Overview of Hepatitis E Virus

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Hepatitis E virus (HEV) is an enterically transmitted virus usually presenting as an acute self-limiting disease. However, mortality increases dramatically from around 1% to 20% in pregnant women. HEV has been the cause of very large outbreaks of hepatitis in developing countries and is also responsible for a significant number of sporadic cases. It is clear that cases occur outside the endemic areas, and new isolates have been identified. HEV-like viruses have also been found in various animal groups, and it is likely that HEV can be regarded as a zoonotic infection. Preventative measures at the moment depend mainly on the provision of clean water supplies, although a vaccine is now undergoing clinical trials.

Introduction

Hepatitis E virus (HEV) is the causative agent of an enterically transmitted type of viral hepatitis. It has been shown to be responsible for outbreaks of hepatitis involving thousands of cases in the developing world. A significant number of sporadic cases, not related to outbreaks, are also reported in endemic areas. The virus is often waterborne so that infection tends to occur where there is inadequate sewage disposal and, consequently, poor water supplies. Outbreaks of infection have not been described in developed countries, although sporadic cases of HEV infection have been identified outside the major endemic areas. Many, but not all of these cases, have been associated with foreign travel, and new isolates have now been identified in these areas. Evidence is accumulating to suggest that HEV is a zoonotic infection. Infections can range from asymptomatic to severe, and the virus has been shown to cause high rates of mortality in pregnant women.

The Virus

The virus causing HEV is a small (approximately 32-nm diameter), spherical, nonenveloped, single-stranded RNA virus. It has three open reading frames (ORF) with most of the structural genes sited in ORF2. All strains appear to belong to the same serotype. It was for some time classed as a member of the Caliciviridae family, although it more closely resembles rubella virus. It is presently unclassified.

Hepatitis E virus was first recognized in the late 1970s following the development of assays for hepatitis A virus (HAV). It was realized that HAV was not the cause of epidemics of acute hepatitis observed in India, since most of those affected could be shown to be immune to HAV [1,2]. It was recognized that this newly described virus was distinct from HAV but it could not be isolated, and the lack of antigens hindered the immediate development of assays. Balayan *et al.* [3] confirmed the viral etiology of the infection when they identified virus-like particles in fecal samples from a volunteer fed with contaminated fecal material. Several assays were developed following the identification of virus-like particles, including immune electron microscopy and a fluorescent antibody-blocking assay [4]. Although useful research tools, these assays did not provide a convenient serologic method.

Attempts to clone the virus failed until Reyes *et al.* [5] managed to identify and characterize the HEV genome using bile from cynomolgus macaques infected with human fecal material obtained from an outbreak in Burma. Bile was shown to have high virus particle counts and was used to construct recombinant cDNA libraries in lambda gt10 phage. These were then screened using cDNA probes prepared from bile from both infected and uninfected animals. The clone ET1.1 was identified and the translated nucleic acid sequence of part of ET1.1 showed a single ORF similar to that seen in other positive strand RNA viruses. Immunoscreening was also carried out using convalescent serum to investigate cDNA libraries prepared in the expression vector, lambda gt11. Expressed proteins reacted with paired human sera from various sources suggesting that there is only one serotype of HEV. Two cDNA clones were identified, 406.3-2 and 406.4-2, which both encode epitopes specific for HEV [6]. These antigens, derived from two isolates, Mexico and Burma, were used to develop prototype serologic assays. The antigens described were used as the basis of an antiglobulin assay that was used to investigate the waterborne outbreak of hepatitis that occurred in Kashmir in 1978 and that had previously been described by Khuroo [1]. Of 45 cases of acute hepatitis, 32 (71%) were positive for anti-HEV IgG, of which 24 were also positive for IgM indicating acute infection. This established the assay as a method of diagnosis for HEV and also confirmed HEV as the cause of this outbreak [7].

The virus was subsequently sequenced [8] and characterized as a single-stranded, positive sense RNA virus with three ORF. ORF1 contains the RNA-dependent RNA polymerase and nucleoside triphosphate binding motifs, and ORF2 contains sequences that code for virus capsid proteins. ORF3 overlaps ORF1 and 2 and until recently its function was unknown. Korkaya *et al*. [9] now suggest that the protein encoded by this region binds to a number of cellular signal transduction pathway proteins and may prepare the host cell for the replication of HEV. Characterization of the virus continues with recent work confirming that the genome is capped [10] and that a capping enzyme, HEV P110, has similar properties to enzymes of a range of other viruses including plant viruses [11]. This suggests that there may be a common origin for what look like distantly related plant and animal viruses.

