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Can a Torque Teno Virus (TTV) Be a Naked DNA Particle Without a Virion Structure?

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INTRODUCTION

Torque teno virus (TTV) is a single-stranded, circular DNA virus, named after a patient (TT), formerly known as transfusion-transmitted virus (TTV), and the naming was just an accidental coincidence. The first TTV genome was detected by Nishizawa et al. in human serum (1). Due to the presence of TTV DNA in the fraction of 1.26 g/cm^3 at the sucrose density gradient, DNase I did not digest this; therefore, it was considered a virus (1). Subsequently, numerous TTV and TTV-related genomes were detected in humans and animals (2–4). According to the ICTV 2020 report, the *Anelloviridae* family contains 31 genera and 155 species. Among these 31 genera, the genus *Alphatorquevirus* has 26 species; other genera include *Betatorquevirus* (TTMV [torque teno mini virus] with 38 species) and *Gammatorquevirus* (TTMDV [torque teno midi virus] with 15 species), which contain TTV that could infect most humans. The TTV infects almost all humans globally with a high prevalence rate/omnipresence (2–5), co-infection of different genotypes of TTVs in an individual is also documented (6, 7), and it could persist in the infected individual for a long time (8). Furthermore, the TTV DNA has been detected in blood, semen, breast milk, saliva, nasal secretions, tears, bile, urine, and feces (3–5). The TTV is likely to transmit through blood, semen, breast milk, saliva, and feces (9–12). Next, the TTV DNA levels in the blood are considered a potential marker for immunological status in solid organ transplantation and immunosuppression drug treatment (13–15). The correlation of TTV DNA levels in pathological conditions like hepatitis, gastroenteritis, periodontitis, multiple sclerosis, and cancer was also documented (16–19). However, the specific pathogenesis of TTV has not been fully established, and it is also believed that it may be a virus that benefits humans (5). Interestingly, the TTV genome in the water is considered an indicator of viral contamination due to its high prevalence in water bodies (20, 21).

ATTEMPT TO VISUALIZE TTV ON ELECTRON MICROSCOPES

Only two attempts have been made to show TTV on electron microscopes so far. HIV-infected patients with a TTV DNA titer of 10^8 copies/ml in the serum samples were used in the first attempt. TTV DNA-rich fractions of 1.31–1.33 g/cm^3 were subjected to electron microscopy and visualized as spherical, virus-like structures with a diameter of 30–32 nm (9). Next, to increase the specificity, immunogold electron microscope analysis for the TTV-positive serum samples was conducted using gold-labeled goat anti-human IgG to demonstrate the TTV (9). For fecal specimens, fractions of human plasma γ-globulin with or without antibodies against TTV were used to prove the virus structure in immunogold electron microscope analysis (9). Although immunogold electron microscope analysis showed possible TTV particles in serum and fecal samples,

some questions remain unanswered. This raises the question of whether the serum fractions of an HIV-infected patient contain only TTV viruses at 1.31–1.33 g/cm^3 with 30–32 nm size and whether there are only TTV antibodies in human serum in this condition. Also, some other viruses with 30–32 nm in the stool sample and their antibodies may have been present in the serum. However, the authors also discussed visualizing probable TTV particles in serum samples and fecal supernatant. Furthermore, they recommended developing a culture system capable of supporting the productive infection of TTV (9). In the second attempt, the TTV-like electron microscopic structure in the cell culture model was demonstrated by the transformation of TTV molecular clones (22). Furthermore, the viral TTV DNA yield was reduced in the subsequent passage of this cell culture supernatant in the TTV molecular clones-based studies (23). However, the authors mentioned the electron microscopic structure as a TTV-like particle (22). Overall, TTV's electron microscopic structure has not yet appeared at the most precise level of hard work and standardization. This raises the question of whether TTV can create a virion structure.

