Hepatitis A virus (HAV) infection occurs worldwide and is an important cause of acute viral hepatitis in the US. In this review, I cover the epidemiology, course of infection, clinical manifestations, serological responses, and prevention of this infection. Although most patients completely recover from this disease, elderly patients have a substantial mortality risk. Recently licensed vaccines are highly efficacious.

Five very different viruses make up the “classical” etiological agents responsible for acute or chronic viral hepatitis in humans. For the most part, these viruses share only a common tropism for the liver, with the hepatocyte representing the dominant site of viral replication and either acute or chronic forms of hepatitis representing the major clinical manifestations associated with infection. These viruses can be considered as two distinct groups, based on several clinically and epidemiologically important characteristics: those viruses that possess a lipid-containing outer viral envelope (hepatitis B, C, and D viruses: HBV, HCV, HDV) and those that do not (hepatitis A and E viruses: HAV, HEV).

HAV and HEV, which lack a lipid envelope, are stable when they are secreted from infected liver cells into the bile, and they gain entry to the intestinal tract via this route. Thus, these viruses typically spread by a fecal–oral mode of transmission, and they can cause extensive common-source outbreaks of disease. However, neither of these viruses causes persistent infection, and neither has been identified as a cause of chronic viral hepatitis. In contrast, HBV, HCV, and HDV all possess lipid envelopes and are likely to be rapidly inactivated by bile. Thus, these viruses are not shed in feces in biologically significant amounts. Their transmission occurs by several other routes, most often involving virus shed from a mucosal surface or by direct percutaneous exposures. In addition, HBV, HCV, and HDV may each cause persistent infection, and each has been shown to be an important etiological agent of chronic viral hepatitis and cirrhosis. Infection with HBV or HCV may lead ultimately to the development of primary hepatocellular carcinoma, often after many years of persistent infection and chronic hepatitis. Although chronic viral hepatitis is not associated with HAV infection, acute hepatitis due to HAV is nonetheless an important public health problem, both in the US and overseas [1, 2].

**Important attributes of HAV**

HAV is a positive-sense, single-stranded RNA virus classified within the genus hepatovirus of the family Picornaviridae [3, 4]. This virus family also includes enteroviruses, such as poliovirus and rhinoviruses, which are frequent causes of the common cold. HAV is thus a positive-strand RNA virus, with genomic RNA that can function as messenger in directing the translation of proteins. A single large polyprotein is expressed from a large open-reading frame that extends through most of the genomic RNA. Translation occurs in a cap-independent fashion under control of a internal ribosome entry segment located within the 5’ untranslated RNA. The polyprotein subsequently undergoes cleavage mediated by a viral protease (3Cpro), resulting in the production of four capsid proteins (VP1–4) and several nonstructural proteins.

Unlike other hepatitis viruses, HAV can be propagated in conventional mammalian cell cultures with reasonable efficiency, usually without any apparent cytopathic effects [3]. African green monkey kidney cells, or fetal rhesus kidney cells, are commonly used for culturing the virus, although many different cell types are suitable. Cell culture isolation of virus is not a useful approach to diagnosis, however, because wild-type virus usually replicates poorly in cell culture. More-efficient replication occurs after a number of passages, when the virus has become adapted to growth in cell culture. Several cell
culture-adapted HAV variants have been shown to be highly attenuated in their ability to cause disease in otherwise susceptible primates, and such viruses have been used for production of both killed (formalin-inactivated) and candidate live (attenuated) HAV vaccines [3]. The mutations responsible for both cell culture adaptation and attenuation in primates have been reasonably well characterized, and are found within the 5’ nontranslated RNA as well as within RNA encoding nonstructural proteins [4]. Thus, these phenotypes probably result from a change in the ability of the virus to utilize cell type-specific host cell factors required for translation and replication of the viral genome.

HAV strains recovered from humans in different regions of the world demonstrate negligible antigenic diversity, leading to the conclusion that only a single serotype of HAV exists, despite the considerable genetic heterogeneity at the nucleotide level. The antigenic structure of the virus is relatively simple, with a restricted number of overlapping epitopes combining to form a single dominant antigenic site that interacts with virus-neutralizing antibodies [4]. These epitopes are highly conformational, and are formed by amino acid residues contributed from more than one capsid protein. Thus, for the most part, antigenicity depends on the assembly of the major capsid proteins into capsid or smaller capsid precursors. Individually expressed capsid proteins do not share antigenic characteristics with the native virus particle. Empty capsids, however, appear to be antigenically indistinguishable from infectious, RNA-containing virions.

