HEPATITIS VIRUSES

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Discovery of a surface antigen closely associated with the agent of viral hepatitis B has helped pave the way for work on a vaccine against this disease. Recent progress on hepatitis A includes recognition of virus particles in patients' feces and development of a radioimmune assay for titrating the virus and its antibody.

Introduction

The World Health Organization has long recognized the public health importance of viral hepatitis and has convened a number of expert committees and scientific groups to deal with the subject. The frequency of these meetings has increased during the past few years as a direct result of the sudden and rapid progress in hepatitis research following the discovery of an antigen (now termed HBsAg) that is closely associated with the etiologic agent of viral hepatitis type B. General information and recommendations concerning hepatitis types A and B are contained in WHO documents published in 1964, 1970, 1973, and 1975 (1-4).

Hepatitis B Antigens

Infection with hepatitis B virus is associated with the appearance of both hepatitis B surface antigen and its homologous antibody in the patient's serum. A second antigen, present in the core of the larger (42-nm) hepatitis B particle, also appears to be intimately related to the infection.

The surface antigen displays complex reactivities. The group-specific antigen has been named a and there are at least four phenotypes: adw, adr, ayw, and ayr. There may be other subdeterminants as well. Subtype ayw is found in a broad geographic area that includes most of Africa, the Eastern Mediterranean countries, and the Middle East and extends to Pakistan. In Northern Europe and the Americas, on the other hand, the predominant phenotype is adw. Both adw and adr are prevalent in Indonesia, Malaysia, New Guinea, and Thailand, while adr is predominant in Southeast Asia and the Far East. In areas with high prevalences of HBs antigenemia, a corresponding high prevalence of hepatoma has been noted.

This surface antigen reactivity is shared by the tubular forms, the small spherical (18- to 22-nm) particles, and the outer shell of the 42-nm particles. An e antigen and antibody system has also been described which seems to be distinct from that of the particles of hepatitis B surface and core antigen. The precise relationships of the e system to hepatitis B virus have not yet been established.

There is accumulating evidence consistent with the view that the 42-nm double-shelled spherical particle may be the complete hepatitis B virus, the core being the nucleocapsid and the surface or outer protein coat being hepatitis B antigen.

Core antibodies are produced in response to replication of the virus in the liver. They appear during or immediately after hepatitis B surface antigenemia, well before the appearance of hepatitis B surface antibody.

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They then persist with a slow decline in their titer. These core antibodies do not signal recovery from infection, they are not correlated with resistance to reinfection, and they are not boosted by reexposure to serum containing hepatitis B surface antigen. Core antibodies are present in persistent carriers of hepatitis B surface antigen.

Isolation and Transmission Efforts

Attempts to isolate hepatitis A or hepatitis B virus in cell or organ culture systems have generally been unsuccessful. Propagation of the type A virus in marmosets has been reported and appears promising. Type A infections among imported chimpanzees are well known as an important cause of hepatitis in animal caretakers. Successful transmission of the type B virus to chimpan-

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3See Hilleman et al. infra.
zees has also been achieved. This latter infection produced serologic, biochemical, and histologic evidence of type B hepatitis. Immunofluorescence and electron microscopy revealed HBsAg in the cytoplasm and viruslike particles with HBcAg in the nuclei of hepatocytes. Serial passage has also been successful. To date no evidence for hepatitis B transmission from chimpanzees to man has been reported.

Detection of Hepatitis A

Immune electron microscopy (IEM) research has recently detected a viruslike particle in fecal extracts obtained from hepatitis A patients. The antigen appeared early in the disease and usually disappeared within three weeks, following the onset of jaundice. Morphologically, the particles were 27 nm in diameter, did not appear to have an envelope, and showed cubic symmetry.

The IEM technique, though useful, requires the expertise of a skilled electron microscopist. A rapid, more sensitive technique that can be applied to large numbers of specimens has been developed. It makes use of a microtiter solid-phase immunoradiometric assay (micro-SPIRA) similar to that employed for detecting hepatitis B core antigen in the sera of patients with posttransfusion hepatitis B. The method for hepatitis A virus involves coupling unlabeled antibody to an insoluble matrix, which can then extract immunologically reactive antigen from the test specimen (liver, stool, or serum). After interaction with a second radiolabeled antibody, the level of specific antigen present is measured. The method seems more reliable than IEM, for it has detected significant levels of hepatitis A antigen in samples that had been judged negative by IEM.

In addition to the foregoing detection of virus in feces, hepatitis A antigen has been observed in sera of infected chimpanzees, thus supporting the hypothesis that viremia exists during the acute stage of the disease. The diagnosis of type A hepatitis by a conventional serologic procedure may now be at hand.