ATTEMPT TO ISOLATE INFECTIOUS TTV

Several research groups attempted to isolate the virus from clinical samples in the cell culture system. The replication of the TTV in liver cells (24) and bone marrow cells (BMCs) collected from clinical specimens (25) was documented. Furthermore, upon stimulation, the replication and release of the TTV in peripheral blood mononuclear cells (PBMCs) collected from clinical samples have been established in the cell culture system (26). The reinfectious nature of supernatants collected from infected PBMCs in healthy PBMCs was also documented (26). However, this infectious nature has only been shown on a single passage level. It has also been observed that TTV in the serum infects the Chang liver and Raji cell lines, thereby releasing TTV DNA into the supernatant (27). But they used infected liver cells for serial passage, and assays using Raji cells showed reinfection in only one passage on cell lines (27). TTV replication did not cooperate best with primary cells at expected levels, so TTV molecular clones were used to conduct molecular and cell biological studies (28). Furthermore, different studies tried to obtain the infectious virus by constructing a full-length TTV clone of different TTV genotypes in several cells and varieties of conditions (22, 23, 29–31). These studies reported successful transfection of the TTV molecular clones and confirmed TTV replication and TTV DNA release in the supernatant. Unfortunately, they could not come up with the infectious virus progeny to infect the healthy cells in continuous passaging (22, 23, 29–31). A few studies have shown that TTV DNA levels decrease with each subsequent passage (23, 29), and some studies have not reported a description of the sequence of successive passages (30, 31). Despite more than 20 years of hard work and the efforts of many research groups to isolate infectious TTV from various cells, it is evident that no one has yet been able to isolate infectious TTV and that no one has demonstrated a scientifically proven electron microscope structure. Some research groups have published what they have tried. But we hope that hundreds of researchers have stopped this study at the stage of DNA detection and sequencing due to the inability to isolate the virus. Similarly, we believe that most TTV pathology studies focus on correlation studies due to a lack of virus isolation. All of these raise the great debate of whether TTV can form the virion structure or whether they are just TTV DNA molecules.

TTV DNA WITHIN THE EXOSOMES

On the other hand, recently, it has been reported that TTV DNA is present in human plasma exosomes (32). Though the electron microscopic structure resembling a TTV-like size inside the exosomes was also demonstrated, the authors are unsure whether it is the TTV particle or any other particles (32). However, it has been scientifically proven that exosomes contain TTV DNA and are not affected by DNAse treatment (32). Furthermore, exosomes have also been found in sucrose density gradients that are almost identical to the sucrose density gradients in which TTV's DNA was detected (33–35). It is well known that the DNA inside the exosomes cannot be digested by DNases (36, 37). Interestingly, both the exosomes and TTV genomes share a common biological environment: blood, semen, breast milk, saliva, nasal secretions, tears, bile, urine, and feces (34, 35). TTV DNA is called a virus because of its presence in a virus-specific sucrose density and because it is not digestible by DNases (1). From these, the question arises as to whether the DNA of TTV is still believed to be a virus because the DNA of TTV within the exosomes is also at a sucrose density gradient and DNases do not digest it.

POSSIBILITIES FOR TTV TO BE A NAKED DNA PARTICLE

The following is a list of possibilities for TTV to be a naked DNA particle that does not have a virion structure or cannot produce a virus structure protein. (i) So far, no one has successfully demonstrated the virion structure of TTV on electron microscopy with strong proven evidence. (ii) Although TTV is found in almost all humans and is ubiquitous, no one has yet been able to isolate a continuous infective virus progeny. (iii) It was also believed that the virus contained structural protein because DNases could not digest from a virus-specific sucrose density gradient. At the same time, the TTV DNA inside the exosomes at the virus-specific sucrose density gradient may not have been digested by the DNases. (iv) Direct clinical samples of BMCs and stimulated PBMCs have been shown to replicate TTV and release TTV DNA. Still, there is no evidence to isolate a continuous, infectious virus progeny. (v) The TTV in the clinical serum samples can also be shown to infect cell lines and release the TTV DNA, which is well documented. Still, there is no evidence to isolate a cell-free virus progeny to infect several subsequent passages. (v) TTV molecular clone-based transfection showed

successful replication within cells and the release of TTV DNA into the supernatant. However, the subsequent viral DNA load was reduced from passage to passage. From all of the above, it is well established that TTV replicates in infected human cells and releases TTV DNA. Yet, the question of the release of the infectious virion with the virus structure protein remains unanswered.

