Raymond S. Koff

Viral Hepatitis

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VIRAL HEPATITIS

Raymond S. Koff, M.D.
Chief, Hepatology Section
Boston VA Hospital and
Boston University School of Medicine
Boston, Massachusetts

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SERIES PREFACE

During the past decade remarkable progress has been made in our understanding of many basic physiological processes related to liver and gastrointestinal tract functions. Much of this information has led to significant improvements in our understanding of clinical diseases that alter normal hepatic and intestinal function and in the therapy of these diseases. Innumerable examples can be cited. For instance, the application of basic principles of physical chemistry has clarified considerably the manner in which cholesterol is solubilized in bile. Related studies have identified the causes of cholesterol gallstones in several large groups of patients, and specific forms of therapy for the prevention or the dissolution of such stones are now available. Other experimental work that relies heavily on basic techniques of immunology and electron microscopy has identified specific infectious agents affecting liver function. These studies, in turn, have provided considerable insight into the different clinical syndromes included under the general heading of viral hepatitis, raising the possibility that effective immunization against these organisms may soon be available. Equally impressive advances have been made in our understanding of the control of gastric secretion and peptic ulcer disease, in the causes of intestinal malabsorption, and in radiographic and endoscopic methods for examining the liver and gastrointestinal tract.

This explosion of knowledge in gastroenterology poses a particularly difficult problem for those interested in the dissemination of new medical information to students, house officers, and medical practitioners. Often advances have come so quickly that the information presented in standard textbooks is outdated before the books become available. Also, it is difficult to revise such texts rapidly because of the large number of authors involved and the long production time necessary for these books. Finally, the space available to authors for extensively reviewing both the basic physiological concepts and their clinical implications is limited in most texts and in more rapidly published medical journals.

This series of volumes published under the general title "Clinical Gastroenterology Monographs" was conceived and designed to overcome many of these difficulties and to bring to the medical practitioner the most current information on the pathophysiology and treatment of major areas of disease affecting the liver and gastrointestinal tract. Each volume covers an important group of related disorders and is sufficiently long to allow for extensive discussion of their

basic pathophysiological, clinical, and therapeutic aspects. New volumes will appear regularly, and a special effort will be made to identify areas for inclusion in the series in which there is a rapidly expanding body of information relevant to the care of patients with a particular gastrointestinal disorder. Existing volumes will be updated and republished frequently where continued advances in information justify such rapid revision.

It is hoped that this series will provide a continuously evolving and current reference source for the broad spectrum of physicians who deal with patients with diseases of the liver and gastrointestinal tract.

John M. Dietschy, M.D.

PREFACE

After several decades of frustration in viral hepatitis research, a large and ever-growing number of investigators in multiple disciplines have been "turned on" by recent advances. Their efforts have resulted in an extraordinary explosion of new information concerning the spectrum of viral hepatitis. In fact, viral hepatitis has become the most extensively written about subject in the recent medical literature. Progress in the field, especially concerning the biophysical and immunologic properties of the hepatitis viruses, has been achieved largely through the ability to identify the agents coupled with experimental transmission of infection to nonhuman primates. It is remarkable that this progress has been achieved despite the repeated failure to grow these viruses in a variety of standard or atypical tissue culture systems.

It is my hope that this monograph will provide the reader with more than a simple statement of the current state of the art. I hope to present a perspective on this data-base which will be of continued usefulness to the reader. New observations should be readily engrafted on this foundation and comprehension of their importance should be facilitated by familiarity with the virology, immunology, epidemiology, and clinical features of viral hepatitis presented here.

I thank Frances Singer for her care in typing this material and the staff and fellows of the Hepatology Section of the Boston VA Hospital for their patience with me during the preparation of this book.

Raymond S. Koff, M.D.