The first isolate from Burma was quickly followed by a second from Mexico which, although distinct, was similar to the original. Cases outside endemic areas have been described and initially these were associated with foreign travel [12]. However, it has become apparent that other isolates are found in areas not regarded as endemic for HEV and which have no connection with foreign travel. The first report followed the identification of a distinct variant from a patient with acute hepatitis in the United States, and sequencing was able to show that this was indeed a new isolate of HEV distinct from the Burmese and Mexican prototypes [13]. There have now been a number of additional isolates described from other nonendemic areas including Italy, Greece, Argentina, Austria, and Spain. The finding of two distinct isolates in Greece shows that, although it was assumed that there was geographic distribution of genotypes, there can be significant diversity between strains in the same region [14]. Kabrane-Lazizi *et al*. [15] have also shown that strains may not be as geographically localized as was once thought. They describe a unique strain of HEV which, while similar to viruses identified in the United States, was isolated from a patient who had returned from Thailand, thus making the origin of the infection uncertain.

Few isolates have been completely sequenced but based on those that have, it has been suggested that HEV comprises at least four major groups [16]. In a recent paper, partial sequence data was analyzed that suggests there may be up to nine groups [17•]. Group I has little genetic diversity and contains the prototype Burmese isolate. Group 2 has the prototype Mexican strain together with several isolates from Nigeria. Group 3 has the human isolates from the United States that are related to a swine isolate. Group 4 consists of the Italian isolate and group 5 an isolate from Greece and two isolates from Spain. Group 6 contains the second Greek isolate and group 7 the Argentinian and Austrian isolates. Finally group 8 and 9 contain the two distinct Chinese isolates. This paper also summarizes the proposed nomenclature for the types and subtypes of HEV.

Infection in Animals

The presence of naturally occurring anti-HEV in a wide range of both wild and domestic animals including pigs, rats, monkeys, and chickens has been demonstrated [18]. These findings have been reported to indicate that HEV is a zoonotic infection. In addition, the initial report of a strain of HEV being found in swine in the United States [19] has been confirmed in other countries [20,21]. Anti-HEV has also been found in rodents [22] in the United States, suggesting that animal reservoirs exist, not only in areas where the virus is endemic, but also in developed countries. Another important finding has been the identification of an HEV-like virus in chickens with hepatitis-splenomegaly syndrome in the United States. This virus was isolated from the bile of chickens with the disease and found to be genetically related to other HEV strains [23].

In addition to finding natural infections in animals, a range of animals can also be experimentally infected with strains of HEV. Animal models for infectious agents are extremely valuable in helping to characterize the infection, define the host range, and develop effective vaccines. Balayan *et al*. [3] first described the transmission of infection to experimental animals; having initially infected himself, he was able to infect cynomolgus monkeys. Other workers have now extended the range of nonprimates that can be infected with HEV to include chimpanzees and other types of monkeys, and in general the course of infection in primates is much like that in humans. In later experiments Balayan *et al*. [24] also reported the experimental infection of pigs and their work with rats, which has more recently been confirmed [25]. Interestingly, although swine-derived HEV and the HEV-US human strain can be transmitted to uninfected pigs [26], human strains isolated in Mexico and Pakistan can not [27].

Further work comparing the pathogenesis in pigs infected with swine and human HEV indicated that the human strain resulted in more severe and persistent lesions than the pig strain [28]. HEV was shed in feces from infected pigs, so exposure to pig feces could be considered to be a risk factor for infection. This work has been extended to look for extrahepatic replication sites [29•]. Using reverse transcriptase polymerase chain reaction, the replicative, negative-strand HEV RNA was found in tissues other than the liver, *ie*, lymph nodes, small intestines, and colon. This provides an important model for further studies on the pathogenesis of HEV and indicates that the use of pig livers and other tissues for xenotransplantation into humans is a potential risk for HEV infection.

The geographical distribution of HEV is show in Figure 1. This shows where the virus is known to have caused epidemics and also where there are substantial numbers of sporadic cases. As described previously, isolates outside these areas have now been described.

The seroprevalence within endemic areas has been shown to vary and is often low compared with that found for other enterically transmitted infections, *eg*, HAV.

