Xenotransplantation and the potential risk of xenogeneic transmission of porcine viruses

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Abstract

The clinical success of allotransplantation and the shortage of donor organs have led to a proposal for the use of animal organs as alternative therapeutic materials for humans. In that regard, swine are preferable to non-human primates as a source of donor organs. While applications for clinical trials for xenotransplantation have not yet been received in Canada, several trials have already been authorized in the United States. A major concern, however, is the potential for xenogeneic transmission of viruses from animals to humans via organ, tissue, or cellular transplantation or via ex vivo exposure of humans to porcine biologic materials. Xenotransplantation allows viruses to bypass the normal immunological defense mechanisms of the recipient. Furthermore, the use of immunosuppressive drugs following transplantation may facilitate the xenogeneic transmission of zoonotic agents. Of porcine viruses, swine hepatitis E virus does not cause any clinical symptoms in the natural host but is a likely zoonotic agent that can infect humans and cause hepatitis. Porcine circovirus type 1 is prevalent in swine populations with no known association with clinical disease, while circovirus type 2 causes post-weaning multi-systemic wasting syndrome. Porcine endogenous retrovirus is integrated into the host chromosomes while porcine cytomegalovirus undergoes latent infection. Two additional porcine herpesviruses have recently been identified in swine and have been named porcine lymphotrophic herpesviruses. These herpesviruses can potentially become reactivated in human recipients after xenotransplantation. All in all, there are a number of viruses in swine that are of primary concern to screen and eliminate from xenotransplantation protocols. Epidemiology and the current knowledge on xenogeneic risk of these viruses are discussed.

Résumé

Le succès clinique des allogreffes et la pénurie de donneurs d'organes ont conduit à une proposition visant à utiliser les organes d'animaux comme alternative thérapeutique chez les humains. À cet égard, les porcs sont préférables aux primates non-humains comme source de donneur d'organes. Bien qu'au Canada aucune demande d'essai clinique de xénogreffe n'ait été déposée, plusieurs essais ont déjà été autorisés aux États-Unis. Une des préoccupations majeures demeure le potentiel de transmission de virus des animaux aux humains via la transplantation d'un organe, tissu ou cellule, ou bien par exposition ex vivo *d'humains à du matériel biologique porcin. La xénogreffe permet aux virus de contourner les mécanismes de défense immunologiques normaux du receveur. De plus, l'utilisation de médicament immunosuppresseur suite à la greffe peut faciliter la transmission d'agents zoonotiques. Parmi les virus porcins, le virus porcin de l'hépatite E ne cause aucune manifestation clinique chez son hôte naturel mais il s'agit fort probablement d'un agent de zoonose capable d'infecter les humains et de causer une hépatite. Le circovirus porcin de type 1 est prévalent dans le populations porcines mais sans association connue avec une maladie clinique alors que le circovirus de type 2 est responsable du syndrome de dépérissement multi-systémique en période post-sevrage. Le rétrovirus endogène porcin et le cytomégalovirus porcin sont intégrés dans les chromosomes de l'hôte et causent des infections latentes. Deux herpèsvirus porcins additionnels ont récemment été identifiés et nommés herpèsvirus porcins lymphotropiques. Ces herpèsvirus peuvent potentiellement être réactivés chez les receveurs humains suite à la xénogreffe. Un certain nombre de virus retrouvés chez le porc sont une préoccupation majeure et nécessite d'être recherchés et éliminés des protocoles de xénogreffes. L'épidémiologie et les connaissances actuelles sur le risque xénogénéique de ces virus est abordé.*

(Traduit par docteur Serge Messier)

Introduction

Recent advances in xenotransplantation technology as a therapeutic approach have the potential to benefit human health. In humans, hepatic failures, such as liver cancer and decompensated liver cirrhosis, may someday be treated by xenograft implantation of porcine liver. Diabetes may potentially be treated by xenotransplantation of pancreatic islets, while neuronal tissues may

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Table I. Infectivity of Porcine Viruses in Humans

	PERV	HEV	PCV1	PCV2	PCMV	PLHV1/2
Known disease in pigs	−	−	−	+	+ / −	−
Transforming ability in pigs	−	−	−	−	−	−
Infection in human cells	+	−[a]	−	−	?	?[c]
Infection in non-human primate cells	−	−[a]	−	−	?	?[c]
Infection in humans	−	+	?[b]	?[b]	−	?[c]
Infection in non-human primates	?	+	−	−	?	?[c]
Known disease in non-human primates	?	+	?	?	?	?[c]
Known disease in humans	−	+	−	−	−	−
Latency	+	−	−	−	+	+

HEV — swine hepatitis E virus; PCMV — porcine cytomegalovirus; PCV1 — porcine circovirus type 1; PCV2 — porcine circovirus type 2; PERV — porcine endogenous retrovirus; PLHV1/2 — porcine lymphotropic herpesvirus types 1 and 2

[a] HEV is not cultivable in vitro in any cells

[b] Only specific antibody has been detected, and no viral sequence has been identified in humans

[c] PLHV virus has not been isolated yet. Only a small part of the genomic sequence has been identified

be implanted for treatment of neurodegenerative diseases such as Parkinson's or Huntington's disease. The pig is the donor animal species of prime interest in clinical xenotransplantation. Although primates seem to be an attractive choice, they are widely considered unsuitable as a source for xenotransplantation, mainly due to ethical issues and the likely transmission of an infectious agent. Pigs are easy to breed and economic to produce. The physiology and the size of porcine organs are similar to those in humans. Transgenic pigs that express human regulators of complement activation on porcine endothelial cells have recently been developed; this can prevent the onset of complement-dependent, hyperacute rejection of pig organs in the human recipient (1,2). It is important to recognize, however, that xenotransplantation may put the human community at risk (3). Transplantation of animal organs to humans will allow microorganisms present in the donor organs to bypass the normal defense mechanisms of the recipient. After transplantation, prolonged contact with the human body may allow the microorganisms to adapt and transmit to the recipient, which is otherwise unable to occur under natural conditions (4). The pathogenic potential and virulence of infectious agents are based mostly on host-pathogen interactions. Therefore, the clinical outcome in recipients cannot be predicted by observation of the agent's effects in the natural host. Through xenogeneic infection, an agent that is non-pathogenic in its natural host may become pathogenic in the recipient. Immunosuppressive drug therapy is common in the transplantation patient, and this immune-suppressed condition may result in unpredicted consequences in the xenograft recipient.

