Hepatitis A

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Hepatitis A is a disease of worldwide distribution which occurs in endemic and epidemic form and is transmitted primarily by person-to-person contact through the fecal-oral route. Common source epidemics due to contamination of food are relatively common, and water-borne epidemics have been described less frequently. The presumed etiologic agent of hepatitis A has now been visualized by immune electron microscopic (IEM) techniques in early acute-illness-phase stools of humans with hepatitis A as well as in chimpanzees experimentally infected with material known to contain hepatitis A virus. In addition, several new serologic tests for the detection of antibody against hepatitis A virus have been described. These include complement fixation and immune adherence techniques. Current data suggest that hepatitis A is caused by a single viral agent lacking the morphologic heterogeneity of hepatitis B viral components and that there may be relative antigenic homogeneity between strains of virus recovered from various parts of the world. Serologic studies to date also indicate that hepatitis A virus is not a major contributing cause in post-transfusion hepatitis.

BACKGROUND

The historical roots of infectious hepatitis as a distinct clinical entity go back, in the United States and Europe, to the late 18th and early 19th centuries, when reports of scattered outbreaks of a syndrome called "infectious," "epidemic," or "catarrhal" jaundice began to appear in the literature. Blumer, in 1923, described 63 epidemics of this disease which occurred in the United States between 1812 and 1920 (1). He differentiated the illness from Weil's disease and characterized it as a disease of childhood and early adult life, having an incubation period of up to 28 days, spread by person-to-person contact, and with greatest incidence in fall and winter months. Williams, in his report of 1923 on epidemic jaundice in New York state, corroborated the findings of Blumer and suggested a viral etiology (2). These early observations established the clinical, epidemiologic, and etiologic basis for a major form of viral hepatitis. In 1947, MacCallum proposed that this entity be called "hepatitis A" to distinguish it clearly from homologous serum jaundice or "hepatitis B" (3).

The existence of two distinct and separate viral etiologies for hepatitis A and B was firmly established during World War II in studies utilizing human volunteers (4-6). The disease was induced through oral feeding of subjects with serum and stool filtrates from acutely ill patients. Existence of homologous immunity was demonstrated through failure to reinduce hepatitis in convalescent volunteers when they were rechallenged with the same inoculum. However, if these individuals were rechallenged with serum or plasma known to cause hepatitis B, clinical hepatitis recurred. These data indicated a lack of cross immunity between the two forms of infection and thus the existence of two etiologic agents.

The human volunteer studies described above were confirmed and expanded by an extensive series of investigations in children, carried out at the Willowbrook State School in New York (7, 8). These studies revealed that the hepatitis A virus was present in feces during the incubation period of the disease, in some cases as early as
2 to 3 weeks prior to onset of jaundice. The shorter incubation period of hepatitis A in contrast to hepatitis B by either oral or percutaneous inoculation was experimentally confirmed, and a pedigreed serum pool containing hepatitis A virus, known as the MS-1 pool, was established. The virus contained in this pool has been commonly referred to as the MS-1 strain of hepatitis A virus. This material was used extensively in further prison volunteer studies at Joliet, Ill., supported by the U.S. Army, which resulted, as will be described later, in the first visualization of the hepatitis A virus.

**Epidemiology**

Hepatitis A is a disease of worldwide distribution which occurs in both endemic and epidemic form. In common with patterns of poliomyelitis before the advent of polio vaccine, the disease classically occurs in children with a demonstrable effect of socioeconomic and sanitation level on overall and agespecific incidence. Thus, in developing areas of the world and in communities in the United States where socioeconomic level is low and sanitation poor, epidemics appear to be cyclic, affecting infants and young children born during interepidemic periods. Since immunity following infection is of long duration, instances of disease in adolescents and adults are rare and usually involve in-migrant susceptibles. As with polio, childhood infection rates are high with the majority of infections being subclinical and clinical disease mild. In areas of higher socioeconomic level and where good sanitation practice exists, environmental barriers and increased host resistance may prevent infection in early childhood. In such areas, there are increased numbers of cases in adults. The geographic aggregation of such susceptibles has resulted in outbreaks in military barracks, colleges, universities, and summer camps. Clinical disease tends to be somewhat more severe in these older age groups and incidence of subclinical infection lower. For the United States as a whole, compilation of national morbidity data since 1952 indicates that type A hepatitis incidence followed a minor cyclic phase with yearly fall and winter peaks. Beginning in 1966, however, this seasonal cyclicity has tended to disappear, and, as shown in Fig. 1, yearly incidence had, by 1973, appeared to reach a plateau.