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INTRODUCTION

HISTORY

Sporadic cases and occasional outbreaks of jaundice, presumably due to acute viral hepatitis, have been recorded in diverse geographic areas throughout history. One suspects that whenever and wherever large groups of people gather under poor sanitary conditions viral hepatitis arises. Hepatitis as a concomitant of institutional life is well-known. It has a prominent position in the history of warfare since epidemics of jaundice were recognized scourges of combatants in antiquity as well as in modern times. Some epidemiologically oriented historians and historically oriented epidemiologists believe that viral hepatitis has played a key role in the outcome of certain military conflicts. McCollum (1) emphasizes the military importance of these diseases and convincingly argues that hepatitis will be a military problem until effective vaccines are developed.

We can understand viral hepatitis best by looking at several historic stages. In the first, which lasted several millennia, the disease was recognized and described in some detail, but nothing was known about etiology or pathophysiology. The pathology was obscured, in fact, by misconceptions promulgated by the giants of pathology in the nineteenth century. They failed to clearly distinguish hepatitis from other common hepatobiliary disorders. As described by MacCallum (2) the second stage began about four decades ago, and great interest in the disease was stimulated by World War II. This was a phase of clinico-epidemiologic and transmission studies which represent milestones in the recognition of distinct etiologic agents, with different incubation periods, routes of transmission, and infectivity. To a great extent these studies were the basis for our present knowledge of the diseases.

The third historical phase of viral hepatitis, the current stage, began about a decade ago with the serendipitous discovery of the surface antigen of the hepatitis B virus which was subsequently associated with the agent. Enormous amounts of new information are appearing at an almost exponential rate. The rubric viral hepatitis has become the most frequently cited topic in Index Medicus. Biophysical and biochemical characterization of the viruses has advanced rapidly, immunologic information has increased, and specific markers and models of infection have been developed. Increased sophistication in the understanding of the epidemiology, clinical course and sequelae, and oncologic implications of hepatitis infections has resulted and prevention has been given a scientific basis.

TERMINOLOGY

The nomenclature of viral hepatitis, the hepatitis viruses, and their antigens and antibodies has changed with newly acquired information about their properties. The following terminology, adopted from the Report of the WHO Expert Committee on Viral Hepatitis (3), is employed throughout this monograph. For ease of reading, however, each term and abbreviation is redefined as necessary.

Hepatitis A

This form of viral hepatitis has also been known as infectious hepatitis, epidemic hepatitis, MS-1 hepatitis, and short-incubation period hepatitis. The responsible agent is the hepatitis A virus, abbreviated HAV. Antibody to HAV is termed anti-HAV.

Hepatitis B

This form of viral hepatitis has been called serum hepatitis, homologous serum jaundice (homologous serum hepatitis), MS-2 hepatitis, long-incubation period hepatitis, and Australia antigen-positive, SH-antigen positive, or hepatitis B surface antigen-positive viral hepatitis. The responsible agent is the hepatitis B virus, abbreviated HBV. The virus was earlier known as the Dane particle. The antigens of HBV and their respective antibodies are as follows:

- Hepatitis B surface antigen, abbreviated HBsAg. Its corresponding antibody is anti-HBs. Subdeterminants of HBsAg are indicated by a set of identifying letters, eg, HBsAg/aywl. The *a* represents a common subdeterminant and *y* and *w*1 represent combinations of subdeterminants.
- hepatitis B core antigen, abbreviated HBcAg. Its antibody is anti-HBc.
- hepatitis B associated e antigen, abbreviated HBeAg. Its corresponding antibody is anti-HBe. The two recognized antigens are written HBeAg/1 and HBeAg/2.

Non-A, Non-B hepatitis

This newly recognized form of viral hepatitis appears to be due to more than one agent. No means of identifying the agents, antigens, or antibodies are available. Some authors suggest the terms hepatitis C and hepatitis D, but these probably should be reserved until specific entities can be identified by serologic techniques.