Xenogeneic risk of porcine viruses

Of the many microorganisms infecting swine, viruses are the major concern since other microorganisms can be greatly suppressed by routine treatment with antibiotics. Of the viruses able to infect swine, those pathogens known to produce apparent disease in pigs should be the primary targets for screening and elimination from donor herds. Viruses that do not produce obvious disease in swine are of additional concern for xenotransplantation, and their pathogenic potentials should be carefully examined. Viruses that cause latent infections by integrating their genome into host cells, those with oncogenic potential, those that can be vertically transmitted, and those that are transmitted from semen are of particular concern. Examples of these are swine hepatitis E virus, porcine endogenous retrovirus, porcine cytomegalovirus, porcine circovirus types 1 and 2, and 2 newly identified herpesviruses. With the exception of porcine circovirus type 2, all of these viruses are generally considered non-pathogenic in pigs. However, swine hepatitis E virus is likely infectious in humans and causes hepatitis. Porcine endogenous retrovirus is integrated into the host cell chromosome while porcine cytomegalovirus and porcine lymphotrophic herpesviruses persist in the nucleus of the cells and may be reactivated in the recipient to produce infectious virus. Some of these viruses (eg, swine hepatitis E virus) have been shown to infect humans and primates in vivo, while some viruses do not even infect human cells in vitro (Table I). The present article reviews the current knowledge regarding these viruses and their potential for xenogeneic transmission in humans upon xenotransplantation. Porcine endogenous retrovirus has been excluded from this review, as several excellent articles are already available (5–7).

Swine hepatitis E virus

Viral hepatitis in humans and hepatitis E

Hepatitis E is one of several types of the recognized viral hepatitis in humans: hepatitis A, B, C, D, E, and possibly G (hepatitis F does not exist). Transmission of hepatitis B, C, and D is mainly through infected blood or blood products. Hepatitis D virus, called Delta agent, is a defective virus and always requires hepatitis B virus as helper virus. In contrast, hepatitis A and E are known as foodborne or water-borne hepatitis. Non-A, non-B hepatitis was a diagnosis by exclusion of hepatitis A and B. Hepatitis G virus has been identified only recently. Many people infected with hepatitis G virus are asymptomatic and, thus, its clinical significance is not clear. Other viruses associated with hepatitis have been identified as

studied in tamarins. GB-A, GB-B, and GB-C viruses were identified from tamarins inoculated with serum from human hepatitis patients. However, GB-A and GB-B viruses appeared to be tamarin viruses, while GB-C virus was found to be a subtype of the hepatitis G virus. Since it is not clear whether GB-C virus is a significant cause of hepatitis in humans, and because its name does not follow the conventional naming for hepatitis, GB-C virus has not yet been officially named.

Hepatitis E virus is excreted in the feces of infected individuals, and, thus, contaminated feces is likely the primary source of infections. Although it is not clear how hepatitis E virus reaches the liver, it is presumed that, following oral ingestion, the virus replicates in the intestinal tract and enters the liver via the portal vein. The virus then replicates in the cytoplasm of the hepatocytes, is released into the bile duct, is secreted back into the intestine, and excreted in the feces. In young adults, clinical features include jaundice, anorexia, abdominal pain, nausea, and hepatomegaly. The mortality rate for hepatitis E is reported to be 1 to 3%, higher than the mortality rate of 0.2% for hepatitis A. Unlike hepatitis B or C, hepatitis E does not progress to chronic hepatitis, liver cirrhosis, hepatocellular carcinoma, or a carrier state, and recovery is always complete. Hepatitis E has been reported to be severe in pregnant women; with high rates of fulminating hepatitis, the case fatality is up to 20%, especially during the third trimester of pregnancy (8).

Epidemics and outbreaks of hepatitis E are usually associated with fecal contamination of drinking water. With some exceptions of foodborne associated epidemics in China, serologically confirmed cases of the hepatitis E epidemics are consistently associated with contaminated water. Consumption of raw and uncooked shellfish has also been linked with cases of sporadic hepatitis. Person-to-person spread has been reported but seems to be uncommon. Hepatitis E-endemic regions include Africa, the Middle East, Asia, and Mexico. In these regions, epidemics have been confirmed serologically and sporadic cases of hepatitis have also been observed. The peak attack rates are seen in young adults (15 to 30 y of age), particularly males. Epidemiological risk factors have been difficult to identify. However, travel to an endemic area is a risk factor reported in a number of cases (9).