Man is the primary reservoir of the hepatitis A virus, although chimpanzees have

![Fig. 1. Incidence of viral hepatitis by 4-week period, U.S. 1966–1974 (source: U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Hepatitis Surveillance Report Number 37, Atlanta, Ga., June 1975).](image-url)
been shown to transmit assumed type A disease to human contacts (9-11) and can be experimentally infected with the virus (12). The most common route of transmission of hepatitis A is by person-to-person contact through the fecal–oral route, and fecal carriers of the virus who are asymptomatic or are in the preclinical stages of disease are major contributors to spread of infection. In addition, common source epidemics due to contamination of water and milk have been described, and food-borne outbreaks of hepatitis A are relatively common. Among specific food items incriminated have been raw oysters and clams, cold meat, green salad, and bakery items.

From the military point of view, the implications of the above epidemiologic features of hepatitis A are evident. The aggregation of large numbers of susceptible recruits into barracks and camps with common eating and sleeping facilities increases the likelihood of dissemination of infection by preclinical-stage fecal carriers of the virus. Similarly, assignment of troops abroad to areas of hepatitis A hyperendemicity provides multiple possibilities for contact with asymptomatic virus carriers. In the military context, food-borne epidemics constitute a particular hazard, the latest large-scale incident having occurred in 1975 at a naval station in Southern California (13).

**RECENT ADVANCES**

In recent years there have been numerous attempts to isolate and characterize the hepatitis A virus in cell culture systems and in nonhuman primate animal models. To date, all attempts at *in vitro* propagation of the virus have ended in failure. More successful were attempts, during the mid-1960's and early 1970's, to reproducibly induce presumed hepatitis A in marmosets (South American monkeys). In 1965, Deinhardt and colleagues reported induction and subpassage of enzymatically and histologically typical hepatitis in several subspecies of these animals (14). One serially transmissible agent, named “Barker” agent after a surgeon who developed acute viral hepatitis and whose acute-illness-phase serum induced hepatitis in the marmosets, was initially advanced as a possible hepatitis A candidate agent. In a separate series of experiments, Holmes, Deinhardt, and colleagues were able to induce hepatitis and subpassage the infection in marmosets inoculated with acute-illness-phase sera from the volunteers in the Joliet prison experiments mentioned previously who developed hepatitis following inoculation with the MS-1 strain of hepatitis A virus (15, 16). Both Lorenz and colleagues, using specimens from the United States (17), and Mascoli and co-workers, using specimens from cases of presumed hepatitis A in Costa Rica (18), were able to confirm the susceptibility of marmosets (specifically of the species *Saguinus mystax*) to infection. In none of the foregoing experiments, however, was a viral agent actually visualized. It was not until 1973 that the presumed etiologic agent was actually visualized by electron microscopie immune aggregation techniques.

The technique of immune electron microscopy (IEM) for direct observation of the interaction between viruses and their specific homologous serum antibodies was first described in 1941 (19) and successfully used by Kapikian and colleagues in 1972 for recovery of a specific enteric virus, “Norwalk agent,” shown to be etiologically responsible for infectious nonbacterial gastroenteritis (20). In 1973, Feinestone and colleagues reported visualization of viruslike particles in the early acute illness phase stools of Joliet prison volunteers who had developed hepatitis following previously described inoculations of the MS-1 strain of hepatitis A virus (21). Soon thereafter, in a report by Gravelle and co-workers (22), morphologically identical viruslike particles were recovered from acute-illness-phase stools from individuals acquiring hepa-
Hepatitis A-associated viral like particles visualized by the IEM technique.

Hepatitis during a common source epidemic of type A disease in Arizona. As described by both groups, these particles are spheres of approximately 27-nm diameter. Figure 2 shows a characteristic group of particles aggregated by convalescent phase serum from a patient with antecedent hepatitis A. The halos surrounding the particles represent specific hepatitis A antibody interacting with virus. Utilizing the ability of the IEM procedure to detect serum antibody as well as antigen, both Feinstone and Gravelle and co-workers detected antibody seroconversions to the particles between preinoculation and convalescent-phase sera in prison volunteers developing hepatitis after inoculation with the MS-1 strain of virus or rises in titer of serum antibody between acute-illness-phase and convalescent sera from cases of hepatitis A from other sources. Both groups failed to detect similar serum antibody rises from cases of hepatitis B.