THE IMPORTANCE OF VIRAL HEPATITIS

Viral hepatitis remains the major unconquered viral disease in the United States. In addition to the immediate morbidity and mortality associated with the disease, a risk of important sequelae with further potential morbidity and mortality has been recognized for type B and non-A, non-B viral hepatitis. Recent estimates of the economic impact of viral hepatitis in the United States (4) do not consider these

sequelae. Nonetheless, they provide a measure of direct costs, which include physician services and hospital, laboratory, and administrative fees and expenses related to preventive treatment, and indirect costs, which include productivity losses due to time off from work and loss of current and future productivity due to premature hepatitis-associated deaths.

For 1970 (4), total direct costs were estimated to be $180 million; indirect costs, $470 million. Long-term sequelae in patients with non-A viral hepatitis, estimated to develop in 10% of cases, increases the economic impact of viral hepatitis strikingly. The social, psychological, and medical impact of this sequence cannot be estimated.

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2

HEPATITIS A VIRUS

THE AGENT

No useful purpose is served by reviewing the long and, until recently, frustrating history of reports of the possible isolation of putative hepatitis A viruses. The first unequivocal demonstration (by visualization) of hepatitis A virus (HAV) resulted from the use of immune electron microscopy (1). This procedure, made possible because antigen is coated and aggregated by homologous antibody, was originally described by Almeida and Waterson (2) who used it in studies of hepatitis B (3). Subsequently it was used to visualize the viral agent responsible for some cases of infectious nonbacterial enteritis (4).

In 1973 Feinstone and co-workers (1) reported the discovery, through the technique of immune electron microscopy, of a virus-like particle in the stools of prison volunteers in whom acute hepatitis developed following inoculation with hepatitis A–containing materials. The demonstration of virus-like particles in fecal specimens was not particularly new or surprising since earlier electron microscopic studies had revealed 18 to 25 nm and 35 to 40 nm particles in the stools of patients with acute hepatitis A (5). No specific relationship of these particles to hepatitis A were demonstrated in that study. Feinstone and associates (1) showed that the particles they found were serologically specific since antibody was present in sera during convalescence but not prior to inoculation. Antibody seroconversion was also found in other hepatitis A cases and antibody was shown to be present in immune serum globulin.

Gravelle and associates (6) subsequently confirmed these observations: they recovered an identical appearing virus-like particle from the stools of patients infected in a foodborne epidemic and demonstrated appropriate changes in serum antibody titers in affected cases. In addition they reported that they successfully infected chimpanzees with concentrated stool specimens from infected patients and recovered morphologically identical particles in the feces of these primates.

These observations have been repeatedly confirmed and extended, and there is no doubt that this virus-like particle, shown in Figure 2-1, causes hepatitis A since it is specifically associated both serologically and temporally with hepatitis A infection. The particle is approximately 27 nm in diameter (1), demonstrates cubic symmetry, and appears to have a surface substructure with 8 to 12 nm capsomeres discerned in some electron microscopic studies (7). In one study, utilizing a modified micromethod for electron microscopy, an internal core-like structure

was seen within the particle with a double-layered coat around the core (8). It is possible that the outer coat represents protein capsomeres while the inner coat represents the nucleocapsid of the virus. Further morphologic characterization of the particle may provide more detailed information concerning the structural components of this agent.

When stained with phosphotungstic acid, the particles appear to be either full (not penetrated by the stain) or empty (penetrated by stain) (9). It has been postulated that the empty particles are deficient in nucleic acid. Although immune aggregates of hepatitis A particles are seen after incubation of filtrates of stool with antibody, in some studies direct electron microscopy has revealed crystalline arrays, containing more than 50 of the 27 nm virus-like particles (7). It is not known whether these arrays are artifacts or represent a unique form of viral packaging or release.

Biochemical and biophysical characterization of HAV also remains incomplete. Three populations have been described when HAV-like particles are banded in cesium chloride gradients (9–11). The major peak with a buoyant density of about 1.28 g/cm^3, appears to be empty when stained with phosphotungstic acid. The intermediate particle may be lost during purification (9). Limited data suggest that the heavy and intermediate particles are found chiefly in the feces, whereas intermediate and low density particles are present in liver tissue and bile. It is not clear whether these differences reflect changes in the particles due to variations in viral replication or to transfer of the particles from the liver to the stool via the biliary tract, or whether they are related to the time when specimens are collected from humans or infected animals. These particles appear to be serologically related, since all react with specific anti-HAV.