The etiologic agent for hepatitis E has been identified and characterized. Hepatitis E virus (HEV) is a small, non-enveloped virus with a positive sense RNA genome of approximately 7.2 kb. The full-length genomic sequence has been determined in several strains isolated from different hepatitis E endemic areas, including Burma, Pakistan, India, China, and Mexico. Partial sequences of other isolates are also available. Although HEV was originally classified in the family *Caliciviridae*, due to the uniqueness of its genome structure, it has been separated from family *Caliciviridae* and designated an unclassified group called 'hepatitis E-like viruses' (10). The viral genome contains only 3 open reading frames (ORFs): ORFs 1, 2, and 3. The ORF1 codes for a non-structural RNA polymerase and ORF2 and ORF3 code for a glycosylated capsid protein and a cytoskeleton-associated phosphoprotein, respectively (11,12). The HEV proteins have not been well characterized for their function, but both the capsid protein and the cytoskeletal protein contain antigenic epitopes, and, therefore, have been used as diagnostic reagents.

Analysis of the genomic sequences of different HEV isolates recovered from different geographical regions has revealed substantial genetic diversity. When the sequences of the strains isolated from countries in Asia were compared, high homologies (93 to 99%) were observed throughout the genome. In contrast, the Mexican strain was distant from Asian strains, and sequence identities between the Mexican strain and Asian strains were approximately 75, 81, and 91% for ORF1, ORF2, and ORF3, respectively. An HEV strain recently isolated from Morocco was homologous with the Asian strains (13). Two additional African HEV strains were isolated from 2 outbreaks of hepatitis E during 1978 to 1980 in Algeria and 1983 to 1984 in Chad (14). The sequence homology of the Chad and Algerian strains was 89 to 95% when compared with the Asian strains. Therefore, the African strains were considered to form a distinct subgroup within the Asian genotype. In conclusion, there are 2 distinct HEV genotypes, Asian and Mexican (Figure 1).

Seroprevalence of hepatitis E in human populations

Recent serological studies in humans in hepatitis E endemic regions indicate that the prevalence of HEV antibodies (3 to 26%) was much lower than anticipated in endemic regions. In contrast, the prevalence of antibodies in non-endemic regions appeared to be much higher than expected. One study indicated that 1–2% of blood donors in the United States (a non-endemic country) were seropositive to hepatitis E (15). Another study demonstrated that 1.2 to 1.4% of 5000 blood donors in northern California were positive to hepatitis E virus. Furthermore, 31 to 38% of the seropositive individuals involved in this study had no history of international travel (16). Studies in Baltimore, Maryland, using serum samples from men who have sex with men, injection drug users, and healthy blood donors, also showed a very high prevalence of anti-HEV (16 to 23% were seropositive) (17). Limited data are also available for Canadian populations. Two independent studies indicated that approximately 2% of the tested sera from Ontario and Saskatchewan were positive (18; personal communication, Dr. M. Fearon, Ontario Ministry of Health). Similar serologic data were reported in other developed countries in Europe, including Spain, Sweden, Germany, Greece, England, Finland, Italy, and The Netherlands (19–23). In the absence of endemic hepatitis E, there was no evidence that the anti-HEV antibody reflected subclinical HEV infection. Although some cases were reported to have been associated with travel to endemic regions, the reason for a relatively high rate of anti-HEV antibodies in the virtual absence of clinically overt hepatitis in these non-endemic areas is largely unknown. In developed countries, hepatitis E is generally considered non-endemic. Although sporadic cases of hepatitis E are occasionally reported in these countries, they are often linked with a history of travel to an endemic region (9). In New Zealand, a hepatitis E case was reported in a young male who had not traveled overseas for the previous 2 y and had not been in contact with any overseas travelers before he was clinically ill (24). In support of the serologic data, hepatitis E cases were also reported in the United States, Italy, the Netherlands, Spain, and Sweden (19–23,25).

The findings of high seroprevalence to anti-HEV in non-endemic areas have led to the speculation that HEV or a closely related agent may exist in animal reservoirs. In fact, besides the seroprevalence in human populations, several serological studies have

