REVIEW

Transfusion transmitted virus: A review on its molecular characteristics and role in medicine

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Abstract

The present review gives an updated overview of transfusion transmitted virus (TTV), a novel agent, in relation to its molecular characteristics, epidemiological features, modes of transmission, tissue tropism, pathogenesis, role in various diseases and its eradication from the body. TTV, a DNA virus, is a single stranded, non-enveloped, 3.8 kb long DNA virus with a small and covalently closed circular genome comprising 3852 bases. It was tentatively designated *Circinoviridae* virus. TTV genome sequence is heterogeneous and reveals the existence of six different genotypes and several subtypes. TTV has been reported to transmit not only *via* parenteral routes, but also *via* alternate routes. This virus has been detected in different non-human primates as well. At present, TTV is detected by polymerase chain reaction (PCR) with no other available diagnostic assays. It shows its presence globally and was detected in high percent populations of healthy persons as well as in various disease groups. Initially it was supposed to have strong association with liver disease; however, there is little evidence to show its liver tropism and contribution in causing liver diseases. It shows high prevalence in hemodialysis patients, pointing towards its significance in renal diseases. In addition, TTV is associated with several infectious and non-infectious diseases. Although its exact pathogenesis is not yet clear, TTV virus possibly resides and multiplies in bone marrow cells and peripheral blood mononuclear cells (PBMCs). Recently, attempts have been made to eradicate this virus with interferon treatment. More information is still needed to extricate various mysteries related to TTV.

INTRODUCTION

Transfusion transmitted virus (TTV) is a recently discovered virus which was suspected to be a causative agent of non-A to non-E hepatitis. TTV was first identified in the serum of a patient who was hospitalized with post transfusion hepatitis of unknown etiology in 1997[1]. Initially, TTV was described as a non-enveloped, 3739 bases long and single stranded DNA virus. Based on its genomic characteristics, it was reported to be a parvovirus-like pathogen. Later, studies on the molecular and biophysical characteristics of TTV demonstrated this novel agent as a non-enveloped virus with a small, covalently closed circular genome of single stranded DNA comprising 3852 bases[2-4]. Its buoyant density is significantly different from that of parvoviruses. Also there was a significant sequence difference between TTV and members of the *Circoviridae*[5]. It was proposed that TTV belongs to a new virus family that was tentatively designated *Circinoviridae*[2] para*circoviridae*[6] or the TTV family[7] by different research groups. TTV isolates have an extremely wide range of sequence divergence[8-10] and were tentatively classified into 23 genotypes with sequence divergence of > 30% from one another[11] or into four major phylogenetic groups[7,11]. TTV like mini virus (TLMV) with 2.8-3.0 kb genomic length was also identified in humans and chimpanzees[12-14].

Since the discovery of TTV, studies have been published describing the prevalence of TTV infection in people with acute or chronic hepatitis as well as in blood donors and drug users and also in healthy persons[15-17]. It is apparent that currently it is not possible to ascribe TTV to any specific diseases. TTV can be transmitted parenterally and has been found in plasma and peripheral blood mononuclear cells. However, non-parenteral transmission is also possible as TTV can be excreted in feces[10]. Molecular and phylogenetic analysis of polymerase

chain reaction (PCR) fragments revealed that TTV could be divided into several genotypes that are found worldwide without any direct correlation with geographical distribution of diseases[2, 18-20]. This is an interesting area to investigate different aspects of TTV and several groups are working to extricate many mysteries related to this agent world over. Until now, an abundance of information has been published on TTV in relation to its molecular form and infectious status.

To have a compilation of information and also have a better understanding of TTV, the present article provides a holistic view on various characteristics of this novel agent with particular emphasis on its molecular characteristics, epidemiological features, endemic behavior and pathogenesis as well as prospects of its eradication on the basis of published information.

MOLECULAR BIOLOGY

TTV, a common virus in humans with high prevalence in the general population[21,22] is a single stranded DNA virus. Its the genome was sequenced by Okamoto *et al*[3] almost entirely on the prototype isolate TA278 encompassing 3739 nucleotides and was temporarily believed to be a linear DNA. However, later studies[2] with the GHI isolate and TA 278 isolate[4] have identified a GC-rich missing link of about 100 nucleotides that complete the TTV genome as a closed circular DNA with a length of 3852 nucleotides (nt) and a particle size of 30-50 nm. Thus, TTV is an unenveloped virus whose genome consists of a circular and single stranded DNA molecule of negative polarity and about 3.8 kb length[2]. TTV has an isopycnic density of 1.31-1.34 g/mL in CsCl[2,4]. The TTV genome has two or three possible open reading frames (ORFs) capable of encoding 770 aa (ORF1), 202 aa (ORF2) and 105 aa (ORF3) polypeptides[4]. Analysis of the TTV transcription pattern in COS-1[23] and bone marrow cells[24] has revealed the existence of at least three species of spliced mRNA molecules of 2.9-3, 1.2, and 1.0 kb in length, with common 5' and 3' termini, leading to the creation of new reading frames (ORF3 and ORF4) in addition to the previously described ORF1 and ORF2[25].

