Hepatitis A

Perspectives and Recent Advances

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The basis for the epidemiologic and etiologic differentiation of two major forms of viral hepatitis, hepatitis A and B, was established in a series of studies undertaken between 1930 and 1970. Final recovery and visualization of the presumed etiologic agent of hepatitis A was not, however, accomplished until the technique of immune electron microscopy was applied to the examination of specimen materials collected from individuals in the early acute stages of infection. Morphologically homogeneous virus-like particles of 27 nm diameter have now been recovered from stools of patients with hepatitis A ill from a variety of sources. Antibody to these particles has been shown to develop during the course of infection with hepatitis A but not with hepatitis B and disease has been induced in nonhuman primates inoculated with purified particle containing fractions. The classification of hepatitis A virus has not been conclusively established, but it would appear to be either a parvovirus or an enterovirus. (Am J Pathol 81:683–694, 1975)

Historical Background

EVIDENCE FOR THE EXISTENCE of a distinct clinical entity characterized by fever and jaundice began accumulating in the late eighteenth and early nineteenth century, when reports of scattered outbreaks of disease, referred to as "infectious," "epidemic," or "catarrhal" jaundice, began to appear in the literature in the United States and Europe. Blumer, in 1923, described 63 epidemics of infectious jaundice in the United States between 1812 and 1920.[1] He carefully differentiated the illness from Weil's disease and further characterized infectious jaundice as a disease of childhood and early adult life, having an incubation period of up to 28 days, spread largely by person-to-person contact, and with greatest incidence in fall and winter months. Williams, in 1923, described the course of epidemic jaundice in New York State and suggested that the disease might be caused by a virus.[2] These early observations established the basis for definition of one of the two current major forms of viral hepatitis, known as hepatitis A.

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That a percutaneously transmitted form of hepatitis might exist was first documented by Lurman in 1885.[3] He described an epidemic of jaundice in Bremen, Germany, in factory workers who became ill after receipt of smallpox vaccine prepared with human lymph. It was not until the late 1930s, however, that the existence of hepatitis transmitted by inoculation of human serum was firmly established. The report, in 1937, of an outbreak of disease in individuals receiving human measles convalescent serum and in British troops given human mumps convalescent plasma, resulted in the classification of these related entities as "homologous serum jundice." [4] In most investigations, the similarity in clinical syndromy between percutaneously transmitted hepatitis and catarrhal or infectious jaundice was noted. It was, therefore, easy to assume that a single etiologic agent was responsible for both forms of the disease. It was not until the extensive work of Fox and colleagues, who, in 1939, investigated an outbreak of jaundice in Brazil following administration of yellow fever vaccine, that the epidemiologic differences between infectious and homologous serum jaundice were clearly described and existence of two distinct etiologic agents postulated.[5] MacCallum, in 1947, proposed that the terms *hepatitis A* and *hepatitis B* be used, respectively, to designate "infectious" and "serum" hepatitis,[6] and these terms have appropriately emerged in current usage.

The viral etiology of hepatitis A was firmly established during World War II in studies involving human volunteers.[7-9] The disease was induced through oral feedings of subjects with serum and stool filtrates from acutely ill patients, but not through feedings of acute illness phase nasopharyngeal washings and urine. Individuals convalescing from hepatitis A did not develop illness when rechallenged with the same virus. If they were rechallenged with material containing the virus of hepatitis B, clinical hepatitis recurred. This data proved that immunity was acquired following Type A infection, but that protection was not conferred against rechallenge with Type B virus.

In 1957, Ward and colleagues successfully induced hepatitis A in pediatric subjects who were fed with pooled, filtered stool suspension obtained from children acutely ill with the disease.[10] Their studies revealed that the virus was present in feces during the incubation period of the disease, some 2 to 3 weeks before onset of jaundice. In 1967, Krugman and colleagues reported that a serum pool collected from a child just prior to onset of hepatitis could induce hepatitis in subjects by both oral and percutaneous inoculation.[11] By either route of inoculation, disease occurred after a short 31- to 38-day incubation period. This pool became

known as the MS-1 pool, with the virus contained in it now commonly referred to as the MS-1 strain of hepatitis A virus.