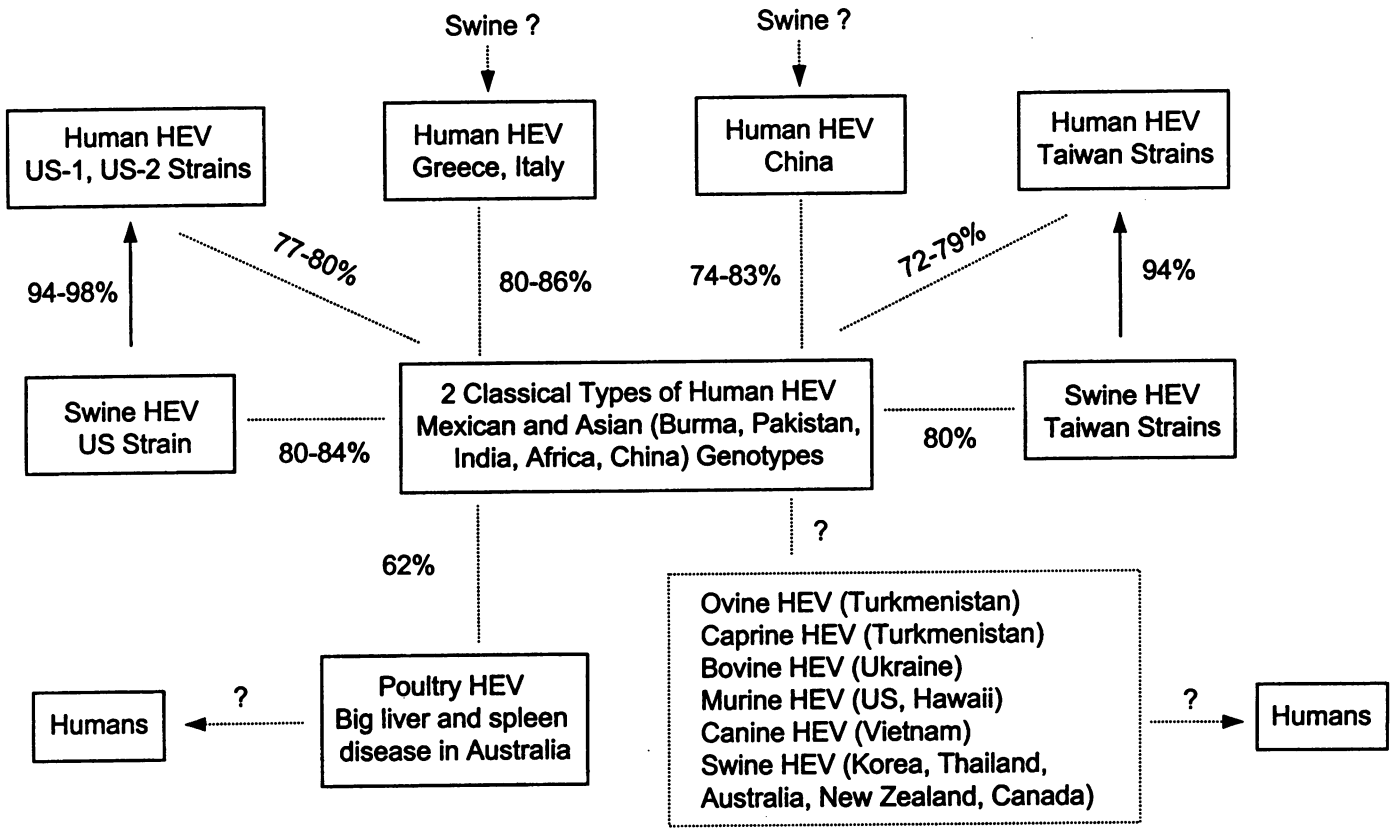

Figure 1. Hepatitis E virus in various animal species and possible cross-species transmissions. The human HEV US-1 and US-2 strains are likely variants of swine HEV US, and, similarly, the human HEV strains isolated in Taiwan are likely variants of swine HEV Taiwan. Sources of human HEV Greek, Italian, and Chinese are unknown, although swine is speculated. The poultry HEV identified in Australia is probably an avian form of HEV, and its association with human infection is unknown. HEV from sheep, goats, rodents, cattle, and pigs from the countries indicated in the dotted box have not yet been isolated, but specific antibodies have been demonstrated in these animal species. Transmission of virus from these animal species to humans is unknown. Numbers indicate the nucleotide sequence similarities. Solid lines with arrow indicate a likelihood of transmission. Dotted lines indicate genetic relatedness by sequence similarities.

been conducted in domestic animals of endemic areas. In the Kathmandu Valley of Nepal, 18 of 55 domestic pigs tested for HEV antibodies were positive (26). In Vietnam, anti-HEV antibodies were detected in 14% of chickens, 36% of pigs, 27% of dogs, and 9% of rats (27,28). Serological data obtained in human populations from both HEV endemic and non-endemic regions strongly suggest that these animals and humans may have been exposed to HEV or closely related agents. However, it was not possible until just recently to draw any valid conclusions, since the probable agent was not recovered from these seropositive animals.

Swine hepatitis E virus and swine hepatitis E-like viruses

In 1997, a new virus was discovered in swine from Illinois in the United States and was designated swine hepatitis E virus (29). The nucleotide sequences of ORF2 and ORF3 of the swine HEV were approximately 80% and 84% homologous, respectively, to the sequences of the known human HEV strains. The phylogenetic analysis of the genomic sequences indicated that the swine HEV was similar to, but distinct from, human HEV. Shortly thereafter, 2 cases of acute hepatitis E were reported in the United States (31,32). A 62-year-old white male from Minnesota was hospitalized following a 3-week history of fever, abdominal pain, and jaundice. Serology and genome detection for hepatitis A, B, and C were negative and no risk factors for hepatitis E were identified. This patient reported no travels outside the United States for over 10 y and no known exposure to untreated drinking water or uncooked shellfish. The patient tested positive for anti-HEV antibodies. Subsequently, viral sequences were cloned from his serum and designated HEV US-1. Surprisingly, the US-1 sequence was substantially divergent from other known human HEV strains, with sequence identities of only 77%. However, the US-1 HEV was rather closely related to the newly identified swine HEV. Sequence homologies of the US-1 strain and the swine HEV were strikingly high, over 94% and 98% at the amino acid level for ORF2 and ORF3, respectively (30). In a second case, a human hepatitis E virus was isolated from a hepatitis patient from Tennessee and was designated US-2 (31). Although this patient had previously traveled to Mexico, the US-2 sequence appeared to be distinct from that of the Mexican strain, but shared 99% identity with the previously isolated US-1 strain. Both US-1 and US-2 strains of human HEV isolated from human hepatitis E patients had remarkable sequence similarity to swine HEV, but only 80% homology with other human stains of HEV identified in other regions of the world. These findings strongly suggest that

swine HEV, or a similar agent, can infect humans and cause hepatitis. Perhaps people can contract hepatitis E from swine (Figure 1).

