sera of patients with infectious or serum hepatitis and in ape sera were immunologically identical. Electron microscopy revealed morphologically similar virus-like particles both in sera from patients with hepatitis and from healthy apes. Australia antigen appears to be hepatitis virus itself, and a single virus group may be responsible for both infectious and serum hepatitis.

Serum from some individuals who have had many blood transfusions contains an antibody that reacts with an antigen initially identified in serum from an Australian aborigine.7 This so-called Australia antigen was found to be rare in sera from presumed healthy American populations, but was more common in association with various leukaemia's, Down's syndrome, nodular (lepromatous) leprosy, and with both infectious and serum hepatitis.7,9 At first, the presence of Australia antigen was thought to be of possible value in the diagnosis of early acute leukemia.7 Later, familial segregation of the antigen in population studies pointed toward an autosomal recessive inheritance.7 However, its appearance during the incubation period of posttransfusion hepatitis7 suggested that the antigen is related to hepatitis virus itself, and electron micrographs of virus-like particles in antigenic serum have been reported.8,9

Previous work has indicated that the antigen occurs in 41% of cases of posttransfusion hepatitis and in 22% of cases of infectious hepatitis; the antigen's presence in sera of chimpanzees, vervets, and squirrel monkeys has been mentioned.9

This communication concerns our observations on the relationship between Australia antigen and hepatitis. Our studies involved 237 patients with infectious or serum hepatitis. Serum samples were collected during acute and convalescent phases of illness in 175 patients, and, in a unique series of 62 patients with serum hepatitis, samples were collected prospectively at weekly intervals following exposure to contaminated blood products. The latter group permitted more precise documentation than heretofore recorded, to our knowledge, of the incidence of Australia antigen in hepatitis, and the temporal relationship between clinical disease and the presence of antigen in the blood. The Australia antigen was also found in chimpanzees and a gibbon.

On electron microscopy, the antigen appears to be a virus-like particle, and the particles found in blood from patients with serum hepatitis or infectious hepatitis and from nonhuman primates were morphologically indistinguishable. All antigens formed a line of identity by agar-gel diffusion. Multiple transfusions were a prerequisite to antibody formation in both man and other primates.

Materials and Methods

The source of antibody to Australia antigen was serum or plasma from patients with hemophilia, who had received multiple transfusions during routine treatment, and plasma from chimpanzees that had been transfused experimentally with chimpanzee blood.10 The specificity of all antibodies was identical to that of the original serum used by Blumberg et al10 to identify the Australia antigen.

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An agar-gel diffusion technique11 with 1% agar in barbital hydrochloride buffer, pH 8.2, containing 0.01M sodium azide was used. The slides were stained first with Sudan black and then with azocarmine to distinguish lipoprotein antigens from the Australia antigen.11

Human sera tested were obtained from four categories of patients. The first category (1) was composed of patients involved in a probable common-source outbreak of infectious hepatitis in the United States in 1968. Serum samples were col-
lected from these patients during the acute and convalescent phases of illness. The second category (2) included patients with infectious hepatitis in west Africa, where the disease is endemic. Serum samples were collected during the acute and convalescent phases of illness in those patients in 1963. The third category (3) consisted of patients with probable posttransfusion hepatitis, from whom serum samples were collected during the course of the disease in 1968. The fourth category (4) was composed of a series of individuals who were exposed to hepatitis-containing blood products, and in whom clinical hepatitis ultimately developed. Sera from these patients were collected prospectively at weekly intervals during the incubation period and the acute and convalescent phases of hepatitis in a study performed from 1952 to 1954.12

Subhuman primate sera were obtained from several sources. All the animals tested were apparently healthy.

We also tested 190 samples of commercial fibrinogen, which has been associated with hepatitis,13 for presence of antigen. This type of fibrinogen is used for the treatment of a fibrinogenemia and factor VIII deficiency. For testing, lyophilized fibrinogen was reconstituted to the recommended volume with diluent and used in the same way as sera in the agar-gel technique.