Many studies have indicated a high degree of genetic diversity of TTV. The entire genome was sequenced for SANBAN and TA 278 isolates[26]. The genetic organization of the genome was similar in two isolates: two open reading frames (ORF1 and ORF2) were sandwiched by the motifs of TATA box and polyadenylation signal, and a GC-rich short stretch resided at the midst of the untranslated region. No other ORFs longer than 300 nt and common to SANBAN and TA278 were found. The overall nucleotide sequence identity between the two isolates was 56.7%, significantly lower than that (93%) between TA278 and GH1[2]. Interestingly, the nucleotide sequence identity was relatively higher in the untranslated region (73.0%) than in the translated region (52.2%). A great degree of genetic diversity for a group of viruses represented by TTV most likely had a long history of evaluation and adaptation to humans. Despite the extensive sequence divergence of TTV and TLMV in coding regions of the genome, three areas of remarkable conservation have been identified in the part of UTR that contains promoters and splice sites[24]. Sequence conservation is found among all known human isolates of TTV and TLMV as well as those recovered from a range of non-human primates.

Based on its physico-chemical and genomic characteristics[2-4], TTV was proposed as a member of new viral family tentatively named the *Circinoviridae*[3]. Although TTV shares some features, such as a negative-stranded circular DNA genome, with members of the *Circoviridae*[1], the genetic organization of its genome is distinctly different from that of viruses that belong to this family. Therefore, TTV was tentatively classified into the virus family, *Circinoviridae*[3].

GENOTYPES

The TTV genome sequence is extremely heterogeneous. Phylogenetic analysis performed on TTV isolates recovered from several parts of the world revealed the existence of 6 different genotypes[3,27]. The sequence heterogeneity of the TTV genome, however, is more complex. One report described the identification of 16 TTV genotypes[28]. Another study identified 5 additional TTV genetic groups[10]. One of these was found to represent an additional TTV genotype, whereas the other 4 additional genetic groups were significantly distinct from TTV and from one another compared to the original TTV genotypes. This observation suggested that these 4 new genetic groups represent closely related, yet different, TTV-like viral species. The existence of genetic divergence between different TTV isolates beyond genotypes was noted by another group of researchers[8]. It has been hypothesized that a whole "swarm" of numerous TTV-like species circulates in the human population worldwide[29]. Despite this extensive sequence diversity, all variants of TTV share a common genomic organization with three predicted encoded proteins of similar length and likely function.

TTV was originally found in humans; however, recent studies showed that TTV can also be identified in serum specimens obtained from domesticated farm animals[29] and from non-human primates[29, 30]. Phylogenetic analysis using the TTV sequence obtained from animals demonstrated that these sequences belong to already known human TTV genotypes[29,30], although some sequences recovered from nonhuman primates remain unclassified[30]. The results of these experiments demonstrated that chimpanzees may be infected with some TTV-related species that have not been found in humans. An additional phylogenetic analysis using all known TTV sequences, some of which were not classified[26,30] or were classified previously as new TTV genotypes[10], suggested the existence of 16 genotypes or 13 different TTV-like species. The prototype TTV-I[8] sequences were classified into eight genotypes found in humans as well as in non-human primates and farm animals. Moreover, genetically distant variants, namely PMV, SANBAN and SEN viruses have been identified. Frequent homologous recombination, which can occur when a subject is co-infected with two or more isolates, is an important multiplier of the TTV genetic diversity. This

phylogenetic tree contains 13 major groups. Each major group represents sequences that are more distant from the other major group sequences. This observation is strongly supported by the analysis of frequency distribution of evolutionary distances. Comparison of sequences from one major group of branches to all other branches demonstrates that each of the 13 major groups of branches may represent different viral species. All of these viruses are closely related to the prototype TTV strains tentatively designated TTV-I. The other viral species identified with each major branch were also designated TTV with the addition of Roman numerals (e.g. TTV-II, TTV-III) as proposed previously[8].

All 13 major groups of branches or viral species may be arranged into four groups. Group A contains viral species I and IX; group B contains II, III, X, XI, XII, and XIII; group C contains IV and V; and group D contains VI, VII, and VIII. The TT viruses found in humans belong to groups A, B, and C. Group C is composed exclusively of viruses found in humans and group D of viruses found only in non-human primates. The primate TT viruses can be also found in groups A and B. Group A includes two viral species. One of these viruses is the prototype TTV-I, which was originally identified in a Japanese patient with parenterally transmitted hepatitis of unknown etiology[1]. Previously, seven TTV-I genotypes were identified[27,28]. However, based on phylogenetic analysis, TTV-I genotypes 2 and 3 were suggested to be combined as genotype 2/3[8]. This suggestion reduces the number of TTV-I genotypes to 6. Earlier, several new TTV variants were found in non-human primates[29,30]. The phylogenetic analysis performed in this study confirmed that sequences Bo-Ho and Bo-De identified by Vergchoor et al belonged to genotype 2/3. However, sequences Ch-Pe and Ch-Br2 constituted two new TTV-1 genotypes[29] whose sequences constituted a new major branch in the phylogenetic tree representing TTV-IX, which is different from, but closely related to the prototype TTV-I. TTV-IX detected in chimpanzees is most closely related to the TTV-I genotype 8 found only in non-human primates[30]. Recently, several new TTV variants were identified in serum specimens from healthy Japanese individuals[31]. Group B contains TTV-II, TTV-III, TTV-X, TTV-XI, TTV-XII and TTV-XIII, which can be found in non-human primates as well as in human specimens. TTV-II, TTV-III, TTV-X and TTV-XIII were found only in humans. Group C is composed of two human viruses, TTV-IV and TTV-V. Group D consists of three new chimpanzee viruses, namely TTV-VI, TTV-VII and TTV-VIII.

