New Perspectives on Hepatitis E

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The infectious agent causing epidemic non-A, non-B hepatitis
was identified in 1983 from a human challenge experiment.
The novel hepatitis E virus (HEV) subsequently was cloned in
1990 and the genome sequenced. HEV transmission is highly
endemic in Asia, the Middle East, and Africa. Fecal contami-
nation of drinking water is the most common mode of
spread. Although usually asymptomatic, HEV infection can
cause fulminant hepatitis. Recent studies indicate that hepati-
tis E may be a zoonotic disease, with pigs and possibly rats
serving as reservoirs for human infection. A recombinant
HEV vaccine is currently in phase III clinical trials. The char-
acterization of the major types of viral hepatitis during the
last 20 years illustrates how modern genetic technology has
revolutionized research in infectious diseases. Within less
than two decades of the discovery of HEV, its epidemiology
has been described, serologic tests have been developed, and
a candidate vaccine has been evaluated in clinical trials.

Introduction

It has been known for decades that there are two major types
of acute hepatitis caused by an infectious agent: one transmit-
ted by percutaneous exposure and one by the oral route. The
leading causes of these two forms of hepatitis—hepatitis A and
hepatitis B—have been recognized since the 1940s. However,
it became clear after the isolation of the hepatitis A (HAV) and
hepatitis B virus (HBV) that there were additional causes of
both the parenteral and enteric types of this disease [1•].

The discovery and characterization of the other major
types of viral hepatitis during the last 20 years illustrate how
modern technology has revolutionized research in infectious
diseases. As a result of these accomplishments, our under-
standing of viral hepatitis has advanced rapidly. Hepatitis E is
one of the most recently identified forms of what could pre-
viously only be called "non-A, non-B hepatitis" [2].

Hepatitis E Virus

An early indication that there was an enterically transmitted
cause of viral hepatitis, other than HAV, came from large
epidemics of acute hepatitis that were observed in the
1950s in the Indian subcontinent [3–5]. These epidemics
apparently were caused by an enterically transmitted agent
based on evidence of recent fecal contamination of water
supplies. However, few outbreak patients had indications of
acute HAV infection, and most had serologic evidence of
prior immunity to hepatitis A. Consequently, another form
of hepatitis was suspected that was initially called epidemic
or enterically transmitted non-A, non-B hepatitis [2].

Confirmation of the existence of a novel hepatitis E
virus (HEV) came in 1983 from a human challenge experi-
ment [6]. A researcher ingested pooled stool extracts from
cases of epidemic non-A, non-B hepatitis. The volunteer
subsequently developed typical icteric hepatitis without
serologic evidence of either hepatitis A or B. Virus-like parti-
cles, 27 to 30 nm in diameter, were identified in the volun-
teer's stool samples by immune electron microscopy (IEM).
Antibodies to these particles were found by IEM in the sera
of other patients who were presumed to have had epidemic
non-A, non-B hepatitis. Cynomolgus monkeys also devel-
oped hepatitis after being inoculated intravenously with the
virus obtained from the volunteer's stool [6••].

HEV was cloned in 1990, and the entire genome was
subsequently sequenced [7–9]. HEV was found to be a sin-
gle-stranded, positive-sense RNA virus, 7.2 kb in length.
The genome contains three overlapping open reading
frames [8]. It is a spherical virus, probably icosahedral in
shape, without an envelope. HEV was originally classified
in the family Caliciviridae, although recent studies have
determined that its genetic sequence is not closely related
to that of any other virus [10]. Currently, there are at least
four major genotypes of HEV that can be divided into nine
groups, but there may be only one serotype [11,12]. Inter-
estingly, strains of this virus tend to be similar genetically
within geographic regions where transmission occurs. Gen-
otype 1 is found in Africa and Asia; genotype 2 was isolated
from an outbreak in Mexico; genotype 3 was obtained
from two patients in the United States; and genotype 4 is
found in China.

Several species of nonhuman primates can be infected
with HEV [13]. In nature, recent evidence indicates that
swine, rats, chickens, and other animals are infected with
viruses related to HEV [14•,15,16]. Importantly, the HEV
strains (genotype 3) from two patients who developed hep-
atitis E in the United States are closely related to swine HEV
[17••]. One of these patients had no history of foreign
travel. The other US patient had traveled to Mexico; this
patient's HEV strain was capable of infecting pigs [18].