Specimens for electron microscopy were prepared by mixing equal volumes of antibody- and antigen-containing sera, incubating the mixture for one half hour at 37 C, and centrifuging at 25,000 g for ten minutes. The resuspended sediment was prepared for electron microscopy by the pseudoreplication technique and negatively stained with uranyl acetate.14,15

Results

Antibody to Australia antigen was found in the blood of eight of 29 hemophiliacs who had received multiple transfusions, and in two chimpanzees which had been transfused repeatedly with chimpanzee blood. Five of eight hemophiliacs with the antibody had a history of clinical hepatitis, but some did not. Antibody was not detected in sera of convalescent patients who had infectious or serum hepatitis and had not received multiple transfusions.

Table 1 summarizes incidences of Australia antigen in the sera of the different categories of patients. In a common-source outbreak of infectious hepatitis (category 1), 2 (5%) of 39 patients had detectable antigen, while 18 (16%) of 112 patients with infectious hepatitis in a hepatitis-endemic area in Africa (category 2) had detectable antigen. In category 3, in which blood samples were infrequently collected from patients with presumed posttransfusion hepatitis, two (8%) of 24 patients had the antigen. The highest incidence of Australia antigen, 74% (in 46 of 62 subjects), was found in category 4, in which blood samples were frequently collected prospectively from individuals exposed to hepatitis-containing blood products.

Of the 46 subjects with serum hepatitis with antigenemia (category 4), all had antigen in their blood between 35 and 120 days after exposure. Table 2 summarizes data from six illustrative cases. The duration of antigenemia after its appearance varied from one week to three months in 42 subjects, but in 4 subjects the antigen was detectable after three months, and in these same individuals was detectable after ten months. All the patients who had jaundice and detectable antigen had the antigen present concomitantly with jaundice. Approximately 15%, however, had the antigen without jaundice (anticteric hepatitis).

Two of 128 chimpanzees, both from the colony at Holloman Air Force Base, NM, and one of 14 gibbons from the Delta Regional Primate Center, Covington, La, had Australia antigen in their sera. The ages of the chimpanzees with the antigen were 6 and 7 years, and the antigen persisted in one of the chimpanzees for at least ten months. No antigen was found in sera from 50 baboons, 33 orangutans, 139 vervets, and 169 rhesus monkeys.

No antigen was found in 191 samples of commercial fibrinogen.

When serum containing Australia antigen was mixed with antibody-containing serum and centrifuged, aggregates of 20-mu virus-like particles were found in the antigen-antibody precipitates. The particles were indistinguishable whether obtained from patients with infectious or serum hepatitis (categories 1, 2, 3, and 4) or from chimpanzee or gibbon sera (Fig 1). Similarly treated sera from normal humans, chimpanzees, and gibbons did not contain virus-like particles.

Antigen found in sera of patients with infectious
hepatitis or serum hepatitis and in simian sera showed a line of identity with no spurring in agar-gel diffusion (Fig 2).

Comment

It is apparent that sampling at weekly or more frequent intervals after the clinical onset of disease is the most effective way of detecting antigen, although it is also clear that some patients may never have antigen detectable by the agar-gel technique. In the prospective study of individuals exposed to hepatitis-containing blood products (category 4) frequent sampling during the acute phase of hepatitis resulted in a high incidence (74%) of detectable antigen. If samples taken during the incubation and convalescent phases in this study were included, the incidence of antigenemia would not be increased. In all cases of hepatitis in which antigen was detected and jaundice occurred, antigen was demonstrated some time while jaundice was present, although eight patients had antigen with anicteric hepatitis. When samples were taken at greater than weekly intervals after the onset of clinical hepatitis, we found only 5% to 16% positive sera, and Blumberg et al. found, at most, 41% antigenemia in posttransfusion hepatitis and 22% in infectious hepatitis.

In all cases of category 4, the antigen appeared between 35 and 120 days after exposure, and, after appearance, was detectable for one week to three months later, with the exception of four patients in whom antigen was detectable more than ten months later. It seems that if antigen persists for more than three months, it may last for an indefinite time, and thus represent the carrier state.