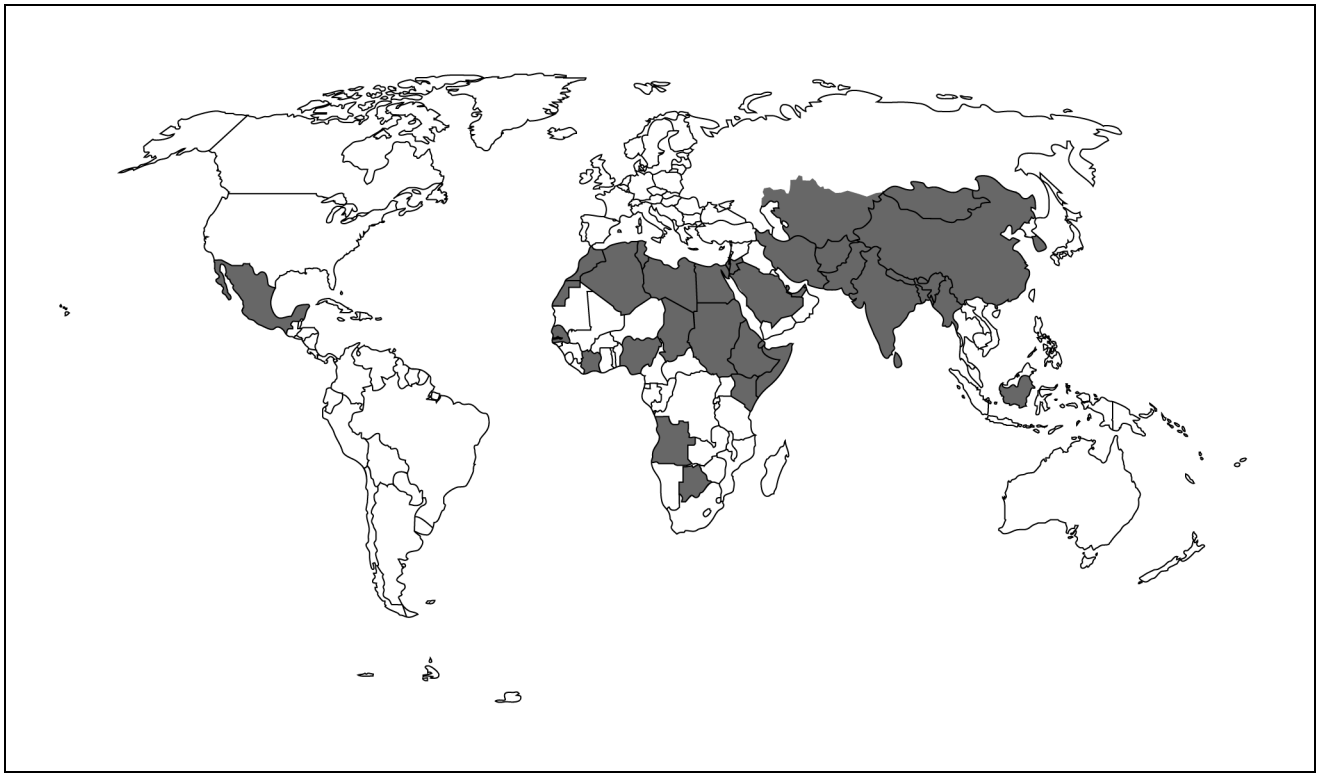

Figure 1. Geographic distribution of hepatitis E. Outbreaks or confirmed infection in greater than 25% of sporadic non-ABC hepatitis. (*From* Centers for Disease Control and Prevention. Accessible at http://www.cdc.gov/ncidod/disease/hepatitis/slideset.)

For example, Hau *et al*. [30] reported seroprevalences for anti-HAV and anti-HEV in Vietnam of 97% and 9%, respectively. This low level of immunity indicates a considerable potential for outbreaks in such areas if the population is exposed to HEV. This has been seen in other studies and therefore, although it may be related to the sensitivity and specificity of the assays used as discussed below, it does seem to be a true characteristic of the epidemiology of HEV.

There has been conjecture about the reasons for the unexpectedly high attack rate observed in young adults. Infections caused by fecal-orally tranmitted agents usually occur during childhood and tend to confer life-long immunity. HEV does infect children as has been shown in many studies, but protective antibody does not seem to be long-lived. This idea has recently been reaffirmed by the work of Mathur *et al*. [31]. Obviously this finding has implications for the success of any future vaccine program.