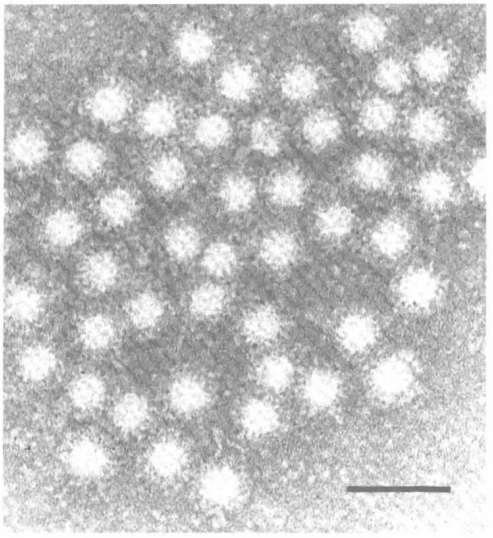

FIGURE 2-1 Antibody-coated aggregate of 27 nm hepatitis A virus (HAV) particles purified from the stool of a patient with acute hepatitis. The bar represents 100 nm. Original magnification × 132,000. (Courtesy of Dr. Jules L. Dienstag.)

The polypeptide composition of HAV has not been elucidated and its nucleic acid type has not been definitively determined. Staining with acridine orange suggests the presence of either RNA or single-stranded DNA (12). Partial inactivation of infectivity by incubation with pancreatic RNAase provided presumptive evidence for the presence of RNA (12). These studies are not confirmed and further characterization is required.

The liver and serum of marmosets and the liver, bile, and feces of experimentally infected chimpanzees have served as sources of HAV but the availability of these materials is severely limited and the quantity of virus obtained is often low, thus limiting research efforts. Attempts to purify HAV have often shown rapid losses of activity which are correlated with the number of purification procedures undertaken, presumably because stabilizing factors are removed (9). These limitations have hindered definitive studies and accurate classification of this virus. Two suggestions have been offered. Feinstone et al (11) postulated that the particle has a high mean buoyant density (on the basis of morphologic appearance) and that it is a parvovirus (it resists acid, ether, and heat as reported by Provost and coworkers [12]). Provost et al (12) and Bradley et al (10) observed an intermediate buoyant density particle; on this evidence and the presumptive evidence for the presence of RNA mentioned above and an intracytoplasmic location of viral particles in infected marmoset hepatocytes, they suggest that the particle is an enterovirus that belongs to the picornavirus group. Additional studies are required to determine the true class to which this virus belongs.

THE ANTIBODY (anti-HAV)

Antibody to hepatitis A virus is detected by immune electron microscopy during the acute phase of hepatitis A when IgM levels are elevated in sera (1). Anti-HAV as detected by immune electron microscopy, increases thereafter, reaching a plateau about 6 weeks after the onset of symptoms. In contrast, anti-HAV detected by the technique of immune adherence hemagglutination is not observed until about three to four weeks after the onset of illness and reaches peak levels about 3 to 11 months after infection (13, 14). It has been suggested that immune adherence hemagglutination detects convalescent phase IgG antibodies whereas immune electron microscopy measures acute phase IgM antibodies directed to hepatitis A virus (HAV) (15). Complement fixation techniques have also been described but are less specific, less sensitive, and more difficult to perform (16). Solid-phase radioimmunoassay procedures appear to be similar in sensitivity to immune eletron microscopy and also detect an IgM acute phase antibody (15, 17, 18). A radioimmunoassay–IgM blocking technique has been reported in which a single specimen obtained in the acute phase of illness may be adequate for serologic diagnosis of hepatitis A (15). In late convalescence sera immune adherence hemagglutination may be the most appropriate serologic technique for detection of anti-HAV (14, 19, 20). Two sets of observations from two distinct sources indicate that the antibody detected by immune adherence persists on followup for at least 5 to 18 years, and probably indefinitely (16, 21, 22).