Hepatitis B Vaccine Development Efforts

Several clinical and seroepidemiologic studies have shown an unusually high incidence of hepatitis B virus infection among selected groups of people: staff members and family contacts of hemodialysis patients, household contacts of asymptomatic or apparently healthy HBsAg carriers, staff members and residents of crowded mental or penal institutions, dentists (especially oral surgeons and their patients), hospital or technical personnel working with blood or its by-products, members of the military. Within these groups a wide spectrum of clinical disease has been observed, ranging from inapparent infection to acute and chronic disease with or without the development of persistent hepatitis B surface antigenemia (HBsAg). The need to provide some form of active immunization for such individuals is apparent.

Progress in the development of a vaccine has been hampered by the very limited success in growing hepatitis B virus in culture and by the lack of a readily available animal model system. Consequently, investigators are turning to healthy carriers of HBsAg as a source of immunogen. This is a new departure in the development of a viral vaccine, and one that must be approached with great care and adequate safeguards.

Studies by several institutions suggest that anti-HBs plays a protective role in the neutralization of infectious HBV in donor blood. That is, blood recipients with preexisting anti-HBs failed to develop anicteric or icteric hepatitis, whereas clinical hepatitis B developed in about 5 per cent of the recipients whose pretransfusion blood was negative for anti-HBs. Unlike anti-
HBs, anti-HBc does not appear to suppress the hepatitis B virus. The highest titers of anti-HBc are found in the chronic carrier state; all chronic HBsAg carriers possess anti-HBc but not anti-HBs.

As already noted, at least four subtypes of HBsAg have been observed: adw, ayw, adr, ayr. They are all thought to be mutually exclusive. This raises the question of whether the antibody induced by a vaccine for one subtype of HBsAg can protect against infection with a second subtype. Fortunately, the evidence to date suggests that cross-immunity does occur, not only naturally in man but also in experimentally infected chimpanzees.

Recent studies using heat-inactivated serum containing hepatitis B virus and antigen have yielded noteworthy results. They showed that this inactivated serum prevented or modified type B hepatitis in subjects who were challenged up to eight months later with an intramuscular inoculum of unheated serum containing the active virus. The apparent success of this crude immunogenic preparation has stimulated research on the possible development of a subunit vaccine which is free of infectious nucleic acid.

Current studies of purified HBsAg subtypes made soluble with mercaptoethanol, sodium dodecyl sulfate, and urea have revealed seven polypeptide subunits for HBsAg/adw and nine polypeptides for HBsAg/ayw. Estimated molecular weights of these polypeptides range from 19,000 to 120,000 daltons. Carbohydrate and lipid moieties are associated with some of these polypeptides. Humoral antibodies and cell-mediated immunity to both the purified polypeptides and the glycoproteins have been produced in guinea pigs. These observations are stimulating further investigations into the potential development of a subunit vaccine (from human serum containing HBsAg) which would exclude genes of a viral or cellular nature and which would not be infectious.

**SUMMARY**

Important advances in hepatitis research have followed discovery of a surface antigen (HBsAg) closely associated with the etiologic agent of viral hepatitis type B. There are four known phenotypes of this antigen, different phenotypes predominating in different geographic regions.

There is also growing evidence to support the view that the largest of three particles (a 42-nm double-shelled spherical particle) showing HBsAg reactivity may be the complete hepatitis B virus.

Although attempts to culture hepatitis A and B viruses have been generally unsuccessful, propagation of hepatitis A virus in marmosets has been reported, and transmission of hepatitis B virus to chimpanzees has been achieved. Also, a virus-like particle 27 nm in diameter has recently been detected in fecal extracts from hepatitis A patients by immune electron microscopy. Another technique (micro-SPIRA) that offers good prospects for detecting hepatitis A virus in large numbers of specimens has recently been developed.

The need to provide some form of active immunization against hepatitis B virus has been pointed up by studies showing an unusually high incidence of hepatitis B infection among selected groups of people: household contacts of HBsAg carriers, hospital or technical personnel working with blood or blood by-products, dentists (especially oral surgeons and their patients), staff members and residents of crowded mental or penal institutions, and members of the military. Unfortunately, despite encouraging findings in several areas, progress in development of a vaccine has been hampered by the very limited success in growing hepatitis B virus in culture and by the lack of a readily available animal model system.
REFERENCES


Efforts to improve nutritional conditions in Latin America and the Caribbean area must include measures to increase the production, marketing, and consumption of foods of high nutritional value.