Evidences and consequences of extra-intestinal spread of rotaviruses in humans and animals

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Abstract Rotavirus is recognized as one of the main diarrheal pathogens in young children and animals. The prevailing central dogma of rotavirus infection states that the infection is confined in the gastrointestinal tract. However, increasing evidences indicate that rotavirus infection is systemic. Clinical case reports of systemic manifestations to rotavirus infection in children has continued to accumulate over the past years. The use of animal models provided pathological and molecular evidences for extra-intestinal infection of rotaviruses. The mechanism correlated with the extra-intestinal spread of rotavirus infection from the intestine is through cell-free and cell-associated viremia. The extent of the extra-intestinal spread of rotavirus infection has not yet been fully elucidated; whether it can only affect a limited number of organs and tissues or capable of involving the body as a whole. Moreover, the influence of systemic rotavirus infections remains to be determined. In this review, combination of previous and new data are outlined to help in better understanding of the extra-intestinal infections of rotaviruses.

Keywords Rotavirus · Extra-intestinal spread · Viremia · Antigenemia

Introduction

Rotavirus (RV), belonging to the *Reoviridae* family, contains 11 segmented, double-stranded RNA genomes within

the triple capsid layers [16]. The RV genomic segments encode six structural proteins (VPs) and six non-structural proteins (NSPs). Severe gastroenteritis caused by RVs has been recognized to occur in children younger than 5 years of age, and results in an approximately 453,000 annual deaths globally [54]. Similarly, RV infections are an economically important disease in livestock such as calves, piglets, kids, and poultry [2]. Occurrence of diarrhea can usually last up to 1 week [8, 9]. In immunocompromised children or young animals, however, RV infection can persist for longer time, leading to more severe dehydration and increased mortality [25]. Due to the prominent diarrhea symptom by RV infection, the RV was once thought to only infect the mature, differentiated enterocytes of the small intestine [12]. Since RV antigen and/or RNA have been detected in blood, immune cells, and other extra-intestinal organs and tissues such as liver, heart, lung, kidney, testes, bladder, and central nervous system of infected children and animals [20, 28, 32–34, 39, 41, 43, 44, 46, 53, 59], these reports proposed that RVs can escape from the gastrointestinal tract and invade other organs and tissues [6, 49, 52]. These observations imply that cell-associated and cell-free viremia may naturally occur during RV infection, inducing the systemic spread of RVs. Extra-intestinal spread of RVs has gained a lot of interest from researchers in order to re-evaluate the prevailing dogma for RV infection. This review summarized former and current studies supporting the extra-intestinal RV infections and focal points for future research questions.

Rotavirus infection

The current knowledge of RV replication is mainly based on results from animal models and cell culture system. In our

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Fig. 1 Schematic representation of intestinal and extra-intestinal spread of rotavirus (RV). RV virion binds the mature enterocyte on the tip intestinal villi, penetrates into the cells, and replicates. As a result of RV replication and release into the lamina propria as well as intestinal lumen, cell-free viruses can directly enter the blood and lymphatic vascular circulation and move into different organs. On the other hand, RV can infect or be engulfed by resident immune cells (macrophages, dendritic cells or lymphocytes), and virus-containing

immune cells migrate into the lymphatics and enter the mesenteric lymph node (cell associated viremia). The MLN may serve as a site for secondary replication for RV. Cell-free and cell-associated viruses may exit out of the MLN and migrate into the extra-intestinal organs. *BL* B lymphocyte, *Br* Brain, *CA* cell-associated, *CF* cell-free, *DC* dendritic cell, *Ki* kidney, *Li* liver, *Lu* lung, *MØ* macrophage, *MLN* mesenteric lymph node, *Pa* pancreas, *RER* rough endoplasmic reticulum, *V* viroplasm