Tissue Culture and Animal Infectivity Studies

In recent years, there have been numerous attempts to isolate the hepatitis A virus in cell culture systems. Although a variety of known viral agents, including adenoviruses, paramyxoviruses, and certain strains of parvoviruses, have been recovered in cell culture following inoculation with presumedly virus-containing materials, none have been shown to be etiologically related to hepatitis A. To date,the hepatitis A virus has eluded all attempts at *in vitro* propagation.

Lack of success in *in vitro* cultivation of the virus lead to a series of attempts to transmit the disease to nonhuman primates. In 1965, Deinhardt and colleagues reported induction of enzymatically and histologically typical hepatitis in several subspecies of marmoset monkeys after inoculation of samples from patients acutely ill with hepatitis.[12] One of these marmoset transmissible agents, recovered following animal inoculation with acute illness phase serum from a surgeon (GB), was subjected to extensive study in several laboratories. Parks and Melnick, after reported recovery of similar agents from marmosets not subject to experimental inoculation, concluded that the GB agent might in fact be a latent agent of marmosets rather than related to human disease,[13] an assertion disputed by Deinhardt and colleagues.[14] In another series of experiments, Holmes, Deinhardt, and colleagues, were able to induce hepatitis and successfully subpassage the infection in marmosets inoculated with acute illness phase sera of volunteers who developed hepatitis following inoculation with the MS-1 strain of hepatitis A virus.[15,16] Both Lorenz and co-workers, utilizing local specimen materials from the United States,[17] and Mascoli and colleagues, utilizing acute illness phase sera from cases of hepatitis A in Costa Rica,[18] were able to confirm the susceptibility of marmosets (specifically of the subspecies *Saguinus mystax*) to infection with human hepatitis A virus.

Recovery and Preliminary Characterization of the Hepatitis A Virus

It was not until recent use of electron microscopic immune aggregation techniques for attempted recovery of the hepatitis A virus that definitive visualization of the etiologic agent became possible. Use of these methods has now allowed serologic analyses and resolution of questions posed by

prior marmoset infectivity studies, and enabled definitive establishment of the chimpanzee as another model of nonhuman primate infectivity for the study of this infection.

The technique of immune electron microscopy (IEM) for direct visualization of interactions of virus and antibody was first described in 1941 [19] and was later elaborated by Almeida and Waterson in 1969.[20] It was also successfully adapted by Bayer *et al.* [21]and later, Almeida *et al.*[22] for the study of hepatitis B virus.

In 1972, Kapikian and colleagues using IEM techniques visualized a virus-like particle in stools of individuals acutely ill with infectious nonbacterial gastroenteritis.[23] This virus (Norwalk agent) was shown to be etiologically responsible for the disease. Workers at the National Institutes of Health correctly inferred, on both epidemiologic and clinical grounds, that the proven enteric transmissibility of hepatitis A should render preacute and early acute illness phase stools from cases as optimum specimen sources for recovery of the etiologic agent. They further postulated, on the basis of analogy with human polio virus infection, that titer of virus shedded in stool would be higher than that present in viremic serum, thus making stool a preferable specimen source for attempted virus visualization by IEM techniques. As a direct consequence of these considerations, Feinstone and colleagues, in 1973,[24] were able to report visualization of a virus-like particles in acute illness phase stools of volunteers who developed hepatitis following inoculation with the MS-1 strain of hepatitis A virus. These volunteers had also provided the primary inocula for the marmoset transmission studies of Holmes, Deinhardt, and colleagues referred to earlier.[15,16] In addition to demonstration, by IEM, of antibody seroconversions to the recovered particles between preinoculation and convalescent serum specimens in the above volunteers, Feinstone *et al.* found similar rises in titer of antibody between acute and convalescent sera in several cases of hepatitis A from other sources but not in similarly collected sera from cases of hepatitis B. In a subsequent report, Feinstone and co-workers [25] found that their particles banded in cesium chloride (CsCl) between 1.36 and 1.43 g/cu cm, with a single peak at 1.40 g/cu cm. On the basis of this and other findings, they concluded that the particles were parvovirus-like.