Epidemiology

HAV is present in a worldwide distribution, the highest prevalence of infection occurring in regions where low standards of sanitation promote transmission of the virus [1]. Risk factors associated with the acquisition of HAV within the US have been determined in the Sentinel Counties Study carried out by the Centers for Disease Control and Prevention over several years [5]. Frequently reported risk factors include having lived in the same household with a patient with hepatitis (~24% of all patients); homosexual activity that might lead to fecal-oral spread of virus through oral-anal contact (~11%); and close contact with young children attending day-care centers (~18%). Several other studies also suggest that preschool day-care centers may at times be important foci for transmission within the US [6]. International travel to regions where hepatitis A is endemic poses a substantial risk for acquiring hepatitis A, but this was reported by only ~4% of patients with acute hepatitis A in the Sentinel Counties Study. In recent years, illicit use of parenteral drugs has been reported as a risk factor by only 2% of patients, but in other studies [7] this has been a much more prevalent risk factor. Significantly, ~40% of patients studied in the Sentinel Counties Study have not reported any apparent risk factor (discounting the ~14% who report having lived in the same household with a child under 5 years of age).

Most cases of hepatitis A can be explained by fecal-oral transmission of the virus. However, the occasional association of hepatitis A with intravenous drug use is interesting. This was first noted in Sweden [7] and later confirmed in the US. It has been suggested that this association may largely reflect general living conditions and poor sanitation, but this may not be the entire story. Acute infection with HAV is associated with a substantial viremia that persists for several weeks [8]. Needle-borne transmission of the virus is certainly a possibility if individuals share nonsterile needles during the prodromal phase of the illness. In addition, HAV is a very stable virus, capable of withstanding substantial heat and drying. In 1992, several outbreaks of hepatitis A were noted among hemophilic patients receiving factor VIII concentrates that had been prepared by a solvent-detergent inactivation process (which does not reduce the infectivity of nonenveloped viruses).

Virologic Events in Acute Hepatitis A

Figure 1 shows the course of events in a New World owl monkey after intravenous inoculation with ~10^4 infectious units of a cell-culture-adapted HAV strain that could be readily isolated in cell culture and measured quantitatively by a modified viral plaque assay [8]. About 4 weeks after inoculation, the animal developed relatively mild chemical hepatitis A, as evidenced by increasing serum alanine aminotransferase (ALT) activities. This was associated with evidence of inflammatory changes in the liver parenchyma. Concordant with the onset of hepatitis was the appearance of antibody, which could be detected either by an ELISA or by a more biologically relevant neutralizing antibody assay that measures the amount of antibody capable of inhibiting the infectivity of the virus particle (see below).

Preceding the onset of chemical hepatitis and the appearance of antibody, there was the typical shedding of infectious virus in feces. Infectious virus may be identified in the feces as early as 4 days after the intravenous injection of infectious material in susceptible primates (7 days in animals fed wild-type virus orally), and continues to increase in magnitude until just before the onset of

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<th>Table 1. Risk factors for hepatitis A.</th>
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<td><strong>Risk Factor</strong></td>
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<td>Household exposure</td>
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<td>Daycare center contact</td>
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<tr>
<td>Male homosexual</td>
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<td>Foreign travel</td>
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<tr>
<td>Parenteral drug abuse</td>
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<td>No apparent risk factor</td>
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Source: Data from US Sentinel Counties Study, Centers for Disease Control and Prevention [2].
chemical evidence of liver disease. Fecal shedding of virus then declines, reflecting a developing immune response to the virus that is not only antibody-mediated but also involves the stimulation of CD8+ virus-specific cytotoxic T cells [9]. The viremia lasts almost as long as the fecal shedding of virus. In a group of six monkeys infected with this virus (a strain that may have been partially attenuated with respect to its ability to replicate in the liver), the average duration of detectable viremia was ~3 weeks [8]. The magnitude of the viremia is perhaps 2–3 log₁₀ less than the fecal shedding of virus but is still typically substantial. Importantly, the viremia is maximal during the prodromal phase, prior to the development of clinical, chemical, or serological manifestations of the infection.

The shedding of the virus in feces comes predominantly from replication of the virus within the liver. However, quite solid data now indicate that the virus also replicates within epithelial cells of the distal ileum. These multifunctional cells are highly polarized. Based on studies with cultured colonic carcinoma cells (Caco-2 cells), the entry and release of HAV from intestinal epithelial cells appear to be restricted to their apical (i.e., lumenal) surface (C Blank and SM Lemon, unpublished data). Thus, it remains unclear how the virus reaches the liver in the initial stages of the infection. However, virus replicated within hepatocytes (which are also highly polarized) is similarly secreted across the apical hepatocyte membrane into the bile, and thence into the gastrointestinal tract. It is likely that the viremia also derives from replication of virus within the hepatocyte, but with spread into the circulation rather than secretion into the bile.