understanding, RV replicates in the mature enterocytes near the tips of the villi, implicating that important factor required for the efficient replication of RV is found in this area (Fig. 1). The initial step in the RV infection is through the binding of the virus to the cell surface receptors, in which the outer capsid proteins VP4 and VP7 play an essential role [31]. The RV entry is a multistep process, involving different cell surface receptors like terminal sialic acids [31] or histo-blood group antigens [29], integrins [26], heat shock proteins [26], and gangliosides [38]. Once the virus binds to the cell surface receptors, the virus particle penetrates into the cytoplasm of the cell through a non-clathrin, non-caveolin-mediated endocytosis [16, 27]. This leads to the release of the double-layered particle (DLP) into the cytosol in which transcription and translation take place (Fig. 1). In RVs, membrane-free cytoplasmic inclusions called viroplasms formed from the interactions of viral proteins NSP2, NSP5, NSP6, VP1, VP2, and VP3 that segregate components needed for genome replication from host defense mechanisms [10, 16]. The subviral DLP containing 11 RV genomic segments generated from the viroplasm moves on the rough endoplasmic reticulum (RER) [16]. The DLP

binds the transmembrane NSP4 protein on the RER, moves into the lumen of the RER, and subsequently acquires capsid proteins VP4 and VP7 to be mature and infectious forms before it buds out from the infected cell through exocytosis or lysis (Fig. 1) [13, 16]. The RV is mainly released into the intestinal lumen during the natural course of infection [16, 25, 52]. In addition, RV might be released into the lamina propria and migrate through lymphatics and blood circulation reaching other organs (Fig. 1) [7, 17, 52].

The RV is known to cause a malabsorptive diarrhea by altering the function of the small intestine [16]. RV-induced diarrhea happens at the early stage of infection before significant histopathological changes can be observed. Histopathological changes such as desquamation of villi epithelial cells, consequent villi atrophy and fusion, and crypt hyperplasia are seen in the later stage of the infection when the virus titer is significantly higher [16, 32]. In the early stage, RV-induced diarrhea may be attributable to NSP4 protein [25]. The enterotoxic activity of NSP4 provides a paracrine mechanism to mediate diarrheagenic changes without damaging the gut and to modulate the levels of intracellular Ca^{2+} stores [50]. NSP4

can target the crypt cells where the increase in Ca^{2+} can activate the secretion of Cl^- which can cause an outflow of water [42]. The expression of NSP4 in polarized epithelial cells can lead to the disruption of the tight junctions that may cause paracellular leakage [16, 25]. Through this mechanism, RV can effectively invade adjacent enterocytes and enhanced its replication. Understanding this process will help in analyzing how RV escapes from the intestinal tract [8, 52].

Extra-intestinal spread of rotavirus infection in children

The question whether RV can escape from the intestine is being acknowledged by case reports in which RV proteins and RNA, and infectious virus are evident in blood and extra-intestinal organs and tissues of infected children (Table 1). The sporadic episodes of these reports denote that systemic dissemination might be a rare event. The detection rate of extra-intestinal RNA in the blood or tissues has varied widely in children and not always in agreement with the antigenemia [9, 21]. Preliminary findings showed that antigenemia is usually detected in a high percentage of children infected with RVs [6, 9, 11, 21]. From these findings, subsequent studies have been conducted to confirm the presence of RV in serum samples of infected children. In one study, RV antigen was diagnosed in 30 of 70 serum samples, and levels of antigen in the sera were significantly increased at the acute phase of the RV infections [21]. Acute sera from children with RV associated encephalopathy were identified to be positive with RV antigen, demonstrating antigenemia; however, the antigen was not detected in the cerebro-spinal fluid [39]. Most of the findings concerning antigenemia were observed during the early phase of infection, where the RV antigen levels in the sera peaked at 2 days post-inoculation (dpi), and no antigen was detected at 6 dpi [48, 56]. Antigenemia was detected in 64 % of children with RV diarrhea and RV RNA was observed in 93 % of the antigen-positive serum specimens [47]. In addition, antigenemia was recognized in 90 % of RV patients who had RV-positive stools and in 89 % of children without diarrhea but with the RV-positive stools [9]. The detection of RV RNA in the sera from infected children was usually associated with high fever [11, 51, 56]. As stated in one article, antigenemia is prognostic of viremia and can be used as marker for the presence of extra-intestinal RV [8].