These techniques have also been used to detect HAV in stool, liver, and, rarely, in serum. Present data indicate that radioimmunoassay can detect lower concent-

rations of HAV than immune electron microscopy but that some false positive reactions may occur (23). Immune adherence is also less sensitive than radioimmunoassay and less specific than radioimmunoassay or immune electron microscopy. The pattern of excretion of HAV (described later in this chapter) is such that for clinical purposes diagnostic screening of stools for antigen with any of these techniques is impractical. Unfortunately routine serologic testing for anti-HAV is not yet a clinically applicable tool simply because of the limited availability of purified antigen for commercial development of a reliable test. Undoubtedly this limitation will be overcome soon.

PATTERNS OF VIRUS APPEARANCE AND DISAPPEARANCE

Viremia

There is undisputed experimental and clinico-epidemiologic evidence that virus is present in blood during the course of hepatitis A infection. Transmission studies with human volunteers suggest that the infectious agent is in blood for two and a half weeks before and about two weeks after the onset of symptoms (24). This interval represents a part of the range for a few patients, rather than the duration of viremia in one patient since sequential studies in an individual patient have not been undertaken. Thus the time course of viremia may vary greatly from one patient to another.

Transmission studies and epidemiologic inferences based on multiple observations suggest that viremia does not usually persist beyond the acute phase of illness. In contrast to hepatitis B, and to non-A, non-B viral hepatitis, a viremic carrier state has not been postulated for hepatitis A.

With the advent of immunoserologic identification of hepatitis A virus (HAV) it should become possible to more clearly define the viremic phase of hepatitis A and to determine whether variations occur in virus titer, time of appearance, and time of disappearance of circulating virus. Virus-like particles observed in the sera of some patients in the acute phase of non-B hepatitis were absent from sera of the convalescent phase (25). In chimpanzees experimentally infected with hepatitis A, virus activity in sera appears close to or preceding the peak serum transaminase levels (17). Further observations are needed to more clearly define the pattern by which virus appears and disappears in blood and to correlate these patterns with virus clearance mechanisms in man and experimental animals.

Virus in Liver

In experimentally infected nonhuman primates, HAV has been recovered from liver tissue and liver has served as a source of antigen for serologic tests and for passage of infection between animals. Virus-like particles of 27 nm have been observed within intracytoplasmic vesicles in hepatocytes of infected marmosets (12) and chimpanzees (9) during the acute phase of illness. Identification of similar 27 nm virus-like particles, immunologically reactive to anti-HAV, in the hepatocytes of patients with hepatitis A remains to be undertaken. The time course and handling of HAV within the liver is largely unknown.

Virus in Bile and Duodenal Fluid

Transmission studies performed many years before HAV was identified suggested that the virus is present in duodenal fluid (26). Although these studies have not been repeated, and HAV has not yet been recovered from duodenal aspirates, it seems likely that the source of virus is bile entering the duodenum, since hepatitis A antigen has been isolated from the bile of chimpanzees during the acute phase of experimentally induced hepatitis A.

Virus in Feces

Fecal excretion of hepatitis A virus (HAV) has been the subject of a number of human transmission studies undertaken before the virus was isolated. These experiments suggested that infective virus is present in stools of patients 14 to 21 days before the onset of symptoms (during the second half of the incubation period), but not during the first phase of the incubation period because stools collected 28 to 35 days prior to the onset of illness were not infective (24). Additional studies indicated that stool specimens obtained eight days after the onset of jaundice were also infectious but fecal samples obtained later were no longer infectious. There is only one report (27) of chronic fecal excretion of HAV (in two children with prolonged hepatic dysfunction), and although the disease associated with this infection had the epidemiologic characteristics of hepatitis A, this was possibly either another form of hepatitis (non-A, non-B) or it represented an unusual occurrence. That the latter is probably the case is suggested by a body of data which indicates that prolonged fecal excretion is not a typical feature of hepatitis A infection.