The first visualization of hepatitis A virus–like particles from a naturally occurring epidemic of the disease was reported by Gravelle and colleagues at our laboratory,[26] who observed 27-nm virus-like particles in stool pools from acutely ill individuals sampled during a common source outbreak of disease in Arizona.[27] As shown in Figure 1, these particles were clearly aggregated by antibody contained in convalescent serum from cases of

Table 1—IEM Levels of Antibody to Phx Ag in Paired Sera of Patients Acquiring Hepatitis A During a Common Source Outbreak

Patient	Serum sample	Level of IEM antibody* against Phx Ag
A	Acute illness	0
	Convalesent	3
B	Acute illness	0
	Convalescent	2
C	Acute illness	1
	Early convalescent	2
	Late convalescent	4
D	Acute illness	1
	Convalescent	3
E	Preacute illness	0
	Acute illness	1
	Convalescent	3
F	Acute illness	1
	Convalsecent	4

*0 = no antibody, 1 to 4 = increasing amounts of antibody.

disease and were morphologically identical to the particles earlier described by Feinstone. The particles recovered in Arizona were designated Phoenix antigen (Phx Ag). Table 1 indicates that increases in serum antibody titer to these particles could be demonstrated by IEM in paired sera of individuals who contracted illness during the outbreak. Such antibody titer increased did not occur in paired sera from cases of hepatitis B. Serologic comparison of Phx Ag with the particles recovered by Feinstone *et al.* showed that the Feinstone particles detected antibody seroconversions in paired sera from the Arizona hepatitis cases, while Phx Ag detected similar seroconversions in paired sera from the MS-1 inoculated human volunteers from whom Feinstone *et al.* made their particle recoveries.

As reported by Maynard *et al.*,[28] and in an attempt to examine infectivity possibly associated with Phx Ag, 3 chimpanzees were inoculated intravenously with stool filtrates containing these particles. An additional animal was inoculated with particle-containing, acute illness phase stool from 1 of the first 3 inoculated animals. All animals were seronegative by IEM for antibody to Phx Ag, and as shown in Table 2, all developed hepatitis following inoculation of the particle-containing stool materials. In 3 of the 4 animals morphologically identical particles were recovered by IEM techniques from stools in the early acute stage of disease. All 4 animals developed IEM antibody to the particles in the inoculum. These data emphasized the close association between particle inoculation, par-

Table 2—Occurrence of Hepatitis in Chimpanzees Inoculated With Hepatitis A-Associated, Virus-Like Particles

Number	Inoculum	Occurrence of hepatitis	Recovery of 27-nm particles acute phase stool	IEM antibody seroconversion to 27-nm particles
0611	Phx Ag	+	0	+
0722	Phx Ag	+	+	+
0754	Phx Ag	+	+	+
0084	722 Ag	+	+	+

ticle excretion, development of serum antibody directed against the particles, and the occurrence of hepatitis.

Results of CsCl banding experiments on the classes of particles recovered in our laboratory were reported by Bradley and colleagues,[29] who found certain differences in banding characteristics between these particles and those recovered by Feinstone *et al.* As shown in Table 3, the particles from both humans and chimpanzees exhibited a heterogeneous banding profile with peak particle counts at 1.32 to 1.33 g/cu cm and minor peaks at buoyant densities of 1.39 to 1.41 g/cu cm. When particle-containing fractions at 1.32 and 1.41 g/cu cm from chimpanzee 0722 were inoculated into two groups of marmosets, animals in both groups developed hepatitis, indicating that infectivity banded heterogeneously as well. Infectivity titrations of the two buoyant density fractions were not done due to lack of available animals. Although these findings would not rule out the parvovirus-like nature of the particles recovered by us, the observation of peak particle counts in the 1.32 to 1.33 g/cu cm buoyant density range is also consistent with the banding characteristics for enteroviruses.