A disease occurring in chimpanzees and other nonhuman primates, similar to human "viral" hepatitis, has been described, and outbreaks of infectious hepatitis have occurred among human handlers of chimpanzees, strongly suggesting that chimpanzees either carry human hepatitis or are susceptible to the same virus. It is therefore of interest that Australia antigen was found in sera of two chimpanzees and a gibbon, and that one chimpanzee had antigen in serum samples drawn ten months apart, suggesting that it had become a carrier. These observations extend a previous report of the presence of Australia antigen in nonhuman primates. When the agar-gel diffusion technique was used, a line of identity formed between Australia antigen found in human sera collected in west Africa and the United States in cases of both infectious and serum hepatitis (categories 1, 2, 3, and 4), as well as in ape sera. Moreover, the morphological and density characteristics of the virus-like particles in antigenic sera were similar (L. S. Barker et al., unpublished data). These findings suggest that a single virus group is responsible for both infectious hepatitis.
and herum hepatitis in a wide geographic distribution and that the same virus group can be carried by nonhuman primates.

Infectious hepatitis, characterized transitively by the oral route, may be transmitted parenterally as well. If the incubation period of hepatitis after parenteral exposure to blood products is 15 to 40 days, the disease is considered to be infectious hepatitis, but if the incubation period is 60 to 160 days, it is called serum hepatitis. However, the virus-like particles found in both infectious and serum hepatitis had identical immunological and morphological characteristics. It is possible, therefore, that the different incubation periods of infectious and serum hepatitis may simply reflect the different portals of entry rather than different etiologic viruses.

Human fibrinogen concentrates have been strongly implicated in the transmission of hepatitis. Of 191 samples of commercial fibrinogen concentrate tested, however, no antigen was detected by agar-gel precipitation. If hepatitis virus and Australia antigen are identical, the negative results in agar gel, no doubt, are due to relatively low virus concentrations in fibrinogen.

Specific floculation of influenza virus, adenoviruses, polioviruses, and coxsackieviruses by antisera have been demonstrated, but the high concentration of antigen necessary for the agar-gel diffusion method has required 100-fold to 300-fold concentration of antigen. The appearance of floculating Australia antigen in unconcentrated sera suggests that these particles, which appear to be hepatitis viruses, are present in very high concentration in most patients with hepatitis. Since a high enough concentration of virus or virus products to produce an agar-gel precipitate is an unusual if not unique finding in viral diseases, it is not surprising that some cases do not give positive results.

It is of interest that no antibody to Australia antigen was found in sera obtained during convalescence in the hepatitis cases studied. The type of antibody that forms agar-gel precipitins apparently results from hyperimmunization through multiple antigenic exposures. Techniques more sensitive than agar-gel diffusion, such as complement fixation, may be expected to increase the incidence of detectable antigen and antibody.

The results of this investigation confirm and extend others previously reported[1-3] and indicate even more strongly that the appearance of the Australia antigen in human sera is related to hepatitis. Moreover, the occurrence of virus-like particles in antigenic sera suggests that the antigen is virus-related and may be part of the hepatitis virus itself. Further evidence, including stability and structural characteristics of the antigen, is consistent with its viral nature (L. S. Barker et al, unpublished data). Its high incidence and persistence in sera from patients with leukemia, Down's syndrome, and leprosy seem to be related to diminished host defenses or unusually frequent exposure, as with transfusions and institutional epidemics. We are currently investigating further the relationship of Australia antigenemia to hepatitis and evaluating more sensitive means of detecting both the antigen and the antibody.

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Primates used in this investigation were obtained from the following sources: (1) Holloman Air Force Base, NM; (2) York Laboratories of Primate Biology, Jacksonville, Fla; (3) Sterling Forest, NY; (4) Delta Regional Primate Center, Covington, La; (5) Tulane University, New Orleans; (6) Minneapolis Medical Research Foundation, Inc; and (7) National Institutes of Health, Bethesda, Md.

The commercial fibrinogen used in this study was obtained from the following sources: (1) E. R. Squibb & Sons, Inc, New York; (2) Merck, Sharp, & Dohme, Division of Merck & Co Inc, West Point, Pa; (3) Hyland Laboratories, Los Angeles; (4) Cutter Laboratories, Berkeley, Calif; (5) Courtland Laboratories, Los Angeles; (6) Michigan State Department of Health, Lansing; and (7) the American Red Cross, Research Laboratories, New York.

References


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