However, human isolates of HEV from Asia and Mexico did not cause disease in pigs in another study [19]. Notably, among herds in the midwestern United States, most swine aged greater than 3 months have been found to be seropositive for antibody to HEV [14•]. Additionally, it has been possible to infect rhesus monkeys and chimpanzees with swine HEV [18]. Taken together, the latest findings suggest that there could be one or more animal reservoirs of HEV. Hepatitis E may therefore be a zoonotic disease [20].

Epidemiology

Because of the unique character and broad host range of this virus, HEV is not readily comparable with other infectious agents. The unusual nature of HEV is further reflected in its distinctive epidemiology.

HEV transmission is highly endemic in south and southeast Asia, the Middle East, and North Africa [2]. Epidemic transmission was also observed in 1986 in two rural villages in Mexico [21], but evidence of transmission in other areas of central and South America has been infrequent [11]. Among the planet's 6 billion people, an estimated 2 billion have been infected with HEV, compared with 5 billion with HAV, the other major enterically transmitted cause of viral hepatitis [13].

HEV, which is found in the feces during active infection, is spread most often through fecal contamination of drinking water [2] and possibly food [22]. Unlike HAV, which is highly infectious, person-to-person or interfamilial transmission is uncommon [23], but HEV may be transmitted vertically from pregnant mothers to their infants [24]. As with HAV, neither sexual nor parenteral contact appears to be an important mode of HEV transmission [25,26].

As noted previously, the existence of HEV first became known from investigations of large epidemics of acute hepatitis. In some endemic regions, recurring epidemics have been a problem [2]. Gross fecal contamination of drinking water has been the major source of epidemic transmission [3,5,27]. Recent studies indicate that HEV transmission increases during both flooding and drought in areas where rivers are used for potable water, washing, and waste disposal [28]. Flooding increases cross-contamination of drinking water with human and animal feces [29], and drought is thought to increase the concentration of infectious particles [30].

Although HEV is a common cause of large waterborne epidemics, sporadic infection probably accounts for more clinical cases in regions that are endemic for HEV transmission [31,32]. In these tropical and semitropical regions, HEV is often the most common cause of acute hepatitis among adults. The major risk factors for sporadic infection have not been clearly determined [2].

One of the most interesting aspects of HEV transmission is that it does not occur in some developing regions, unlike HAV infection, which can be found everywhere. Most notably, HEV infection has been documented infrequently in Latin America, where other enteric infections are prevalent [2]. Another mystery is why the prevalence of antibody to HEV (anti-HEV), which indicates past infection, is much lower than the prevalence of HAV infection in regions that are endemic for both hepatic diseases. Unlike a very high prevalence of anti-HAV starting in early childhood, the prevalence of anti-HEV increases slowly among children, rises rapidly during young adulthood, and levels out among adults at around 10% to 40% [33•]. It is unclear why HEV antibody prevalence does not steadily increase among older individuals, who presumably are exposed to contaminated water and food along with the rest of the local population. In some highly endemic areas the prevalence of anti-HEV can be greater than 60%, but the level of infection does not reach that of HAV [34].

Transmission of HEV has been found infrequently within developed countries [2,35]. Correspondingly, the prevalence of antibody to HEV is much lower (0.5% to 4%) in developed countries than in endemic regions [33•]. However, the prevalence of anti-HEV is not zero, despite the fact that endemic HEV transmission has not been confirmed in North America or Europe. Although some HEV infections may have been acquired by individuals during travel to endemic regions [36,37], there is often no history of foreign travel among anti-HEV–positive residents of Western countries. Therefore, it is unclear whether these antibodies represent prior asymptomatic HEV infection, cross-reactivity with other infectious agents, or false-positive test results [2]. The two reported cases of acute HEV infection acquired within the United States with viral strains genetically similar to swine HEV indicate cross-infection with animals [17••]. In support of this possibility, recent studies indicate that the prevalence of antibody to HEV is higher among individuals who work closely with swine [38].