Corwin *et al*. [32] have described the ecology of HEV transmission in southeast Asia and discussed the events that may lead to outbreaks. They showed that there is probably a dose-response effect since Indonesian communities living upstream—where it is assumed there is a greater concentration of virus in the shallow, slow moving water—experienced higher attack rates than communities living downstream. In addition, in areas where reduced dilution is caused by low rainfall, there seemed to be an increased risk of infection. Corwin *et al*. [32] described two scenarios that favor the occurrence of epidemics. The first is where flooding occurs, causing raw sewage to contaminate water used for drinking; many outbreaks have been reported in the rainy season. The second is during dry conditions when the concentration of HEV is undiluted and gives rise to infection.

One major difference between HEV and HAV is the finding that secondary cases of HEV are rare: only 2% of contacts compared with 15% for HAV. It seems that large doses of virus are needed to cause illness since water implicated in outbreaks has often been described as grossly contaminated. A dose response effect has been described in experimentally infected animals [33], and this may be the same for humans as suggested by Corwin *et al*. [32]. Where significant person-to-person spread has been reported, continuing water contamination has to be considered as a more likely explanation [34].

Clinical Disease and Diagnosis

Hepatitis E virus infection has many features in common with hepatitis A including the same route of transmission and the fact that both usually cause acute self-limiting disease with no chronic sequelae. The incubation period for HEV is 30 to 40 days, although this can vary between 15 and 60 days. In one volunteer study, symptoms were observed at day 30 with jaundice developing on day 38. Viremia was detected at day 22 and virus was present in

stools at day 34, therefore preceding the onset of jaundice [35]. This means that, as with hepatitis A, patients are infectious before the onset of specific symptoms. Since there is a viremia during the illness, a theoretical possibility exists of transmission by blood, although current evidence would suggest that this is not an important route of infection.

Symptoms cannot be distinguished from those caused by other hepatitis viruses, and infection can result in jaundice, malaise, hepatomegaly, and anorexia, although asymptomatic cases are also seen. Cholestatic jaundice is a common finding, and on biopsy characteristic pseudoglandular structures have been described.

Hepatitis E virus has been shown to be the cause of fulminant hepatitis in both epidemics and sporadic cases in endemic areas [1,36], and the overall mortality is 1% to 2%. This rises to 15% to 20% in pregnant women, particularly those who become infected in the third trimester [37]. A high mortality rate associated with such cases has long been recognized, but was thought to be due to the virus responsible for infectious hepatitis in developed countries, *ie*, hepatitis A. It is obvious from early papers that the outcome of infection seen in developing countries is different from that seen in the developed world, and provides a hint that there may be two etiologies. There has been controversy about the role of HEV in pregnant women. However, in a recent paper by Jaiswal *et al*. [38•] the high mortality rate is confirmed. They report on the outcome of 273 women with viral hepatitis of whom 127 were pregnant. The incidence of HEV infection was not significantly different in the two groups: 57% in pregnant women and 46% in nonpregnant women. However, the highest mortality rate (56%) was found in the HEV-infected fulminant hepatitis cases occurring in the third trimester of pregnancy. The course of acute hepatitis E was similar in nonpregnant and pregnant women, but the presentation of fulminant hepatitis was more common during pregnancy, suggesting that pregnancy increases the severity rather than the susceptibility to infection.

Several cases of severe disease in women returning from the Indian subcontinent have been reported in the United Kingdom [39]. In this study, spontaneous labor resulted in the birth of a healthy child and the recovery of the mother. Awareness of HEV as a possible diagnosis is urged, particularly in patients returning from endemic areas, since early recognition and intensive treatment of acute liver failure are instrumental in patient survival.

Reasons for the high mortality in pregnant women has not been adequately explained, although recent work by Endy *et al*. [40•] may provide some answers. These authors investigated the immune response in pregnant women with HEV, and suggested that the elevated levels of soluble Fas that were observed may be related to the severe degree of liver damage seen. They also report depressed levels of leukemia inhibitory factor that may account for the fetal loss reported in some studies. Khuroo *et al*. [41] investigated a group of pregnant women with HEV. Two of eight babies died, one with hepatic necrosis. HEV detected by polymerase chain reaction in cord blood or postnatal samples suggests that intrauterine infection occurs, but more work needs to be done in this area.