Seroprevalence of hepatitis E virus in swine populations

The newly identified swine hepatitis E virus appears to be common in pig populations. Studies have assessed the prevalence of anti-swine HEV antibodies in pigs from 4 countries, 2 endemic (China and Thailand) and 2 non-endemic (Canada and Korea) (32). It was found that swine herds in all 4 countries were seropositive for HEV. Other studies have also demonstrated that anti-swine HEV antibodies are prevalent in swine populations of non-endemic countries. A large percentage of commercial pigs in the United States are seropositive to swine HEV. In a retrospective study of 15 commercial swine herds in the mid-western United States, pigs on all 15 farms tested were found to be positive for swine HEV. Maternal antibodies in suckling piglets born to seropositive sows waned by 2 mo of age, and most of those pigs seroconverted to swine HEV by 4–5 mo of age (29). The antibody prevalence in these pigs was over 80%. The prevalence rate was higher in sows, where 19 of 21 sows examined in this study were found to be positive. In Canada, the authors' laboratory has studied the seroprevalence of anti-swine HEV antibodies in swine across the country. Of more than 400 samples evenly representing swine farms in the provinces of Saskatchewan and Alberta, approximately 45% of pigs at 6 mo of age were found to be seropositive to HEV (18). The prevalence of anti-swine HEV in pigs from Quebec was 38 to 56% (depending on the tested area), which was similar to the prevalence of the western provinces (unpublished data). In Taiwan (a non-endemic region of HEV), about 37% of pigs were seropositive for anti-HEV (33). In Australia, 30% of random samples collected from 2 commercial piggeries were positive, and over 90% of pigs from 2 other piggeries were positive by the age of 4 mo (34). Similar results were observed in New Zealand pigs (personal communication, Dr. Garkavenko, Auckland General Hospital, Auckland, New Zealand). These studies indicate that HEV is prevalent in swine populations, regardless of whether hepatitis E is endemic in the respective human populations.

Pigs in HEV-endemic countries have also been examined for anti-swine HEV antibodies. In China, about 40% of pigs older than 4 mo of age were seropositive, and in Thailand, 20 to 90% of pigs older than 3 mo of age were found to be positive, depending on the farms examined (32). Animals other than swine in both HEV endemic and non-endemic countries have also been demonstrated to be positive for anti-HEV antibodies. Twenty-nine to 62% of cows tested were positive in Somalia, Tajikistan, and Turkmenistan, and 42 to 67% of sheep and goats in Turkmenistan were positive (35,36). In the Ukraine (a non-endemic country), 12% of cattle appeared to be positive (36). Wild-caught pigs in Australia were positive (34). In the United States, 77, 90, and 44% of wild rats were positive in Maryland, Hawaii, and Louisiana, respectively (36,37).

Recently, a similar type of the HEV isolate has been obtained from chickens. Big liver and spleen disease, characterized by hepatomegaly and splenomegaly, has been recognized in Australia (38). The disease has been considered the most economically significant in Australia, causing a decrease in egg production and an increase in mortality in broiler breeder flocks. The etiology of this disease has been presumed to be a virus. Payne et al (39) have obtained a partial amino acid sequence of a viral protein and have designed a pair of degenerate primers. By using the primers deduced from the amino acid sequences, a specific fragment was amplified by polymerase chain reaction (PCR) from liver extracts of infected chickens. It appears that the DNA sequence of the PCR product was 62% homologous to the sequence of a human HEV. The finding of sequence homology between the human HEV and the big liver and spleen disease virus is intriguing. It needs to be confirmed by sequencing other regions of the viral genome, as the identified region represents the helicase gene found in other positive RNA viruses. It is, however, tempting to speculate that big liver and spleen disease virus may be an avian form of HEV (Figure 1).

Other swine hepatitis E-like viruses

Although hepatitis A, B, and C are endemic in humans in Taiwan, hepatitis E has never been reported. However, in 10 to 20% of cases of acute hepatitis, the etiology was undefined. Recently, 4 different HEV isolates were recovered from acute hepatitis patients (with unknown etiology), and nucleotide sequences were obtained. The 4 HEV isolates were almost identical to each other, but appeared to be distinct from all known human HEV strains isolated from Mexico, Burma, India, Pakistan, and Africa. Sequence homologies between the Taiwan isolates and other known human HEV were only 72 to 79% (40). None of these patients had history of traveling off the island and the source of transmission remained unclear. When pigs in Taiwan were examined for anti-HEV antibodies, 37% of the tested pigs were found to be positive (33). Attempts were made to isolate virus from pigs and the viral sequences were subsequently obtained. Phylogenetic analysis comparing the sequences obtained from the humans and those from swine revealed a homology of up to 95% (41). Furthermore Taiwan HEV isolated from both humans and swine form a distinct branch divergent from all other known strains of HEV, including US-1, US-2, and US swine HEV strains (Figure 1).

In addition to Taiwan and the United States, new strains of HEV have been identified from other industrialized non-endemic countries. A new strain of HEV has been isolated from a hepatitis E patient in Italy who had no history of traveling to endemic areas. The sequence of the Italian HEV was only 80 to 85% similar to other known HEV strains (42). Three other strains of HEV were identified from China, Greece, and Italy, and the genomic sequences of these strains were significantly divergent from other known HEV (43,44). The 2 Greek strains were distinct from each other and also distinct from the Italian HEV or the 2 US strains. Similarly, sequences of the Chinese strains were distinct from known human HEV sequences (43). Thus, to date, at least 7 distinct genotypes seem to exist worldwide for hepatitis E virus isolated in humans: Mexican, Asian, US-1 and -2, Taiwanese, Chinese, Greek, and Italian. Among these, the US-1 and -2 strains and the Taiwanese strain likely originated in swine, while sources of the Greek, Chinese, and Italian HEV remain unclear.