Travelers from developing countries are seldom infected with HEV, even when living for long periods of time in endemic regions [33•,39]. Also, no outbreak of HEV has ever been documented among deployed US military personnel [40]. HEV has been a problem for some French forces but has been less of a problem for other European troops deployed to endemic areas [41,42].

Clearly, the epidemiology of hepatitis E differs substantially from that of HAV infection, the other major hepatitis virus spread by the fecal-oral route. In contrast to HAV, which infects nearly all young children in developing countries and results in protective immunity during adulthood, a large proportion of the adult population remains susceptible to HEV infection in endemic regions. Additionally, travelers to endemic regions are at much lower risk of hepatitis E than of hepatitis A. These epidemiologic characteristics of HEV transmission indicate that this virus cannot spread as readily as HAV, possibly because less virus is excreted in the stool, a relatively larger infective dose is required, or HEV is less able to survive in the environment than is HAV [2].

Pathogenesis and Immunity

HEV causes an acute, self-limited infection, which is most often asymptomatic. The pathogenesis of the liver disease when clinical infection develops is not well understood. In a study of HEV-infected cynomolgus monkeys, morphologic analysis indicated that hepatocytolysis was immune-mediated and not caused by a direct cytopathic effect of the virus [43]. Histologic examination of the liver during HEV infection generally shows changes similar to those with other types of viral hepatitis with no zonal pattern of hepatocyte injury; many patients have a cholestatic type of hepatitis [2].

The mean incubation period from exposure to symptomatic disease is about 40 to 45 days (range, 4 to 10 weeks) [2,33]. Unusual for viral causes of hepatitis, larger infective doses of HEV may shorten the incubation period, increase the likelihood of developing clinical disease, and lead to a higher case fatality rate [27,44]. Although children can develop symptomatic infection [45,46], acute hepatitis E is most often observed among young adults in the second to fourth decade of life [3,5,27]. In hepatitis E outbreaks, attack rates have varied from 3% to 30% among adults and from less than 1% to 10% among children [2]. It is unclear why clinical disease appears to be more common among young adults.

The illness caused by HEV infection is similar to other types of viral hepatitis [21]. The most prominent symptoms and signs include flulike complaints, nausea, abdominal pain, jaundice, and an enlarged, tender liver. Typically, there is a rise in serum levels of conjugated bilirubin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase just before symptoms begin. After individuals develop symptomatic infection, their recovery usually occurs within 2 to 8 weeks [2,33•]. Chronic infection and liver disease have not been observed following HEV infection. In addition, viral shedding in stool usually lasts for only a brief period: HEV is excreted beginning about 1 week prior to the onset of symptoms, and it persists for only about 2 to 4 weeks [2]. HEV probably reaches the gastrointestinal tract in bile, which contains infectious virus during active replication of HEV [2].

The mortality rate from HEV infection is less than 1% [2]. HEV infection can, however, cause fulminant hepatitis [47]. Severe disease develops most often in pregnant women in their third trimester [27,31]. The mortality rate is as high as 20% among pregnant women [5]. This is unique among the various types of viral hepatitis, which generally do not cause fulminant disease during pregnancy [48]. Infants infected during pregnancy appear to have a high mortality rate as well [24].

The nature of the immune response following HEV infection is not as well understood as for other types of viral hepatitis. IgM antibody to HEV is detectable about 3 to 8 weeks following infection, at around the same time that clinical disease develops [2]. IgG anti-HEV develops a few days after IgM antibody. Anti-HEV IgM lasts for several months, and anti-HEV IgG is detectable for at least several years following infection [49,50].

Although recent infection appears to confer protection from disease, it is unknown how long immunity persists. The repeated occurrence of epidemics in certain endemic regions indicates either that immunity is not long-lasting or that a minority of the population has been infected with HEV at any one time [2]. In studies of cynomolgus monkeys, protection against hepatitis E was provided by passive immunity with plasma containing HEV antibodies [51,52]. The effectiveness of passive immunity indicates that humoral immunity is important in preventing disease and possibly infection. However, the titers of IgG anti-HEV drop relatively quickly after natural infection, which may indicate loss of immunity. The role of cell-mediated immunity is unknown.