CLINICAL MANIFESTATIONS OF HAV INFECTION
The onset of hepatitis A is characteristically abrupt. Early symptoms include malaise, fatigue, nausea, vomiting, and discomfort in the right upper quadrant of the torso. Although fever occurs in about half of all patients and may be impressive in early stages of the disease, it is usually absent by the time the patient seeks medical attention. The first specific evidence that the illness relates to an intrahepatic process may be any of the following: the onset of dark urine, light clay-colored stool, or icterus of the conjunctival covering of the sclera. The urine typically acquires the color of cola soda and is characteristically frothy. Icterus may be noted when the serum bilirubin exceeds 25–30 mg/L. These signs typically appear during the first few days of illness, but are usually absent at the onset. Diarrhea occurs in about one-half of all infected children but is uncommon in adults.

The most striking laboratory findings include increases of serum aminotransferase activities, serum bilirubin (both total and direct), and serum alkaline phosphatase activity. Serum ALT is usually increased more than serum aspartate aminotransferase. These enzyme activities may be minimally increased, or in severe cases may be >100-fold the upper reference limit. Serum aminotransferase activities usually increase before serum bilirubin does. Alkaline phosphatase activity also rises relatively late in the course of the infection, and tends to parallel serum bilirubin increases. Other laboratory findings include nonspecific increases of acute-phase reactants, immunoglobulins, and the erythrocyte sedimentation rate. Concentrations of total hemolytic complement may be depressed in acute hepatitis A, possibly reflecting the presence of circulating immune complexes as described by some workers. Rheumatoid factor (IgM anti-IgG) is often present.

The entire acute illness may last from 1 to several weeks. Resolution is marked by a return in the patient’s general sense of well-being and appropriate declines in
abnormal serum chemistries. Typically, the aminotransferase activities may begin to resolve before the serum bilirubin. However, aminotransferase activities may remain abnormal for several months after acute HAV infection, although usually at relatively low values. Abnormal ALT activity may persist after the serum bilirubin has returned to normal. However, it is probably safe to say that abnormalities in serum chemistries are seldom present >6 months after the acute infection and virtually never after 1 year.

Extrahepatic manifestations of hepatitis A are uncommon. Urticarial rash and acute arthritis, such as may occur with acute hepatitis B, are not found in acute hepatitis A. Concomitant meningoencephalitis has been reported in several cases, possibly reflecting the fact that HAV is a picornavirus and thus relatively closely related to the enteroviruses, common causes of viral meningitis and meningoencephalitis in humans. Acute renal failure has been occasionally reported in association with acute hepatitis A.

Children younger than 2 years rarely manifest symptoms and signs of acute viral hepatitis when they are infected. However, the severity of disease progressively increases with the age at the time of infection. In studies done in American military populations, ~70% of soldiers who became infected with HAV developed specific clinical signs of hepatitis A, including jaundice [10]. Hepatitis A in adults is usually an illness of several weeks’ duration, with perhaps a few months of extended convalescence. It is, however, occasionally a fatal disease, particularly in older persons. Fulminant hepatitis and death due to hepatitis A occur almost exclusively in individuals who are infected after age 50 (Fig. 2). Overall, ~70–80 deaths from hepatitis A infection are reported in the US each year. Severe manifestations of hepatitis A infection are more likely in individuals with underlying alcoholic liver disease or with chronic hepatitis caused by other viral agents.

**SEROLOGICAL MEASURES OF HAV INFECTION**

The development of antibody to HAV, as measured by a solid-phase competitive inhibition blocking assay or a virus neutralization assay, coincides with a marked diminution in the quantity of viremia and fecal shedding of virus [8, 11]. Detection of this acute-phase antibody response is the mainstay of diagnosis. IgM antibodies to the virus are present in >99% of individuals at the time of their initial presentation. The IgM anti-HAV response
usually peaks within the first month of illness and declines to nondetectable amounts within 12 (usually 6) months. Virus-specific IgM antibody usually is detected by very sensitive and specific solid-phase antibody-capture immunoassays and typically is found against a background of nonspecific increases in IgM.