Aside for the occurrence of antigenemia and viremia during RV infection, other clinical manifestations are being linked to RV infection in humans. Several reports have indicated that RV antigen or RNA were detected in the spleen, heart, kidneys, testes, bladder, liver, cells or secretions from the respiratory tract, and endothelial cells

from RV infected children and adult [9]. It may seem that RV can move to these organs via viremia, but there is still no indication whether it can replicate efficiently outside the intestine. Increasing reports presented detection of RV antigen or RNA in the cerebrospinal fluid of diarrheic children [23, 30, 37, 39, 45]. Previously, reported cases showed the presence of RV nucleic acid in cerebrospinal fluid of children with acute cerebellitis which was attributed to cerebellar seizures [14, 47]. The use of magnetic resonance imaging makes way for identifying cerebellar foci and residual cerebellar atrophy in cases where RV RNA was detected in cerebrospinal fluid [57]. One rare case reported the manifestations of acute encephalopathy and rhabdomyolysis following RV gastroenteritis [40]. Transient acute flaccid paralysis and seizures were reported in a case of a three-year-old child with RV gastroenteritis in Turkey [48]. Although RV has been identified in the central nervous system, the incidence of its occurrence is said to be rare [56]. Also noted is the increasing incidence of acute pancreatitis in children with RV infection which usually subside within 5–10 days [22]. The occurrence of acute pancreatitis during RV infection was correlated to the presence of inflammatory edema with infiltrates of neutrophils and mononuclear cells, degranulation of acinar cells, and dissociation of lobules and acini with necrosis [36]. The occurrence of antigenemia is also being associated with immunocompetent children with RV diarrhea, implying that antigenemia could be the center of the pathogenesis of various extra-intestinal infections with RV [1]. Even though evidences of medically relevant extra-intestinal diseases such as acute encephalopathy, acute cerebellitis, rhabdomyolysis, and acute pancreatitis are now being documented, it is still unclear on how RV infections influence the development and significance of such diseases. The limited organ and tissue samples from humans are also an obstacle to determine the extent of extra-intestinal spread of RV in humans. The causal role of extra-intestinal manifestations of RV in fatal cases has not been clearly established [37]. On the other hand, if systemic disease occurred in only one in 10,000 (0.01 %) of 111 million RV-infected children each year, approximately 111,000 cases of systemic disease would be expected annually [52]. Therefore, the total impact of RV viremia and extra-intestinal infections for systemic diseases should be further addressed.

Extra-intestinal spread of rotavirus infection in animals

Experimental animal models

Recent publications have provided concrete evidences that RV can escape from the intestine. Since there are

Table 1 Summary of reported extra-intestinal spread in humans (selected studies)