Diagnosis

Beginning in the 1980s, several serologic tests were developed to identify antibody to HEV. Generally, the diagnosis of acute HEV infection is based on the detection of IgM anti-HEV; past infection is determined by the presence of IgG anti-HEV. The first serologic test for anti-HEV utilized IEM [6]. Later, more practical tests were developed, including an immunofluorescence assay [53], which was accompanied by the development of the Western blot [54,55] and enzyme-linked immunosorbent assay [56] based on synthetic peptides and recombinant expressed proteins. Soon after HEV was identified, polymerase chain reaction assays for HEV RNA were developed that have detected HEV genetic material in both stool and serum specimens during active infection [57,58]. HEV does not grown well in cell culture [59].

None of these tests for HEV infection are commercially available in the United States. The lack of a licensed test undoubtedly has led to under-reporting of this infection in North America. Serologic tests for anti-HEV are marketed in Europe and in some countries in Asia but are not routinely employed. They are too expensive for use in many developing regions, adding to the under-reporting [60]. Furthermore, HEV infection may have been underdiagnosed because of the low sensitivity of many serologic tests, which is more of a problem in evaluation of populations in nonendemic countries [61].

Prophylaxis and Treatment

There is no proven prophylaxis for HEV infection. Pooled immune serum globulin has not proven effective in humans, even when produced from donors residing in endemic countries [62]. The best personal preventive measure is avoidance of fecally contaminated water and food. Absent a vaccine, community prevention and control efforts in endemic areas depend on the development of infrastructure to provide potable water [63]. Once hepatitis E develops, medical care is supportive for this self-limited infectious disease.

Vaccine development

In primate models, HEV infection was found to be associated with immunity to reexposure with different isolates over a period of several years [64–66]. Humans also appear to be protected from hepatitis E after a prior HEV infection [49,50]. These findings indicate that it might be possible to develop a vaccine against hepatitis E. In fact, the two most dynamic areas of current HEV research are vaccine development [67••] and the study of possible zoonotic reservoirs of HEV infection [68].

Because HEV does not replicate well in cell culture, vaccine development has focused on recombinant vaccines. Candidate vaccines against hepatitis E have been formulated that consist of capsid proteins (from open reading frame 2) expressed in *Escherichia coli* and insect cells. In early studies, cynomolgus monkeys were protected against hepatitis E by active immunization [51,69]. In further studies of nonhuman primates, baculovirus-expressed capsid protein vaccines protected against hepatitis following heterologous challenge but not always against high-dose challenge [70•,71]. One candidate vaccine was not effective when given 48 hours after HEV exposure [70•].

A baculovirus-expressed capsid vaccine has undergone phase I clinical trials and has now entered phase II/III testing [67••]. In initial testing, the vaccine was immunogenic and did not cause serious side effects. Based on studies in nonhuman primates, the vaccine should provide protective immunity against disease and possibly against infection. The duration of immunity after an initial series of immunizations is a critical factor that will have to be determined in future studies. Another critical factor will be the efficacy of the vaccine against heterologous strains of HEV.

Conclusions

The study of hepatitis E has been an instructive story of recent advancements in medical science. Substantial progress has been made in a short period of time through both epidemiologic studies and basic research, which has relied on modern cloning and recombinant technology. New tools have clearly accelerated the pace of research. Within less than two decades of the discovery of HEV, the major epidemiologic features of this unique pathogen have been described, serologic tests have been developed, and a candidate vaccine has been evaluated in an initial clinical trial. Nevertheless, there are still many mysteries about the epidemiology, pathogenesis, and immune response of HEV infection.

The unknown aspects of hepatitis E could have a major impact on vaccine development and use. However, vaccine research could also illuminate the immunologic and epidemiologic characteristics of this unique infectious disease. The development of an animal model of HEV infection in the laboratory would aid research efforts substantially.

It is likely that a HEV vaccine will be developed that provides at least short-term protection from disease. Who will make and buy this vaccine? Because the incidence of acute HEV infection among travelers from the developed world to endemic countries has been estimated to be less than one in a million [33•], the market for this vaccine in nonendemic countries will be limited [72]. Pregnant women living in endemic regions may derive the greatest benefit from an effective HEV vaccine. Whether developing countries would establish vaccination programs that target only a small segment of their population is unknown. Much will depend on whether manufacturers can produce an affordable vaccine for use in countries with limited health-care budgets [63].

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