The relationship of the particles recovered by Feinstone *et al.* [24] and by us to MS-1 hepatitis A in marmosets was examined in collaborative studies between our laboratory, the Bureau of Biologics, Food and Drug Administration, and the National Institute of Allergy and Infectious Diseases.

Table 3—Isopycnic Banding in CsCl of Hepatitis A-Associated Virus-Like Particles Derived From Stool

Particle source	Buoyant density peaks (g/cu cm)	
	Primary	Secondary
Human		
Phx Ag	1.32	1.41
Chimpanzee		
0722	1.32	1.41
0084	1.33	1.39

These experiments utilized *S. mystax* marmosets inoculated with serum containing the MS-1 hepatitis A virus provided by Dr. S. Krugman. Hepatitis occurred in 9 of 20 animals inoculated with MS-1 serum, and hepatitis was induced through 3 subsequent animal passages utilizing pooled sera collected from marmosets in each preceding subpassage at and just prior to serum enzyme elevations. Animals with hepatitis at each passage level developed IEM antibody against the virus-like particles described above, and liver homogenates from 3 animals in the fourth passage experiment, who died in the acute phase of hepatitis, revealed particles morphologically indistinguishable from those recovered by Feinstone *et al.*[24] and ourselves.

Additionally, although hepatitis could be consistently induced in marmosets inoculated with the GB agent recovered by Deinhardt and colleagues, antibody rises to the hepatitis A–associated, virus-like particles could not be detected in these animals.

The foregoing results suggest that the virus-like particles recovered by the NIH workers and ourselves constitute the virus of hepatitis A of the MS-1 prototype. They also confirm and extend observations on the susceptibility of marmosets and chimpanzees to infection with this agent. It would now appear evident that the GB agent is not related to hepatitis A.

Classification and Strain Characterization of the Hepatitis A Virus

In addition to particles recovered during the above mentioned outbreak in Arizona we have now found morphologically identical particles in stools from cases of hepatitis A from several areas of the United States ranging from Georgia to Alaska. Locarnini and colleagues have recently recovered similar particles from stools of acute cases of hepatitis A in Australia.[30] Also, Provost and colleagues have now described the virus-like particles recovered from the marmosets infected with specimens from individuals acutely ill with hepatitis A in Costa Rica as morphologically similar to the previously described classes of particles.[31] The experimental linking of all these particle classes to MS-1 hepatitis A, together with the consistency in worldwide epidemiologic patterns for the disease, suggest that hepatitis A is caused by a single viral agent lacking the morphologic heterogeneity characteristic of the structural components of hepatitis B virus. It also seems reasonable to hypothesize that there will be relatively little major antigenic heterogeneity between strains of viruses recovered from different geographic areas. More definitive strain characterization of this virus must await the development of additional serologic tests for antigen and antibody detection. The recent communication of Hill-

eman,[32] reporting the development of complement fixation and immune adherence tests using viral antigens prepared from infected marmoset liver, promises an important new laboratory tool for further seroepidemiologic study of hepatitis A.

The classification of the hepatitis A virus has not been conclusively established. Provost *et al.*[31] have provisionally classified their Costa Rican isolate as an enterovirus, based primarily on buoyant density analysis (peak particle density at 1.34 g/cu cm), acridine orange staining characteristics, and intracytoplasmic visualization of virus in marmoset hepatocytes. The finding by Feinstone *et al.*[24] of highest concentration of particles at buoyant densities (1.40 g/cu cm) more characteristic of parvoviruses argues against an enterovirus classification. Our findings indicate heterogeneous banding of the virus with peak particle counts in a range consistent with that reported by Provost and co-workers. Final classification of this agent must await the results of further studies now in progress.