HUMAN TRANSMISSION

TTV was first characterized as a blood-borne virus and was thus referred to as a transfusion-transmitted virus (TTV)[3,32]. However, later studies suggested the existence of other routes of transmission also. In fact the mechanism of TTV transmission has not yet been elucidated. The higher prevalence of TTV in persons treated with blood[3] or blood products[32] has suggested parenteral transmission as a frequent route of TTV infection. TTV is common in patients who have an increased risk of infection with blood-borne viruses, such as hemophiliacs (68%), patients on maintenance hemodialysis (46%), and abusers of intravenous drugs (40%)[3]. There is a high prevalence of TTV in blood products. TTV contamination was found in 10 of 18 batches (56%) of factor VIII and IX concentrates manufactured from non-remunerated donors, and in 7 (44%) of 16 batches of commercially available products[32]. These observations suggest that TTV is transmitted by blood and blood products. However, infrequent detection of TTV-DNA in serum samples from prostitutes and homosexual men[17] and the findings of fecal[33] and bile[34] excretion of TTV indicate that TTV may have characteristics different from other blood-borne viruses. The prevalence of TTV in blood donors in different countries varies between 1.9% to 62%[3,22,27,32,35]: 1.9% in England[32], 9.1%-12.8% in United States[27], 12% in Japan[3], 36% in Thailand[35] and 62% in Brazil[22]. Moreover, the majority of TTV infected people had no history of blood and/or blood products transfusion. The relatively high prevalence of TTV in blood donors and the large proportion of TTV infected patients with no history of transfusion of blood and blood products also suggest that alternative routes of transmission of TTV infection may exist[36]. TTV DNA has also been detected in saliva[36], throat swabs[37], breast milk[38], semen[39] and vaginal fluid[40] thus, supporting routes of transmission other than blood and blood products[33,37].

Excretion of TTV in feces of infected individuals suggests of possible fecal-oral transmission[33]. Some studies have reported placental transmission of TTV[41-43], whereas others have not detected TTV in cord blood and amniotic fluid[44-45]. These studies show the absence of transuterine transmission of TTV. Since children of TTV-infected mothers apparently tend to get infected more often and earlier after birth than children of TTV negative mothers, the role of postnatal transmission of TTV is being considered. Postnatal route of transmission from mother to child and infection via frequent social contacts seem to be very important modes of transmission in children[46-48]. Furthermore, variation in the TTV prevalence in children from 5.1% in Japan[49] to 54% in the Democratic Republic of Congo[50] is also suggestive of the possible involvement of some specific environmental factors in the acquisition of TTV infection. The sexual mode of transmission is likely of low effectiveness[51].

ANIMAL TRANSMISSION

Infection with both TTV and TLMV has been detected in various non-human primates[2,52,53]. At the same time, there are reports showing cross-species transmission of TTV genotypes. Human TTV variants can infect chimpanzees and macaques[13,54]. Beyond primates, host range of TTV and TLMV is uncertain. One study has demonstrated frequent TTV infection of domestic animals such as cows, pigs, sheep and chickens[47]. However, it is not known how these species acquire TTV infection. Recently, highly divergent TTV like viruses were detected in pigs, cats and dogs, distinct from those found using the N22 primers[55] suggesting that this virus family may indeed be widely distributed in the mammalian order. There are reports showing high prevalence of TTV infection in captured

chimpanzees and crab eating macaques[56]. These findings suggest that TTV is widespread among wild Chimpanzees living in West Africa. However, this TTV infection was found non pathogenic. Based on analysis of full-length sequence data, this TTV may represent a new TTV-like viral species or genus, although it is closely related to human TTV[56].

TARGET ORGANS

Regarding target organs for TTV infection, TTV-DNA has been detected by both PCR and in situ hybridization in liver and peripheral blood mononuclear cells (PBMC)[57,58]. However, these studies have shown that TTV replicates in liver, but not in PBMC. Simultaneously, TTV was detected and found with replicative intermediates in bone marrow cells from TTV infected patients[24]. This finding and few other studies[59] indicate that TTV-DNA in PBMC corresponds to viral particles passively attached to cell membrane and TTV infects hematopoietic cells but only replicate when these cells are activated. Since the percentage of circulating activated cells is very low, this may be the cause for the lack of detection of TTV replicative intermediates in freshly isolated PBMC from TTV infected patients.