Diagnosis

Tests to detect both anti-HEV IgM, and IgG are available as commercial enzyme-linked immunosorbent assays in some, but not all parts of the world, and various research assays have also been described. The presence of specific IgM indicates an acute or recent infection, and this usually disappears around 3 months postinfection. A positive IgG test usually suggests past infection. However, using two independent assays, Thomas *et al*. [42] found an unexpectedly high level of seropositivity in a series of US blood samples. The anti-HEV in this study seemed to bind to an HEV capsid protein but did not relate to prior infection or to a nonspecific reaction. The authors urged caution when interpreting the results of seroprevalence studies carried out in nonendemic areas. Various antigens have been used in attempts to produce the optimal test in terms of sensitivity and specificity, and in general tests that include antigens derived from ORF2 are better than those based on ORF3 [43]. Molecular tests such as polymerase chain reaction have been employed to study HEV infections as in the volunteer study described above, and have also proved useful in transmission studies. No doubt this approach will provide valuable information in the future and may be useful in a diagnostic setting.

Prevention

Any improvements in water quality and sanitation will be helpful in preventing transmission of HEV since most of the outbreaks described can be attributed to contaminated water supplies. Improvement should be implemented wherever possible. Although passive protection has been demonstrated in animals [44], when administration of immune globulin has been tried in humans it has met with disappointing results. Such preparations are particularly of little value if they are produced in countries where HEV is not endemic and there is little immunity to the virus. Khuroo and Dar [45] and Arankalle *et al*. [46] looked at the efficacy of an Indian preparation of immune serum globulin. There was no protective effect observed in the first study and only a marginal beneficial effect in the second.

Successful propagation of HEV in tissue culture has been achieved [47], and this has provided an important experimental means to study the replicative process of the virus and the mode of virus neutralization. However, there is no efficient method of producing virus and progress in vaccine development is now being made using recombinant technology. Characterization of the neutralization

epitopes, which is important for effective vaccine development, continues and Meng *et al*. [48] have recently described the minimal size fragment that can model such an epitope. Antibodies to this fragment, designated pB166, can cross-neutralize three different genotypes suggesting that a common neutralization epitope may exist and that the proteins described could act as potential vaccines. Progress towards an effective vaccine has already been made, and Emerson and Purcell [49•] provide a review of the current situation. They describe the early work in which protection of cynomolgus macaques was achieved using a fusion protein consisting of part of the ORF2 protein expressed in *Escherichia coli*. Following this, a recombinant capsid protein expressed from a baculovirus vector in insect cells and derived from the Pakistani strain was used to vaccinate monkeys with a range of doses. Animals were protected from hepatitis as determined by liver histology and liver function tests, and those animals that were vaccinated with the highest dose were also protected against challenge with the Mexico strain, the strain most genetically distinct from the Pakistani strain. Control animals all developed hepatitis on challenge.

Using this approach a recombinant HEV vaccine was produced by DynCorp (Rockville, MD) in the United States for trials in humans. The antigen used was a purified polypeptide produced in insect cells infected with recombinant baculoviruses containing truncated HEV genomic sequences encoding the viral capsid antigen ORF2 isolated from an outbreak in Pakistan. Preliminary results were encouraging; the vaccine was well tolerated and elicited antibody titers when given at a sufficient dose. Initial seroconversion appears to occur in at least half of the recipients after the second dose and titers are boosted after the third dose [50]. Phase II-III efficacy trials are now being conducted by Walter Reed Armed Forces Research Institute of Medical Sciences and GSK (GlaxoSmithKline) in Nepal. It seems that recombinant HEV antigens are immunogenic and can induce antibodies. However, there are still outstanding questions to be answered as pointed out by Emerson and Purcell [49•]. These include whether a vaccine will induce sterilizing long-term immunity, whether a monovalent vaccine will protect against all strains, and how many neutralization epitopes exist. It is obvious that when an effective vaccine becomes available it will be a valuable tool in preventing infection in endemic areas, particularly in pregnant women, and it will also provide protection for travelers to these regions.

Finally, as alluded to earlier, there is no specific treatment for HEV. Patients are treated symptomatically and, as described for cases in pregnant women, may need intensive care.

Conclusions

Progress continues to be made in our understanding of HEV. The existence of isolates outside endemic areas has now been convincingly demonstrated and the relationship of infections in humans and animals is being clarified. Important animal work is highlighting potential problems associated with xenotransplantation and elucidating the pathogenesis of the virus. The development of a vaccine has reached the clinical trials stage; certainly an effective vaccine would be very welcome in areas where outbreaks of infection give rise to considerable morbidity and mortality.

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