Subjects	Age	Prior health status	Blood	Clinical findings related to extra-intestinal spread	Ref.
4	4–8 Months	Immunodeficiency	NT	Rotavirus antigen was detected in the liver and kidney; elevated liver aminotransferase	[23]
2	6 Years old	Healthy	NT	Rotavirus RNA was detected in the cerebrospinal fluid of patients who developed encephalopathy	[37]
1	2.5 Years old	Healthy	Negative	Cerebellitis might be associated with rotavirus infection	[47]
40	3 Years old	Healthy	NT	Incidence of afebrile seizure following rotavirus gastroenteritis was 2.06 %; more than half (52.5 %) of patients experience seizures on the third day of diarrhea	[30]
8	<9 Years old	Healthy	5 out 8 children with antigenemia	Five of 8 children with rotavirus gastroenteritis had significant levels of rotavirus antigen in their acute phase sera; CSF was negative for rotavirus antigen	[45]
70	<12 Years of age	–	30 of 70 acute-phase serum with antigenemia	Antigenemia was associated with the outbreak of rotavirus gastroenteritis	[21]
54	<49 Months	Immunocompetent	Viremia	Rotavirus antigen was detected in the blood of children with stool positive with rotavirus	[11]
102	≤3 Years old	Healthy	Antigenemia (63 % with rotavirus diarrhea; 3 % nondiarrheal)	Rotaviral antigen was detected in the sera of children having rotaviral diarrhea; antigenemia was associated with G1 serotype	[53]
83	–	–	Viremia	Sixteen of 83 blood samples were positive for rotavirus RNA (19.3 %); incidence of liver damage, rash, lower respiratory tract infection and CNS involvement in the blood RV-RNA positive group were significantly higher than those in blood RV-RNA negative group	[28]
124 children 28 adults	–	Healthy	Sera positive for rotavirus antigen	Rotavirus antigenemia was detected in 90 % (51/57) of children with rotavirus-positive stool, in 89 % (8/9) of children without diarrhea but with rotavirus positive stool, in 12 % (2/17) of children with bronchitis without gastroenteritis, and in 12 % (5/41) of children with gastroenteritis but with rotavirus negative stool. Antigenemia was not detected in healthy adults	[8]
1	6 Months	Healthy	NT	Acute encephalopathy and rhabdomyolysis after rotavirus gastroenteritis	[40]
60	1.4 ± 1.4 Years old	Healthy	Antigenemia	Serum rotavirus antigen levels peaked on day 2 of the illness, followed by gradual decrease by day 6; higher antigen level was observed in patients with fever	[56]
1	3.5 Years old	Healthy	NT	CSF was positive for rotavirus by PCR; rotavirus associated with meningoencephalitis	[14]
199	–	Healthy	Antigenemia	Antigenemia was significantly higher among children with rotavirus diarrhea	[51]
1	27 Months old	Healthy	Rotaviral RNA in CSF	RT-PCR and nucleotide sequencing detected G1P [8] strain in the CSF	[39]
1	2.5 Years old	Healthy	Rotavirus antigen detected in the sera	Rotaviral antigens (VP6, NSP1, NSP3, and NSP4) were detected in the brain, heart, and liver. VP6 and NSP4 genes were detected in the liver and brain by RT-PCR	[46]
62	3 Months–18 years old	Healthy	Antigenemia	Rotavirus antigen was detected in blood of 31/68 (45.6 %) patients. Upper respiratory tract symptoms were the major extra-intestinal symptoms in patients	[59]
1	4 years old	Healthy	Rotavirus RNA detected in blood	Severe cerebellitis in association with rotaviral gastroenteritis. Rotavirus nucleic acid was demonstrated in both serum and CSF	[57]
24	<3 Years old	Healthy	Antigenemia, RNAemia	Presence of rotavirus antigen in 33.3 % and almost full-length NSP4 gene in 70.8 % of acute-phase plasma and PBMC; RNAemia in 4.2 % of convalescent-phase PBMC	[41]
1	2 Years old	Healthy	NT	Acute pancreatitis following rotavirus gastroenteritis	[22]
250	<5 Years old	Healthy	Antigenemia	Antigenemia was present 162/250 (64.8 %) samples from children with rotavirus diarrhea; genomic dsRNA was detected free in sera and not inside virus capsid protein	[1]

NT not tested

limitations for studying on RV infections in humans, establishment of animal models may provide important insights on the extra-intestinal spread of this pathogen (Table 2). The first study was carried out in 1958 using infant mice inoculated with EDIM strain [35]. In this study, the detection of RV in body fluids (blood and urine) and several organs and tissues (brain, liver, spleen, kidney, lungs, and urinary bladder) were evident in different post-inoculation time [35]. Much later on it was followed by another study using severe combined immunodeficiency (SCID) and normal BALB/c mice showing the development of hepatitis in mice inoculated with heterologous rhesus RV strain (RRV) MMU 18006, but not in mice inoculated with bovine RV strain WC3 [58]. The study also showed the presence of RRV antigen in the liver of SCID mice, suggesting altered tissue tropism of the virus that was once demonstrated to be exclusively detected in the intestine [58]. Brown and Offit [5] reported that specific RV proteins were detected in the Payer's patches, mesenteric lymph node or inguinal lymph node after inoculation of EDIM in mice. By determining the surface markers on cells containing RV-specific proteins collected from the lymphoid population, RV specific proteins were primarily detected in macrophages and to some extent in the B cell population [5].