DIAGNOSTIC ASSAYS

The development of sensitive and reliable polymerase chain reaction (PCR) protocols allowed the detection of TTV DNA at a very high prevalence in sera of healthy populations around the world[21,22,29]. Currently, the heteroduplex mobility assay to detect multiple infections with isolates of TTV belonging to different genotypes or subtypes has also been developed. In the simplest application of heteroduplex mobility assay, heteroduplexes are formed by denaturing and reannealing mixtures of PCR amplified DNA fragments from divergent isolates of the same virus. When these products are separated on polyacrylamide gels a homoduplex band plus two slow moving heteroduplex bands are observed. The mobility of heteroduplexes is related to the genetic distance between two strands. This technique was applied earlier to HIV isolates, measles virus, CMV and hepatitis C virus. While detecting TTV DNA by PCR, it was found that repeated freezing and thawing of serum did not have much effect on stability of TTV DNA. Specimens are not required to be aliquoted for repeated testing and retrospective studies[60].

Both TTV and TLMV have sequence heterogeneity. Some TTV subtypes have less than 50% sequence identity. However, there are certain conserved regions. Primers were designed in such a way that most of the subtypes could be detected[61]. Recently, real time PCR based methods with either SYBR Green or TaqMan Probe, designed to quantitate selectively TTV and TLMV, have also been used[61].

EPIDEMIOLOGY

Epidemiological studies have shown that TTV is described worldwide in various populations. The prevalence of TTV viremia in healthy adults of developed countries is in the range of 1%-34%. Prevalence reported from third world countries was found to be higher, typically 40%-70%. In people who have received multiple blood transfusions the virus is almost universally present with more than one subtype in each individual. Table 1 demonstrates the countrywide prevalence of TTV infection in different categories of populations including both healthy persons and patients with various types of diseases[62-75].

TTV INFECTION IN LIVER DISEASES

From preliminary reports two characteristics of TTV infection have emerged rendering it as a potential cause of liver disease. First, Okamoto *et al*[3] demonstrated that TTV-DNA levels in liver tissue were equal to or 10-100 times higher than those in serum, suggesting that this virus replicated in the liver. Second, Nishizawa *et al*[1] reported the appearance of TTV-DNA in the sera of patients with post transfusion hepatitis of unknown etiology to display close correlation with ALT levels. Neither one of these characteristics, hepatotropism or correlation of viral titres with serum ALT, had previously been demonstrated for HGV. However, most subsequent investigations could not confirm their significance in the development of fulminant hepatitis, cryptogenic chronic liver disease and HCC. In addition, the implications of coinfection with TTV in the natural history of chronic HBV or HCV infection are also far from clear. Although TTV can be transmitted by parenteral route, its role in causing posttransfusion hepatitis has not been established[76-79]. The majority of individuals who become TTV-DNA-positive after blood transfusion usually have normal ALT and do not develop chronic hepatitis, although TTV viremia frequently persists for several years. Patients who develop chronic hepatitis are invariably coinfected with HBV or HCV and chronic hepatitis is closely correlated with HBV or HCV infection. This raises the possibility that TTV is merely an innocent bystander rather than a primary hepatitis virus.

In one of the studies[79], the rate of TTV infections was found to be significantly higher among transfused than among non-transfused patients (26.4% and 4.7%, respectively) and the risk of infection increased with the number of units transfused. The rate of TTV infections with non A-E hepatitis (23.2%) was almost identical to the rate among patients who had been transfused, but did not develop hepatitis (21.8%). Of those patients with acute hepatitis C, 40.0% were simultaneously infected with TTV and TTV did not worsen either biochemical severity or persistence of hepatitis C. In non-A-E cases, the mean ALT was comparable among those positive for TTV and those negative. Neither was there a consistent relationship between ALT and TTV-DNA level among these patients[80].

The role of TTV in acute hepatitis is another unresolved issue. In two Japanese studies[81,82] TTV-DNA was identified in 13.6%-43% of cases of non-A-E community-acquired acute hepatitis. However, these positive rates of TTV do not differ statistically from either those obtained among patients with other types of viral

Table 1 Global prevalence of TTV infection in normal subjects and patient populations