Total antibody to HAV, on the other hand, is measured in a separate competitive inhibition (blocking) ELISA. Total antibody to HAV consists predominantly of IgG antibody, except immediately after acute HAV infection, when IgM and IgA antibodies represent a greater proportion of the virus-specific antibody response. Quantitative estimates of the IgG anti-HAV content of sera are commonly based on a comparison with a World Health Organization anti-HAV Reference Immunoglobulin Reagent, and are reported in terms of mIU/mL. Like tests for IgM anti-HAV, tests for total anti-HAV are almost always positive at the onset of acute hepatitis A. Thus, a negative test for total antibodies to hepatitis A effectively excludes a diagnosis of acute HAV infection. However, a positive competitive inhibition test is unable to distinguish between acute (recent) or long past infection. Because of its high positive and negative predictive value, IgM anti-HAV is the test of choice when confronted with a patient who might have acute HAV infection. Competitive inhibition immunoassays for total anti-HAV antibodies usually remain positive for life following acute infection and are a good marker of immunity. Commercially available immunoassays for total anti-HAV antibodies are relatively insensitive, however, and may not detect the protective antibody responses present after a single dose of inactivated HAV vaccine [12]. The minimal protective amount of antibody is <10 mIU/mL; unfortunately, 100 mIU/mL is the approximate minimum amount of antibody detectable in commercial antibody tests. Some investigators have successfully circumvented this problem by modifying the commercial test and increasing the volume of serum added to the competitive inhibition reaction.

Commercially available tests for IgM anti-HAV and for total anti-HAV are not influenced by passive administration of immune globulin given in normal doses. Thus, the administration of immune globulin does not complicate subsequent efforts at serological diagnosis.

**PREVENTION**

Formalin-killed whole-virus vaccines are highly effective in prevention of hepatitis A [13, 14]. Two such vaccines have been recently licensed within the US: Havrix (Smith-Kline Beecham) and Vaqta (Merck & Co.) [15]. Both of these vaccines contain inactivated whole-virus particles and empty capsids produced in infected cell cultures, and both are adsorbed to alum in an effort to enhance immunogenicity. However, these vaccines vary with respect to the methods by which the amount of viral antigen in each vaccine dose is determined, as well as the relative purity of the viral proteins within the vaccine. Nonetheless, both vaccines appear to be comparably immunogenic. They are generally given as a single primary immunization, followed by a booster dose 6–12 months later. Pediatric formulations contain about one-half the antigenic mass of the adult formulation and are recommended for individuals younger than 17–18 years. Thus far, no significant clinical differences have been documented with respect to the adverse events reported after immunization with these two vaccines. In general, these are very safe vaccines, with reactogenicity similar to that of hepatitis B vaccines. However, a few reports of anaphylaxis after administration of inactivated HAV vaccines mandate that vaccine should be administered only in settings where epinephrine is immediately available. Guillain–Barré syndrome has occurred in several immunized persons, but its relation to the immunization is uncertain.

Immunization should be a priority for individuals at increased risk of acquiring hepatitis A, or who are at increased risk of fatal, fulminant disease if they contract hepatitis A [2, 15]. The former include children in communities where hepatitis A is traditionally endemic, as well as travellers going abroad to developing nations where HAV can be highly endemic. Other high-risk persons include male homosexuals and persons using illicit parenteral drugs [5, 7]. Persons with underlying chronic liver disease from any cause, particularly if they older than 45–50, are at increased risk of fulminant hepatitis A and should be immunized. Immunization of foodhandlers could prevent common-source outbreaks of hepatitis A, but the cost-effectiveness of such a strategy is not known [2]. Given the large proportion of cases with no defined risk factor for hepatitis A, universal immunization likely would be required to successfully control hepatitis A. The high costs and limited availability of the vaccine precludes such a recommendation at present, however.

Immunization with inactivated HAV vaccines results in demonstrable titers of IgG anti-HAV, and occasionally low amounts of IgM antibody. The titers of vaccine-induced antibody are usually much lower than those present after natural infection, yet these lower antibody amounts confer good protection against disease (and probably infection as well). The minimal protective antibody amount has not been defined, but several lines of evidence suggest that it is <10 mIU/mL. The most sensitive method for detection of vaccine-induced antibodies is a radioimmunoprecipitation assay using metabolically labeled virus [5]. Differences in the relative titers of neutralizing and immunoprecipitating antibodies in the vaccinee’s serum vs that in sera from naturally infected persons suggest that the vaccine-induced antibodies are of much lower affinity. This does not seem to be due to formalin-induced alterations in the antigenicity of the virus (which have not been demonstrated), but more likely reflects the method of presentation and the antigenic mass.

Limited evidence indicates that immunization with
inactivated HAV vaccines may delay the appearance of IgM antibody to the virus, should an individual subsequently become infected with HAV (certainly an unusual situation). When considering this possibility in an immunized person, therefore, it may be useful to obtain a late serological specimen for IgM antibody, or to look for increases in total antibody concentrations. Preliminary results with immunoassays that utilize nonstructural HAV proteins as antigen suggest that it may be possible to develop assays capable of effectively discriminating between antibody response to immunization vs infection.

References