In an attempt to examine possible infectivity associated with these particles, Maynard and colleagues were able to induce and successfully subpassage enzymatically and histologically confirmed hepatitis in chimpanzees inoculated with human stool filtrates containing the particles (12). Animals excreted morphologically identical particles during the early acute stage of hepatitis and developed serum antibody directed against the particles, further supporting the inference that the particles themselves, now referred to as hepatitis A antigen (HA Ag), constitute the virus of hepatitis A. The link between HA Ag and experimentally induced hepatitis A in marmosets was provided in a collaborative study between the Center for Disease Control, Food and Drug Administration, and National Institutes of Health, in which S. mystax marmosets developing hepatitis after inoculation with the MS-1 strain of hepatitis A virus developed antibody seroconversions to HA Ag (23). In addition, liver homogenates from animals sacrificed in the acute stage of infection contained HA Ag. Provost and colleagues have also recovered 27-nm-diameter viruslike particles from the livers of S. mystax marmosets which developed hepatitis after inocula-
tion with specimens from individuals acutely ill with hepatitis A in Costa Rica (24). Krugman and co-workers have demonstrated that children developing hepatitis A in the Willowbrook studies developed convalescent-phase antibody against these particles, thus relating the Costa Rican-derived particles as well as the previously described classes of HA Ag to MS-1 hepatitis A (25).

Using IEM techniques with HA Ag as antigen source to examine the possible relationship of Barker agent-induced marmoset hepatitis to hepatitis A, it has been observed that antibody seroconversions to HA Ag do not occur in animals infected with Barker agent (26). These data indicate that the Barker agent is not related to hepatitis A.

Of major importance has been the development of several additional laboratory techniques for the diagnosis and seroepidemiologic investigation of hepatitis A. Hilleman and colleagues have recently reported development of complement fixation and immune adherence tests for detection of hepatitis A antigen and antibody, utilizing HA Ag purified from the livers of marmosets infected with hepatitis A virus (27). Utilizing immune adherence, they presented preliminary data indicating that approximately 30% of adults from a small sample of American workers and blood donors were hepatitis A antibody positive, in contrast to 85% in a small sample of adults in rural Costa Rica. They were also able to characterize 24 commercial lots of immune serum globulin in regard to hepatitis A antibody content. Further development of the marmoset as a source of diagnostic reagents for hepatitis A antibody testing has, however, been severely limited by the general unavailability of these animals. The recent success of workers at the National Institutes of Health in purifying HA Ag from human stool for use as reagent in immune adherence testing suggests another possible source of reagent material. However, the logistic difficulty of obtaining sufficient early acute-illness-phase stool on a regular basis from hepatitis patients may severely limit the general availability of this material. Hollinger and colleagues have recently described adaptation of radioimmune assay techniques for detection of hepatitis A antigen and antibody, utilizing antigen purified from stool of infected chimpanzees (28). It is hoped that the chimpanzee may provide a more reproducible source of hepatitis A diagnostic reagents until a suitable in vitro cell culture system for propagation of the virus is found.

Hepatitis A antigen has now been found in stools from naturally occurring cases of hepatitis A in several areas of the United States, ranging from Georgia to Arizona, California, and Alaska. Dienstag and co-workers were able to recently demonstrate the particles in stool of naval recruits involved in a large foodborne outbreak of hepatitis A in San Diego (13). In addition to the particles recovered by Provost et al. from Costa Rican cases (24), Locarnini and colleagues have recovered HA Ag from acute cases of hepatitis A in Australia (29). The morphologic and immunologic similarity of the various classes of particles referred to as HA Ag, together with the consistency in worldwide epidemiologic patterns for the disease, suggest that hepatitis A is caused by a single viral agent lacking the heterogeneity of hepatitis B virus structural components. It seems reasonable to infer that there will be little major antigenic heterogeneity between strains of virus recovered from different geographic areas, thus accounting for the passive protection conferred by United States-derived immune serum globulin in other parts of the world.

From the military viewpoint, risk of infection should be viewed within the context of enteric transmission, since the viremic state for hepatitis A, as with polio, is presumed to be transient. Recent serologic studies by Alter et al. (30) and Feinstone and colleagues (31) indicate that non-hepatitis B-associated post-transfusion hep-
titis is also not associated with hepatitis A. This presumed lack of a hepatitis A vi-
remic carrier state also has significant implications for the possible early develop-
ment of hepatitis A vaccines. Unlike hepatitis B vaccine, which can be produced from
human hepatitis B antigen carriers without requirement for \textit{in vitro} cultivation of the
virus, production of a hepatitis A vaccine will most probably require a breakthrough
in technology for \textit{in vitro} replication of hepatitis A virus.

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