S.No.	Country	Group	No. tested	No. Positive (%)	Reference No.
1	Italy	Patients with different clinical diagnosis			62
		Unselected pathologies	221	110 (50)	
		Hemophillia A	33	24 (73)	
		Hemodialysis	36	19 (53)	
		HCV positive patients			
		Normal ALT	30	17 (57)	
		Abnormal ALT	50	24 (48)	
		Cirrhosis	30	9 (30)	
		HCC	13	8 (62)	
		HCV negative patients			
		Non-A non G hepatitis	23	11 (48)	
		Autoimmune hepatitis	11	4 (36)	
		Primary liver diseases	17	11 (65)	
		Cryptogenic extrahepatic diseases			
		Systemic lupus erythematosus (SLE)	34	19 (56)	
		Psoriasis	102	56 (55)	
		Rheumatoid arthritis	60	17 (28)	
2	Italy	Healthy blood donors	100	22 (22)	63
		Hemophiliacs	178	123 (69)	
3	Italy	Patients			64
		HIV I infected mothers	83	29 (34.9)	
		- Intravenous drug users	46	21 (45.6)	
		- Non-intravenous drug users	37	8 (21.6)	
		Uninfected			
		- Infants born to TTV	29	8 (27.5)	
		infected mothers			
4	Italy	HIV Negative			65
		- Blood donors	104	91 (87.5)	
		- Chronic hepatitis C	106	99 (93.4)	
		- Hemodialysis patients	100	100 (100)	
		- Thalassemic patients	36	36 (100)	
		- IVDUs	37	31 (83.8)	
		HIV Positive			
		- IVDUs	102	102 (100)	
		- Homosexuals	58	52 (89.7)	
		- Heterosexuals	50	44 (88.0)	
5	Italy	Haemophiliacs	217	204 (94)	66
6	China	Healthy persons	136	29 (21.3)	67
		Prostitutes	140	46 (32.9)	
7	China	Intravenous drug users	50	14 (28)	68
		- Hemophilics	50	35 (70)	
		- Thalassemics	40	27 (67.5)	
		- Hemodialysis patients	50	13 (26)	
		Household contacts			
		- Spouse	40	3 (7.5)	
		- Non spouse	57	7 (12.3)	
		Acute hepatitis A	52	4 (7.7)	
		Non A-E hepatitis			
		- Acute	12	5 (41.6)	
		- Chronic	9	2 (22.2)	
		- Fulminant	11	5 (45.4)	
		Hepatitis B carriers	200	30 (15)	
		Hepatitis C carriers	100	36 (36)	
		Healthy adults	100	10 (10)	
8	China				69
		Healthy children	122	33 (2.7)	
		Non A-E hepatitis	19	8 (42.1)	
		- Acute	13	6 (46.1)	
		- Chronic	3	1 (33.3)	
		- Fulminant	3	1 (33.3)	
		Thalassemic children	64	47 (73.4)	

S.No.	Country	Group	No. tested	No. Positive (%)	Reference No.
		- Transfused during cardiac surgery	80	37 (46.3)	
		- Chronic HBV carrier	30	10 (33.3)	
		- Biliary atresia	32	5 (15.6)	
9	USA (Minnesota)	Healthy donors with elevated ALT	99	5 (5)	70
		Healthy donors with normal ALT	146	1 (0.7)	
10	Japan	Patients with chronic liver disease of unknown etiology	69	57 (83)	71
		Volunteer blood donors	50	40 (80)	
11	Tanzania	Rural women	156	115 (74)	72
12	India	Sewage water	63	8 (12.7)	73
13	Brazil	Patients sera	184	48 (26)	74
		Patients saliva	167	49 (46)	
14	Norway	Blood donors	201	180 (98.6)	75

HCC: Hepatocellular carcinoma; IVDUs: Intravenous drug use.

hepatitis or among healthy volunteers. In addition, the ALT levels do not show any difference between TTV-positive and TTV-negative patients. Furthermore, the presence of TTV infection had no apparent effect on the clinical course of patients with hepatitis A, B or C. Thus, according to these studies no correlation appears to exist between TTV infection and the clinical features of sporadic hepatitis. Contrasting another Japanese study[83], TTV-DNA was detected in 2 out of 7 (29%) patients with acute hepatitis of unknown etiology, but in none of the 4 patients with acute HCV-associated hepatitis. At least half of all cases of fulminant hepatitis are seronegative for hepatitis A-E viruses. TTV has been fond in 27%-50% of patients with fulminant hepatitis[3,16,84], but such patients probably received multiple transfusion before testing and recent TTV infection has not always been established. Therefore, it is unclear whether TTV infection is secondary to transfusion or plays an etiologic role in fulminant hepatitis.

Current data suggest that TTV is not the causative agent of chronic liver disease of unknown etiology and neither does it affect the degree of liver damage when present as a coinfection with HBV or HCV[16,77,84-86]. According to our previous study[86], for example, TTV-DNA was detected in 20% of the HBV-positive and 19.5% of the HCV-positive chronic liver disease patients, in 8.3% of seronegative chronic liver disease patients, in 8.3% of seronegative chronic hepatitis/cirrhosis patients and 7% blood donors. Yet, no significant differences between TTV infected and non-infected patients were found as to demographic data, assumed source of infection, biochemical abnormalities, or severity of liver histology. Thus, regarding etiology and progression towards serious chronic liver disease, its contribution seems to be minor if not altogether non-existent. Concerning antiviral therapy, there are no data or treatment of patients who are infected with TTV alone since the role of TTV as a cause of chronic hepatitis has yet to be determined. Studies of patients infected with both HCV and TTV who were treated with interferon showed that the responsiveness to therapy was correlated with HCV alone. In addition, certain genotypes of TTV were quite resistant to interferon although interferon effectively reduced HCV in the same patients[87].