Cross-species transmission of swine hepatitis E virus

Since HEV is able to infect pigs, chickens, primates, cows, and rodents, and the new genotypes identified in human hepatitis E

patients are very similar to the virus isolated from pigs, it is possible that the virus may have been transmitted from pigs to humans. Limited but convincing evidence is available for the cross-species transmission of swine HEV. Meng et al (45) have shown that swine HEV is indeed able to infect primates. Rhesus monkeys (*Macaca mulatta*) seroconverted upon intravenous infection with swine hepatitis E virus and the virus was detected in the blood and fecal excretions. Viremia lasted for up to 5 wk after virus inoculation. Serum levels of liver enzymes alanine aminotransferase and isocitrate dehydrogenase were elevated. Mild, focal, necroinflammatory pathological changes were detected in the liver. A chimpanzee (*Pan troglodytes*) infected intravenously with swine HEV also seroconverted and excreted the virus in the feces, but did not show viremia or pathological changes in the liver. Pigs are susceptible to the US-2 strain of human HEV (with a high degree of sequence homology to swine HEV). Pigs inoculated with the US-2 human HEV were clinically normal but seroconverted 2 wk post-infection and the virus was detected in the feces. In contrast, other types of human HEV, namely Pakistan HEV and Mexican HEV, were not able to infect pigs (45–47).

Zoonotic potential of swine hepatitis E virus

Seroprevalence of anti-HEV antibodies in the human risk groups was assessed. In Taiwan, pig handlers and pork dealers had higher seroprevalence than the control group (27, 15, and 8% respectively; 35). In Iowa, anti-HEV antibody titers were determined among selected populations including 204 hepatitis patients who had no association with hepatitis A, B, and C, 87 field staff of the Department of Natural Resources, and 332 healthy blood donors. Non-A to -C hepatitis patients (4.9%) and field workers (5.7%) showed significantly higher prevalence of anti-HEV antibodies than normal blood donors (48). Pig handlers in China and Thailand also had high seroprevalence to HEV (71 to 100%), although this study did not include a control group (34). These findings strongly suggest transmission of swine hepatitis E virus to humans and the induction of hepatitis in infected individuals.

Ample evidence has been accumulated suggesting that swine HEV is likely a zoonotic agent. Swine HEV is genetically similar to the human HEV isolated from HEV non-endemic areas (2 US strains and the Taiwan strain), but is distinct from HEV isolated from hepatitis E endemic areas (the Asian strains and the Mexican strain). The swine HEV is able to infect primates and cause hepatitis. Conversely, human HEV that is genetically similar to swine HEV will infect pigs, but human HEV genetically distinct from swine HEV cannot infect pigs. Genetically similar HEV in both swine and human populations co-exist in the same geographical regions. Various other animal species have been shown to have specific antibodies for HEV and a specific HEV sequence has been demonstrated in chickens (39).

It seems that HEV is common among pigs, is excreted in feces, and then infects humans by feco-oral contamination. The transmission may be by direct contact or through food or water contaminated with feces containing HEV. The HEV infection may lead to the development of acute hepatitis in humans, or may be asymptomatic. In either case, infected individuals will seroconvert. However, it is unclear why epidemics of acute hepatitis in non-endemic countries have not occurred despite the virus being commonly present in swine populations. It was shown in pigs that the swine HEV infection was dose-dependent (45); a lower dose did not result in liver enzyme elevation, liver pathology, or virus excretion in the feces. Perhaps the swine HEV infection and disease induction in humans may also be dose-dependent. It is also likely that different strains or genetic variants of HEV may exist. Different animal species may serve as different reservoirs for different strains of HEV. Certain strains of HEV may cross the species barrier, but the cross-species infection may be inefficient, resulting in abortive or asymptomatic infections. Among the different strains of HEV, swine HEV may be the most efficient at infecting humans. This cross-species infection may also be dose-dependent. The potential for cross-species infection by HEV raises a public health concern. Risk groups include swine practitioners, pig farmers and handlers, meat handlers, those involved in manure disposal, and others in close contact with swine.

Since swine have attracted a major interest for xenotransplantation, swine HEV is a major concern as a potential xenogeneic agent. Swine HEV replicates in the livers of pigs and likely infects humans by crossing the species barrier. Xenografts of tissues, organs, and cells from pigs to humans will allow the direct transmission of swine HEV to humans. Although swine HEV infects pigs and primates subclinically, it is not known if the swine HEV can become pathogenic in humans, especially in immunosuppressed recipients. This virus should be considered as a potential xenogeneic agent with the possibility for vertical transmission.

Porcine circovirus

Circoviruses have been identified in several birds and plants. In birds, psittacine beak and feather disease virus, pigeon circovirus, and chicken anemia virus are the most commonly recognized circoviruses. A mammalian circovirus was first discovered as a persistent contaminant of the porcine kidney cell line PK-15 (ATCC-CCL33) and was named porcine circovirus. Circoviruses are non-enveloped viruses with icosahedral symmetry ranging from 15 to 22 nm in diameter. Its genome is one of the smallest of any known viruses, consisting of single-stranded, circular DNA with only 1759 nucleotides with a negative polarity (55). Animal circoviruses share some minor similarities, while plant circoviruses have limited similarities to animal circoviruses. The DNA replicates in the nucleus of the infected cell and is able to produce at least 4 viral proteins. To date, 2 types of circoviruses have been identified, types 1 and 2.

Porcine circovirus type 1

In the first seroepidemiological study on the prevalence of porcine circovirus, now designated PCV type 1, approximately 60% of German slaughter pigs were found to be positive (49). Independent studies also indicated that a high percentage of pigs in Germany, Northern Ireland, and Canada were seropositive to PCV (50–53). In Canada, 26% of slaughter hogs and 55% of sows in commercial herds were found to be positive, with similar results obtained in the United States and the United Kingdom (53% and 86% seroconversion, respectively). It is, therefore, generally believed that PCV1 is ubiquitous in pig populations worldwide. However, no disease has been reported to be associated with PCV1.