Overall, we believe that TTV is likely to be a naked DNA particle that cannot produce viral structure protein. TTV may be a virus that cannot make capsids, such as the recently classified families *Endornaviridae* (38), *Hypoviridae* (39), and *Narnaviridae* (40) viruses, or it may be like the *Amalgaviridae* virus that is capable of encrypting the capsid gene but not capable of producing virions (40, 41). The putative capsid protein of *Amalgaviridae* viruses with double-stranded (ds) RNA genomes is homologous with the nucleocapsid protein of negative-stranded RNA viruses of the genera *Phlebovirus* (*Bunyaviridae*) and *Tenuivirus* (41). However, it has been reported that *Amalgaviridae* viruses cannot produce virions (40, 41). Similarly, since the ORF1 N-terminal of TTV has arginine-rich regions similar to the capsid proteins of circoviruses viruses, it is believed that the ORF1 protein of TTV may be the capsid protein (5, 42). It is noted that the replication-competent circular DNA (rccDNA) includes the bovine meat and milk factors (BMMF) and sphinx infective DNA molecules and that capsid proteins do not form fully mature viral structural proteins and only remain as infective and replicative circular ssDNA molecules (43–45). Capsid protein (virion) in viruses acts as a physical barrier to protect the virus genome, recognizes the host cell receptors and entry, causes systemic infections in the host, and facilitates transmission (40). Interestingly, the viruses without capsid (virion) such as the *Endornaviridae*, *Hypoviridae*, *Narnaviridae*, and *Amalgaviridae* families of viruses are transmitted through pollen grains, gametes, seeds, spores, and hyphal anastomosis, and they are often not associated with a significant pathological condition; furthermore, they can persist for a long time in the host (38, 39, 46). However, the cell-free transmission of these viruses is not fully established (38, 39, 46). Similarly, TTV is also present globally with a high prevalence rate, co-infection of multiple genotypes in an individual occurs, and the virus persists in an individual for a long time (2–4, 6, 8). Moreover, the cell-free transmission of TTV has also not yet been proved. However, TTV-infected cell culture supernatant has been shown to establish infection for very few serial passages (23, 26, 27, 29). From these findings, it can be inferred that TTV may have established infection through exosomes in the cell culture supernatant. Although the molecular mechanisms have not been fully identified, the potential for DNA viruses such as herpes simplex type 1, human herpesvirus 6, Epstein–Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (47); adenovirus (48); and RNA viruses such as hepatitis C virus (49), dengue virus (50), Zika virus (51), Seneca valley virus (52), and respiratory syncytial virus (53), to become intercellularly transmitted/spread through exosomes has been established. It has also been found that the rice dwarf virus (54), tick-borne Langat virus (55), and dengue virus (56) are transmitted from insects to plant phloem/human cells through the exosomes of insects. Since TTV DNA is present in human plasma exosomes (32), it is speculated that TTV may be able to spread intercellularly through these exosomes and that it may be transmitted from one human to another through these exosomes during blood transfusion. However, in-depth future studies are needed to prove the exosomes-mediated spread/transmission of TTV. The fact that TTV can express virus-specific proteins (57–60) and that antibodies against the virus-specific proteins expressed are detected in the human serum (57, 59, 61–63) confirms that TTV is a foreign material (virus). Since the detection of TTV's DNA in biological specimens such as serum (9, 64–67), saliva (64, 66, 67), umbilical cord blood (64, 65), breast milk (64, 65), semen (66), feces (9), and nasal secretions (11), it can be assumed that TTV is transmitted through these biological specimens, although the extent to which biological specimens can cause infections is not widely known.

CONCLUSION

In conclusion, it can be inferred that TTV may be a naked DNA particle without a virion structure, although further research is needed to establish this hypothesis. It is also likely to be a plasmid-like structure within human cells because human-specific TTV only affects humans, and almost all humans are positive to it, regardless of age, gender, pathology/physiological status, geographical location, and season. Only the TTV DNA load in the serum fluctuates under different pathological conditions. It is imperative to determine how TTV spreads within the host and whether exosomes play a role in this spread. We also need to look at how TTV is transmitted from one person to another and whether there is functional importance of the live infected cell or exosome or cell-free TTV/TTV DNA for this transmission. It is imperative to determine how this live infected cell or exosome or cell-free TTV/TTV DNA recognizes the host cell and how it enters the cell. Can TTV DNA in exosomes be isolated from clinical samples and infect cell lines? If so, we need to scrutinize what kind of exosomes are and from what cell types the exosomes come from. When transfecting intact TTV DNA extracted from clinical samples, it is necessary to examine whether it successfully replicates and releases TTV DNA and in what cells it occurs and under what conditions. The importance of TTV in the direction of naked DNA or naked DNA within the exosomes needs to be explored to understand whether TTV has a beneficial or adverse effect on the host in cancer, organ transplantation, immunosuppression, and other pathological conditions.

AUTHOR CONTRIBUTIONS

PD, KN, and KD wrote the first draft of the manuscript, conceived the study, and wrote the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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