More studies had been conducted to prove the assumption that RV can escape from the intestine and spread to other organs and tissues. Studies on extra-intestinal spread using neonatal mouse model conducted by Mossel and Ramig [44] manifested the presence of RV in the liver, mesenteric lymph node, spleen, and lung. The number of animals that manifested extra-intestinal infection varies with the different inoculated strains [43, 44]. For example, liver positive for RV in CD-1 mice were only detected in 1 % of CD-1 mice inoculated with SA11-C14 and 26 % of CD-1 mice inoculated with RRV [43]. Sera and plasma fractions of blood from the mice infected with murine RV strain EDIM and rhesus RV strain RRV were positive for RV RNA and virion, which also suggests that RV antigenemia is not RV strain dependent [7]. In a study conducted on immunocompetent mice, rats, rabbits, and calves inoculation of homologous or heterologous RV results in the detection of RV antigen in the sera [6]. The neonatal rat model with rhesus RV strain and human HAL1166 showed the presence of RV antigens and infectious virus in liver, lung, spleen, kidney, pancreas, thymus, and bladder [12]. A non-human primate animal model using juvenile macaques demonstrated the presence of RV in the plasma and cerebrospinal fluids by real-time PCR [60]. A more thorough investigation is needed to further evaluate the rate of occurrence on extra-intestinal manifestation in RV infection.

Established mouse models are at present being utilized to examine whether RV can induce systemic diseases. One good example of this is the association of RV in causing

biliary atresia. Earlier studies had delineated that RVs can cause extrahepatic biliary obstruction in newborn mice orally inoculated with HCR-3 and RRV strains [55]. Another study on RV-induced murine biliary atresia correlated this phenomenon with an active expression of NF- κ B [18]. Allen et al. [3] had demonstrated that induction of biliary atresia in mice is strain-specific, in which it may dictate tropism of RVs for cells of hepatobiliary origin. Inoculation of serum from mice or children positive with RV has been shown to cause biliary atresia in neonatal mice [7]. Insights in the murine model of RV-infection provide us that RV can induce biliary atresia.

Even thorough studies on RV infection in animal models are being conducted; the question on how RV spread to other organs still remains to be clarified. Based on the reports, however, it can be postulated that both cell-associated and cell-free viremia can instigate the movement of RV to extra-intestinal organs and tissues (Fig. 1). As pointed out of most reports, high percentage of RV antigen has been detected in the mesenteric lymph node and Payer's patches (Fig. 1) [5, 17, 43, 52], both involve in the digestive tract immune system, of which is often referred to as gut associated lymphoid tissue. These findings denoted that RV replication can be associated with immunosurveillance cells such as macrophages and dendritic cells which can migrate to regional lymph nodes via the lymphatics as well in the blood vascular system [5, 12, 19, 44, 52]. Additionally, detection of RV in the mesenteric lymph node may serve as a secondary replication site and help prolong the infection (Fig. 1) [17, 32, 33, 52]. By these routes, RV can travel throughout all tissues and organs of the body via the blood and lymphatic vessels (Fig. 1). In addition, infection of RV of these cells may be associated with the presence of cellular receptors that allow the entry of RV. Identifying specific cell receptors of these cells and other extra-intestinal organs may help understand the tropism of RV and course of infection in the body.

Cell-free viremia may contribute in a high percentage to the extra-intestinal spread of RV rather than the cell-associated viremia on the account that almost all sera were positive for RV antigen, RNA or infectious virion [4, 8, 9, 32–34]. The possible mechanism is that replication of RVs led infected enterocytes being lysed and shed from villi, and eventually underlying lamina propria of villi are disclosed. Since the intestine has plenty of blood and lymphatic vessels in the lamina propria of villi, a lot of RVs can readily enter these vascular systems and can spread to extra-intestinal organs and tissues particularly through the portal vein (Fig. 1) [15, 52]. Once the blood is drained from the gastrointestinal tract, it moves into the liver [15]. In most reports of RV extra-intestinal spread in children and young animals, the liver is the major organ with a high frequency of RV detection [17, 21, 33]. It may concur that upon RV infected blood from the

Table 2 Summary of reported extra-intestinal spread in animal models (selected studies)