Porcine circovirus type 2

A post-weaning multi-systemic wasting syndrome (PMWS) in pigs was first recognized in western Canada in 1991 (54). The disease was characterized by progressive weight loss, respiratory signs (tachypnea and dyspnea), and jaundice in nursery pigs. Porcine circovirus type 1-like antigens and DNA were demonstrated from the diseased pigs, and the new type of porcine circovirus was designated PCV type 2. The full-length genomic sequence of 2 independent PCV2 isolates was determined (55,56). Porcine circovirus types 1 and 2 share overall genomic sequence homology of only 68%, indicating that the 2 types of PCV are closely related, yet distinct. Based on the genomic sequence differences between the 2 types of PCV, it is now possible to differentiate them. It appears that PCV2 is widely spread throughout the world, including Spain, Germany, France, Canada, Japan, and Korea (57–60).

Porcine circovirus type 1 is non-pathogenic in experimentally infected pigs. Miniature pigs or colostrum-deprived piglets infected with PCV1 became positive for the virus (especially in the lung, liver, spleen, and thymus), but no significant clinical signs were observed (50,61). In contrast, PCV2 has been associated with PMWS in pigs with a nursery mortality of 7%. In all cases of PMWS reported to date, PCV2 antigen and the PCV2 genomic DNA have been demonstrated in multi-systemic lesions. Other infectious agents, including porcine parvovirus and porcine reproductive and respiratory syndrome virus, have also been demonstrated from these cases, but not consistently. Attempts were made to reproduce the syndrome experimentally, but it had not been possible to fulfill Koch's postulates. Recently, lesions typical of PMWS were finally demonstrated in both colostrum-deprived piglets and conventional pigs by PCV2 inoculation (62,63), although only a limited number of animals showed clinical signs.

It is worthy to note that PCV1 has the potential to transform primary porcine cells (64). Cells infected with PCV1 showed biological characteristics similar to cells transformed by simian virus 40 large tumor (SV40 large T) antigen. Primary porcine cells transformed by PCV1 lost contact inhibition and formed cell colonies. These cells survived up to 16 passages. It is not known if the PCV DNA is integrated into the chromosome or if PCV can infect any human cells and retain this transforming ability. Therefore, the potential risk of porcine circovirus for transmission to humans via xenotransplantation remains unclear.

Circovirus in humans and other animal species

Although PCV1 is the only circovirus isolated from mammals to date, PCV1-specific antibodies were demonstrated in humans in Germany (65). Approximately 20% of the healthy adults were positive for PCV-like antigen, and the number increased to 30% in sera of hospitalized patients. Similar serologic results were obtained in Canadian populations. Twenty-four per cent of randomly selected samples from hospitalized patients in western Canada were shown to be positive to PCV antigen by ELISA using a recombinant PCV antigen (unpublished data; personal communication, Dr. P. Willson, University of Saskatchewan, Saskatoon, Saskatchewan). Mice and cattle in Germany were also found to be positive to PCV (65). However, neither virus nor the viral genome has been detected ye from any mammalian species, including humans, other than pigs. The antibody responses found in humans and other animal species may not have resulted from an active virus infection, since it has not been possible to detect PCV-specific nucleotide sequences from antibody positive subjects (personal communication Dr. P. Willson, University of Saskatchewan). There is no evidence that humans have been infected with PCV during normal contact with swine and swine products. Therefore, it remains unknown whether immunosuppressed xenograft recipients will be at a risk of infection by porcine circovirus. However, swine herds should be screened for the virus and positive herds excluded from the xenotransplantation protocols.

Porcine herpesviruses

Herpesviruses are widespread in nature and are found in insects, reptiles, amphibians, as well as every species of birds and mammals, including humans and primates. The virions are large and spherical with a diameter of 150 to 200 nm. The herpesvirus genome consists of a single molecule of linear, double-stranded DNA, 125 to 230 kb in length. The herpesvirus has the second largest viral genome, next to that of the poxvirus. A hallmark of herpesvirus infection is that the virus remains persistent in the infected host for a lifetime. The herpesvirus genomic DNA may be integrated into the host chromosome (rarely) or exists as an episome and undergoes a latent state. The virus is frequently reactivated and shed. The herpesviruses are grouped into 3 subfamilies, *Alpha-*, *Beta-*, and *Gammaherpesvirinae*, based on the phylogenetic trees and biological properties. In pigs, 4 herpesviruses have been identified: pseudorabies virus, porcine cytomegalovirus, and the 2 recently identified lymphotrophic herpesviruses. Pseudorabies virus belongs to the subfamily *Alphaherpesvirinae*, and porcine cytomegalovirus belongs to *Betaherpesvirinae*. Canada has remained free of pseudorabies for many years. Since pseudorabies infection in pigs is clinically apparent, its xenogenic risk is diminished and of less concern in xenotransplantation.

Porcine cytomegalovirus

Porcine cytomegalovirus (PCMV) causes rhinitis in pigs less than 10 wk of age. In older pigs, the infection is subclinical. Porcine CMV often produces enlarged cells, from which the name cytomegalovirus was designated. Large intracellular inclusion bodies are found in the PCMV-infected cells of mucous glands of the turbinate mucosa, and, hence, the disease is called 'inclusion body rhinitis.' Similar to human cytomegalovirus, PCMV crosses the placenta and infects fetuses, resulting in congenital infections (66–68). The infected fetuses may die or may suffer from generalized disease after birth. Porcine CMV is endemic worldwide. In the United Kingdom, 50% of herds were infected, while the infection rate in Iowa in the United States was 12%. The seroprevalence in Japan and The Netherlands was extremely high, with more than 99% and 93% of the tested pigs positive, respectively (70,71). A PCR-based method of detection of the virus in pigs has recently been applied to PCMV and confirmed that the virus is endemic in many commercial herds (71–73). Prevalence of PCMV in Canadian pigs was reported to be 59% by PCR (71).