Because the prevalence of TTV is high among patients with chronic viral hepatitis and cryptogenic liver disease, a similar situation has been anticipated to persist among patients with HCC. However, the prevalence of TTV in Thai patients with HCC has shown a wide range of divergence, for example, 6%-60% and 5%-50% in cases with HBV and HCV markers respectively, and 1%-67% in cases without HBV and HCV markers[27,88]. Our study demonstrated the majority of TTV infected HCC to harbor double or triple infections with HBV and/or HCV and the prevalence of TTV infection was comparable to that of healthy volunteers[89]. Contrasting that, another group reported TTV-DNA to occur more frequently in patients with liver cirrhosis and HCC than in those with chronic hepatitis[27]. Using a case-control study to compare the prevalence of 174 Italian patients and matched controls, it was demonstrated that individuals infected with TTV did not exhibit an increased relative risk for developing HCC[90]. Furthermore, it has been demonstrated that the TTV genome is not found integrated into host hepatocyte DNA[91], the one process that might represent a potential risk factor in the development of HCC.

TTV INFECTION IN RENAL DISEASES

Using the polymerase chain reaction (PCR), epidemiological studies have indicated a worldwide distribution of this virus, with prevalence surveys in the general population reporting values of 12% to 19% in Japan[3,92], 36% in Thailand[27], 2% to 10% in European countries[32,85] and 1% in the USA[16]. In patients on maintenance hemodialysis (HD), who are at an increased risk of parenterally transmitted hepatitis virus infection, a high prevalence (32%-53%) of TTV infection has been reported[93,94]. However, the transmission route of the virus is still unknown and the question of any association between duration of HD or previous transfusion and TTV infection is still a matter of controversy[77,95]. There is also little information about the occupational risk of TTV infection in HD unit workers. By using logistic regression analysis, it was shown that a prior blood transfusion and time on HD were not predictors of the presence of TTV-

DNA, so that TTV may have a transmission route not shared by HBV, HCV or HGV. The possibility of TTV transmission, *via* a nosocomial route in HD units, must be considered[77]. One of the possible routes of transmission in an HD unit is direct from person to person. For this reason, the healthcare staff in the HD unit was suspected as being at high risk for TTV infection. Although a low risk of TTV infection was suggested in hospital staff, there is little information on HD unit workers[96]. TTV infection rate was not influenced by age, sex, or mean duration of dialysis[77,79,97]. Nosocomial transmission may account for TTV infection in some patients on hemodialysis[98].

TTV-DNA genotype 1 (G1) was found to be the main TTV DNA genotype in hemodialysis patients. The fact that hemodialysis patients are polytransfused makes it likely that they are at risk of multiple exposures. Therefore, it was interesting that a significant number of patients were apparently coinfected with different strains of TTV. Sequence analysis of clones from two patients with apparent mixed infections showed that TTV strains belonging to two different major genotypes could coexist in a single patient. It was suggested that infection with one TTV type does not protect against infection with another TTV type[99]. The preliminary data suggested that TTV is transmitted mainly *via* a parenteral route[3,85]. When TTV infection was studied in hemodialysis patients who were monitored for HCV infection, co-infection was found in 48% at enrollment. The follow-up of the renal transplant patients revealed, that the persistence of single TTV variants over a long period after organ transplant was common. Considering the heterogeneity of TTV isolates, this finding is against frequent infection with different nucleotide sequences and so horizontal spread of certain variants could have been expected. Nevertheless, in the examined patient group, permanent infections, with only single nucleotide changes in the consecutive samples of the same patient could be observed and the TTV variant detected in one patient was usually remotely related to the TTV variants infecting the others with 58%-97% nucleotide sequence identity between the variants. As a highly variable region of the TTV genome, the N22 region is a candidate to carry humoral epitopes on the surface of the virions. Mutations in this region were, however, infrequently detected. The host's immunity is a plausible evolutionary driving force of the development of TTV genotypes and variants, which process affects most probably the hypervariable genomic regions. If so, the iatrogenic immunosuppression of the transplant recipients can contribute to the long persistence of one variant, while novel infections are infrequent[100].

TTV COINFECTION

Accumulating molecular and clinical evidence indicated that the effects of HIV infection can be modified by coinfection with other viruses[101-104]. However, limited information is available about the prevalence and possible pathogenic role of TTV in HIV infected patients with or without AIDS in relation to specific risk factors, and about the viral titres of TTV. Puig-Basagoiti *et al*[105] reported no influence of TTV infection on CD4 T cell counts

and clinical or immune status in HIV-infected patients. In a recent study by Martinez *et al*[106], no relationship was found between TTV DNA detection and HIV category, CD4 count, HBV and HCV infection or demography features. By contrast, Christensen *et al*[101] reported correlation between a low CD4 T cell count and high TTV titre in Danish patients with HIV infection as well as a possible prognostic significance of TTV viral load in immunocompromised patients. High TTV viremia levels were found to be associated with decreased survival rates. A significantly higher rate of TTV positivity was noted in HIV-infected patients than in HIV-negative healthy individuals by two distinct PCR methods. Although the detectability of TTV by either of the two PCR features was as in previous studies[105,106], the relative rate of TTV DNA in the patients studied was found to be associated with the HIV viral load and CD4 cell level as well as the development of AIDS.