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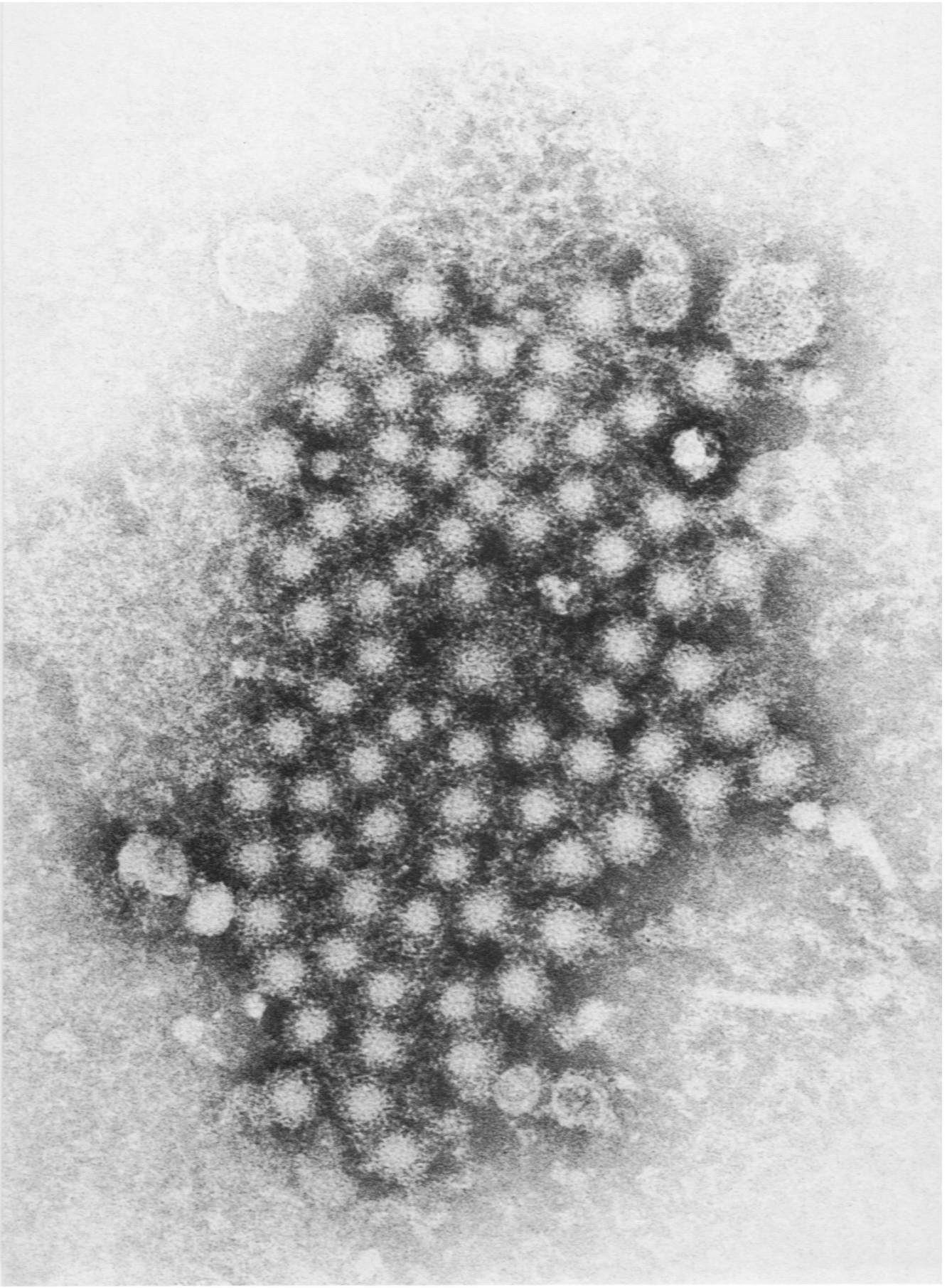
Figure 1—Hepatitis A-associated virus-like particles in pooled stool filtrate from acutely ill patients coated by antibody in convalescent phase serum from a patient.



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