Since immunoserologic methods have been developed to detect HAV, the pattern of fecal excretion of HAV both in man and the nonhuman primate has been studied more intensively. In marmosets, whether infected parenterally or orally, fecal excretion of hepatitis A can be demonstrated when serum enzymes increase. Whereas the virus is excreted in the feces for several days after oral inoculation of virus, and is then followed by a period of negative virus recovery studies, before it reappears, early fecal excretion does not occur after parenteral inoculation in this animal, (28). However, in chimpanzees fecal excretion of hepatitis A has been reported as early as nine days after parenteral inoculation (29). In other studies, utilizing chimpanzees, virus was recovered in stools from the onset of illness until the peak SGPT was reached (30). Other investigators report that, in chimpanzees, peaks in fecal HAV excretion have occurred simultaneously with peak SGPT levels but excretion persisted for 7 to 18 days after peak enzyme levels were recorded (17).

Studies of hepatitis A in man have revealed that the 27 nm particles are present in stool five days before serum transaminase levels increase but are not present after peak transaminase levels are reached (31). Other studies indicated fecal excretion of virus a few days before or on the first day of abnormal transaminase values but in some children virus was not detected during the one to eight days preceding peak transaminase levels (32). Much variation in fecal excretion seems likely, however, since in another study the presence of virus in stool has been reported as early as 21 days before peak SGPT levels and as late as 14 days

after peak SGPT values (23). Maximal fecal excretion of HAV in this study occurred 5 to 15 days before peak SGPT levels. In another report, HAV was observed in approximately one third of stool specimens obtained between the second and third weeks after the onset of dark urine (33).

Although an early study suggests that virus is not present in the feces of anicteric patients (34), more recent data indicates that HAV can be found with equal frequency in anicteric and icteric patients (23). Epidemiologic evidence indicates that anicteric patients are as infectious as icteric patients.

Virus in Other Body Fluids

The presence of virus in urine and in nasopharyngeal washings has been suggested but recovery or isolation of the agent outside of blood, the liver, bile, and feces is not yet reported. It might be expected that hepatitis A virus (HAV) is present in other fluids during the viremic phase, but proof is not available.

PERIOD OF CONTAGIOUSNESS

Since immune electron microscopy and human transmission studies indicate that infectious hepatitis A particles are excreted in the feces from the latter half of the incubation period through the early acute phase of illness, it is assumed that patients are contagious throughout this interval. Epidemiologic evidence suggests that while feces collected during this period may be infectious under experimental conditions, contagiousness varies considerably. Relatively few outbreaks of hepatitis A have been attributed to persons who are incubating the disease (35-37) and studies of household transmission suggest that most infections acquired by household contacts result from exposure a few days before or at the onset of symptoms in the index case (32).

These observations suggest that the disease is most contagious just before or at the onset of disease. It seems likely that this phenomenon reflects increased or peak infectious particle excretion at this time. The pathophysiologic mechanism responsible for the increased synthesis or release of a larger number of infectious particles remains to be determined.

Studies in chimpanzees infected experimentally with hepatitis A virus (HAV) indicate that fecal excretion of viral particles may be cyclical with peaks at 9 to 11, 14 to 15, and 20 to 21 days after inoculation (29). The particles obtained from the earliest period were hollow and had a buoyant density of 1.30 g/cm^3, whereas particles in the later periods appeared to be full and banded bimodally with buoyant densities of either 1.29 and 1.33 or 1.33 and 1.40 g/cm^3. Thus there appear to be cyclical variations in the pattern of fecal excretion and in the biophysical nature of the particles excreted. The full particles obtained at day 14 to 15 are probably intact virus since they have been shown to be infectious. Whether the earlier appearing hollow particles are infectious is not known. It is likely that they do not represent intact virus and are therefore noninfective. It is not known whether the same phenomenon applies to man infected with hepatitis A.

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