As is the case with human CMV infection, PCMV may be recovered from urine and cervical fluids. Porcine CMV has been isolated from the testis and epididymis of infected pigs (74), suggesting that PCMV may be secreted into semen. Despite the difficulty of in vitro cultivation of the virus, PCMV has been shown to infect porcine primary pulmonary macrophages and epithelial-like and fibroblast-like cells derived from porcine fallopian tubes (75). The ability of PCMV to infect lung macrophages raises some concerns that PCMV may modify host defense mechanisms and alter the pathogenic consequences in the host. This immune modulation may enhance the disease outcome by opportunistic infection, as occurs in both murine and human CMV. Recently, however, sequence analysis of the DNA polymerase gene locus amplified by PCR indicated that PCMV is genetically more similar to human herpesvirus types 6 and 7 than to human or murine CMV (72,73). Human herpesvirus types 6 and 7 are lymphotropic herpesviruses and infect predominantly CD4+ T lymphocytes both in vitro and in vivo. Herpesvirus types 6 and 7 share the same receptor molecule on T cells with human immunodeficiency virus (HIV) and have been isolated from AIDS patients, as well as from healthy adults. Further studies need to be done on the pathogenic potential of the porcine CMV in humans.

The reactivation of PCMV has been demonstrated by 2 independent studies (76,77). After administration of corticosteroids, gnotobiotic pigs excreted the virus, demonstrating PCMV reactivation. Porcine CMV seems to be host-specific both in vivo and in vitro. In regards to concerns of the possible PCMV transmission from pigs to humans via xenografts, attempts have been made to determine if PCMV can infect human cells. In this study, human B cells (RAJI cells) or human embryonic kidney epithelial cells (HEK-293) were co-cultivated with porcine alveolar macrophages infected with PCMV as a source of virus, and it was found that the PCMV DNA was not detectable by PCR from the cells of human origin (78). It is not known if PCMV infects other human cell types, such as T lymphocytes or macrophages. It is important to resolve this issue, as the cultivation of the virus in porcine cells is difficult and shows limited tropism (75). Previous attempts to infect other animal species with PCMV, including rabbits, mice, hamsters, and cattle, were not successful (79). Despite its potential importance for xenogeneic infection, little is known about the PCMV pathogenesis and cell tropisms. No data is available on human exposures to PCMV.

Porcine lymphotropic herpesvirus types 1 and 2 — Besides pseudorabies virus and PCMV, 2 additional herpesviruses have recently been identified in pigs from Germany and Spain (80). Two specific sequences of the herpesvirus DNA polymerase gene were detected by PCR from spleens and peripheral blood mononuclear cells of pigs. The 2 sequences were distinct by 8%. Furthermore, these sequences were distant from those of already known porcine herpesviruses sequences (41% homology) but somewhat closer to the sequences of gammaherpesviruses (68% homology). A study indicates the prevalence of the 2 new types of herpesviruses to be as high as 90% in domestic pigs in Germany. Based on the limited sequence information, these 2 new viruses were tentatively designated porcine lymphotropic herpesvirus types 1 and 2 (PLHV-1 and -2) (81) and were assigned to the subfamily *Gammaherpesvirinae*. These sequences were also found in wild pigs in Germany. In vitro, all members of gammaherpesviruses replicate in lymphoblastoid cells with their specificity being for either T or B lymphocytes. In vivo, the experimental host range of gammaherpesviruses is limited to the family to which the natural host belongs. Despite the identification of the specific sequences for the 2 new porcine herpesviruses and the designation as lymphotropic viruses, actual virus isolation has not yet been reported, and, therefore, their tropisms for other animal species, tissues, or lymphocytes are virtually unknown. Basic properties of the virus are yet to be delineated.

Conclusion

To date, about 25 different viruses have been identified in pigs. Most of these viruses do not cause disease in humans, with the exceptions of Nipha virus, which caused recent outbreaks in Malaysia (82), and swine hepatitis E virus (Table I, Figure 1). Viruses that cause apparent diseases in pigs are relatively easy to eliminate from donor herds and, therefore, are of less concern in xenotransplantation. Viruses that are asymptomatic in pigs and those that undergo latency are more difficult to eliminate. These viruses need to be carefully screened because organ transplantation may provide unique opportunities for 'species jumping' of viruses. The list of tests available for known viruses in pigs should be comprehensive, and the sensitivity and specificity of the diagnostic tests should be maximized. In this regard, screening for donor pigs should include both PCR and serologic assays whenever possible. Research to detect unknown viruses of potential concern in xenotransplantation should be promoted. The development and use of animal models will provide the best opportunity to understand the basis for species jumping and viral pathogenesis. Policy development will be necessary to set up an appropriate national system for screening and monitoring animal sources and recipients for known viruses, discovery of new viruses, and development of new and better diagnostic methods. Reference diagnostic laboratories need to be established for individual viruses to provide reliable screening information. At a recent workshop held in March 2000, organized by Centre for Infectious Disease Prevention and Control of Health Canada, general strategies for national surveillance and the international coordination on xenotransplantation and xenozoonosis were discussed. Draft guidelines for national registry of patients, testing infectious agents, and archiving samples from donors and recipients are anticipated. The guidelines will reassure the principles on xenogeneic safety in regards to individual and societal risks as well as benefits and future directions of xenotransplantation.

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