A series of recent reports has indicated that viral infections can influence the pattern of autoantibody expression in patients with autoimmune diseases. Neidhart and coworkers[107] demonstrated differences in the autoantibody pattern of patients with SSc with antibodies to cytomegalovirus. Other investigations showed a significantly lower prevalence of rheumatoid factors in RA patients infected with TTV, in comparison with non-infected patients. Hajeer *et al*[108] reported a negative correlation between anti-parvovirus B19 antibodies and rheumatoid factors in patients with RA. In several studies, the autoantibody pattern found in patients with SSc was found to be similar[109-111]. Comparison of the autoantibody patterns in virus infected and non-infected patients with SSc showed that continuing GBV-C or TTV infection or both, have no evident effect on the manifestation of autoantibodies. In conclusion, various reports showed neither a higher prevalence of GBV-C RNA and/or TTV DNA, nor changes in the pattern of expression of autoantibodies in patients with SSc. Therefore, these data provided no evidence for an association between GBV-C and/or TTV infections and SSc.

Not only the clinical significance and the pathogenesis of TTV infection but also the association between TTV infection and raised ALT values have been controversial[1,3,113]. It was found that the raised ALT values were independently related to TTV viraemia among Taiwanese who were not infected with HBV and HCV. TTV infection seemed to be responsible for raised ALT values and to hint positive hepatopathic effects. Tuveri *et al* suggested that TTV might be implicated in a few cases of acute and chronic non A-non G hepatitis[113]. However, Nakano *et al* reported that TTV was not the main causative agent of cryptogenic liver disease[114]. Further efforts at confirming the pathogenecity of hepatocyte damage by TTV are necessary. As for direct correlations between TTV and *H pylori* infection, TTV DNA was detected at a similar rate in patients with and without *H pylori* infection, and *H pylori* infection was detected at a similar rate in patients with and without TTV infection. Similarities of prevalence of TTV between patients with and without infection by *H pylori,* and prevalence of *H pylori* between patients with and without infection by TTV, as well as the discrepancy in

age distributions between prevalence of TTV and *H pylori* in our patients with peptic ulcer disease indicate that no correlation exists between TTV and *H pylori* infection, even though the two agents have similar age distributions in the general population and similar routes of transmission have been suggested for the two agents[115]. According to one report, the high prevalence of genogroup 1 TT virus infection in patients with laryngeal cancer and its striking co-prevalence with human papilloma virus infection is biologically important in the progression of squamous cell carcinoma of the larynx[116].

TTV might replicate in the respiratory tract[117]. Also, although we found no evidence that TTV might be the direct cause of ARD, TTV loads in both nasal swabs and plasma samples were substantially higher in subjects with bronchopneumonia (BP) than in the subjects with milder ARD (laryngitis, bronchitis, and bronchiolitis), suggesting among other possibilities that TTV could be locally or systemically immunosuppressive and aggravate disease induced by other agents[117]. However, there is no information on this matter except for recent report showing an inverse relationship between TTV burdens and CD4 cell counts in patients with human immunodeficiency virus type[101,118,119].

PATHOGENESIS

Although liver tropism has been suggested, TTV also has been found in other organs including kidneys, prostate, mammary glands, brain, bone marrow cells (BMCs) and peripheral blood mononuclear cells (PBMCs)[3,59,120,121]. Although it is not known precisely in which cell(s) TTV replicates, TTV DNA has been detected frequently in the PBMCs[58,120] and it has also been suggested to infect and replicate in hematopoietic cells in the bone marrow[122,123]. Earlier reports had revealed that there was a higher TTV genome load in the PBMCs of cancer patients than in healthy controls (blood donors)[124]. This could have been related to immune abnormality in cancer patients when compared with the controls, thereby allowing increased TTV replication in the former.

It was demonstrated that TTV is present in the nucleus and cytoplasm of some of the PBMCs. It is possible that infection of immune cells could facilitate escape of the virus from the immune response. Concealed as a "Trojan horse", TTV in PBMCs might serve as a reservoir of TTV for chronicity of the infection and transmission in some clinical and epidemiological settings. The observation that TTV-negative PBMCs bound considerable virus *in vitro* suggests that at least some TTV found associated with *ex vivo* derived PBMCs might be of plasma origin rather than produced by the cells themselves. We have, however, obtained evidence indicating that PHA-stimulated PBMCs support TTV replication *in vitro* and release substantial titres of virus into the culture fluid. TTV-related single-stranded DNA viruses such as circoviruses and parvoviruses require actively multiplying cells for productive replication[125,126]. That proliferating hematopoietic cells might be an important source for the TTV that circulates in infected individuals is suggested by findings showing that baseline TTV viremia decreased markedly in virus-positive prospective bone marrow transplant recipients after myelosuppression with cyclophosphamide and total body irradiation[122]. Whether TTV is also dependent on cell cycling for active replication remains to be formally established.

It is well known that some viruses can be activated by immune stimulation, most notably HIV, which requires lymphocyte activation for optimum replication[127,128]. In this regard, it has been shown recently that an animal circovirus related to TTV, porcine circovirus type 2 (PCV-2), can replicate to higher levels in piglets as a result of immunization with a bacterial porcine vaccine[129]. One major distinction between PCV-2 stimulated in this study and TTV is that the porcine virus is capable of causing a wasting syndrome and may do so more effectively following immune stimulation. In addition, immune suppression due to drugs or induced stress resulted in a transient increase in TTV titers in humans[130,131]. This indicated that resting PBMCs could not produce TTV, but that mitogen activated cells could be stimulated in vitro to replicate TTV. Thus, there is a suggestion that TTV levels may be related to the state of activation of the host immune system as postulated in an earlier publication[132].

Recently, replicative circular double-stranded intermediates of TTV DNA have been reported in liver and BMC[24,133]. TTV DNA has been detected by in situ hybridization in nuclei of hepatocytes from experimentally infected rhesus monkeys, indicating that TTV truly infects hepatocytes[134]. However, TTV DNA titers in sera of TTV-infected patients decreased to undetectable levels during immunosuppression following bone marrow transplantation[122]. Moreover, liver cells have been found to contain only TTV DNA and not mRNA[123], suggesting that TTV replicates in hematopoietic cells rather than liver. Southern blot analysis argues against integration of the TTV genome into the genomes of human hematopoietic tumor cells, obtained from bone marrow aspirates, lymph nodes, or human hepatocellular carcinoma[91,135]. Although, TTV DNA is frequently detected in PBMC of infected individuals[58,120], double-stranded replicative intermediates have not been detected in PBMC.

TTV, a new member of the *Circoviridae* family, has not been cultured *in vitro* and its pathogenic potential is still not clear. A cell line for isolating and cultivating TTV will significantly accelerate the research on TTV. Human lymphoblastoid cells, particularly B cells, transformed with oncovirus such as Epstein-Barr virus, may be useful for culturing TTV. Because DNA viruses are often found integrated into host genomic DNA as has been reported for hepatitis B virus[136-139], the possible integration of TTV DNA into the genome of hepatic cells was investigated. When a 2.2 kb TTV probe was used in a Southern blot analysis of liver genomic DNA, no signal was obtained. Thus, TTV DNA was not found to be integrated into hepatocyte chromosomes, and the liver apparently was not the site of TTV replication for this particular case. Similarly, Yamamoto *et al*[91] have reported the absence of viral replication in hepatocytes of TTV infected cases of hepatocellular carcinoma. These data, taken together, suggest that the site of TTV replication occurs in the bone marrow rather than in the hepatocytes, and that TTV

infection was the cause of the aplastic anemia. Similar findings suggesting TTV replication in bone marrow have been obtained by other researchers[88].

ANTI VIRAL THERAPY

After IFN-alpha administration with a regimen of 6 MU thrice a week for 24 wk followed by 3 MU thrice a week for 12 wk, 24 of 50 (48%) concurrent TTV-infected patients achieved complete clearance of TTV DNA 6 mo after the cessation of therapy, with 7%-8% TTV spontaneous clearance rate reported annually in previous reports[77]. Other studies indicated that IFN-alpha has a potential antiviral effect on TTV. In previous studies, IFN therapy was effective against TTV with an eradication rate of 45%-55%[140,141]. Transient disappearance of TTV viremia during IFN-alpha therapy was observed in some reports, demonstrating the direct antiviral effects of IFN-alpha on the suppression of TTV. Nevertheless, delayed TTV clearance (TTV DNA positive at E/T and negative after cessation of therapy) that had not been reported previously occurred in few patients. Delayed complete virological response was observed in chronic hepatitis B patients after the end of therapy with Thymosin alpha l[142] or IFN[143] that revealed immune modulation effects. Since TTV is a DNA virus as HBV, delayed clearance of TTV after IFN therapy may indicate that immune modulation plays an important role. The findings in the present study implied that both antiviral effects and immunomodulatory actions of IFN-alpha are important on the eradication of TTV. Further studies are needed to investigate and clarify the actual mechanism of responsiveness of TTV to IFN-alpha.

In evaluating the clinical characteristics and virological features related to clearance of TTV after IFN-alpha therapy, the viral clearance at the E/T was the only important factor associated with clearance of TTV viremia. Neither the pretreatment ALT levels nor the histopathology were predictors for TTV clearance. Besides, there was no correlation between response of HCV and TTV. All the results may indicate the difference in virologic kinetics and mechanism of IFN effects between TTV and HCV that influence the response and resistance to IFN-alpha. The ALT levels at E/T or 6 mo after cessation of treatment were not related to TTV but HCV viremia also denied the hepatopathic effects of TTV infection.

OUTLOOK ON TTV

From the compilation of published reports on this newly characterized virus and its global status, it is evident that TTV is prevalent in several countries of the world. As such, it is not involved in causation of a serious problem in the body and simply acts as a bystander without much impact of its single or co-infection with other viruses. Of course, attempts are still going on to find out exact clinical implications of TTV infection. Much is already known about the molecular biology of the virus, yet there still remains a need to develop simple techniques based on molecular and immunodiagnostics to diagnose TTV infection in all categories of laboratories. This will facilitate studies on TTV in more detail and at several places to unravel mysteries related to this infection.

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