Species	Virus/es	Blood	Clinical findings related to extra-intestinal spread	Ref.
Mice	EDIM	Viremia	Rotavirus was detected in blood after 22 h post-inoculation; rotavirus was also detected in the urine, liver, kidney, spleen, brain, lungs, bladder	[35]
Mice	Rhesus rotavirus strain (RRV), WC3	NT	Rotavirus was detected in the liver tissue inoculated with rhesus rotavirus strain but not in the WC3; development of hepatitis in immunodeficient and normal mice inoculated with RRV	[58]
Mice	HCR-3, RRV	NT	Active virus replication was seen as early as 48 h post-inoculation in biliary tract and liver	[55]
Mice	SA11-C14, RRV	NT	Virus was detected in the liver	[43]
Mice	SA11-clone 4, RRV	Low titer of rotavirus present in the blood	Virus was found in the mesenteric lymph node suggesting rotavirus can spread by the lymphatic pathway. Rotavirus was also found in Payer's patches, liver, spleen, lungs, and blood	[44]
Juvenile Macaques	Simian rotavirus strain TUCH	Very low quantities of detected viral RNA (~20 copies/ng of total RNA)	The virus TUCH strain was detected in the plasma and cerebrospinal fluids (CSF) of six infected macaques	[60]
Piglet	Wa strain (virulent human rotavirus)	Transient viremia	Inoculated piglets showed virus in the serum; upper respiratory tract infection (rotaviral nasal shedding)	[4]
Mice	RRV	NT	Rotavirus-induced murine biliary atresia	[18]
Rat	RRV, HAL1166	Viremia	Rotavirus antigens and infectious virus were detected in various organs [liver, lungs (macrophages)], spleen, kidneys, pancreas, thymus, and bladder	[12]
Mice	Homologous EC strain, heterologous Rhesus rotavirus strain	RNA copy numbers in the blood were very low	Both viruses were detected in mesenteric lymph nodes, livers, lungs, blood, and kidneys; dendritic cells and potentially B cells and macrophages might support rotavirus replication in the MLN	[17]
Mice	EC strain, EDIM, RRV	Viremia, Antigenemia	Rotavirus RNA and infectious virus were present in the sera and associated plasma fraction of the blood in all infected mice.	[7]
Mice	RRV, SA11 (cause systemic infection) NCDV, OSU, EW (murine strain)	NT	Rotavirus was found in the liver, pancreas, mesenteric lymph node, and bile duct of mice inoculated with RRV strain	[19]
Mice	RRV	Infectious virus in blood cells	Rotavirus antigen was detected in pancreatic macrophages and infectious virus was found in the blood cells, pancreas, spleen, and liver. Extra-intestinal spread of rotavirus was found in both infant and adult mice inoculated with RRV	[24]
Calves, piglets	Bovine reassortant strain	Viral RNA detected in the sera	Viral RNA was detected in sera of infected calves. Viral RNA was also found in the mesenteric lymph node, lungs, liver, choroid plexus, and cerebrospinal fluid in infected calves	[32]
Mouse	RRV, UK	NT	Rotavirus was detected in the biliary tract of mice inoculated with RRV and UK	[20]
Calves, piglets	Bovine triple reassortant strain	Viral RNA detected in the sera	Rotavirus antigen was found in the mesenteric lymph node, choroid plexus, lungs, and livers in inoculated calves and piglets	[33]
Piglets	G9P [23], G9P [7]	Sera was positive for viral RNA	Rotavirus antigen was detected in the mesenteric lymph node, liver, lung, and choroid plexus	[34]

NT not tested

gastrointestinal tract drains into the liver; the blood that will return into the blood stream will carry RV and can be distributed in the other organs and tissues in the body. The manner how RV can survive in this process remains to be unfolded and further studies are needed to clarify this concept.

Livestock animals

The RV was first isolated in 1969 in diarrheic calves and from thereafter, similar events were reported in other domestic mammalian species and domestic fowls [52]. However, the demonstration of extra-intestinal dissemination in a natural setting has not yet been fully exemplified. Most of the detection of RV in farms was done using fecal samples, while antibody titers were tested in serum samples. The occurrence of extra-intestinal spreading in the naturally RV infected animals may have been overlooked since the focus is mainly on the enteric aspect of the disease. The use of colostrums-deprived piglets and calves has helped to analyze the pathogenesis and extra-intestinal spread of different RVs. In particular, the use of colostrums-deprived piglets have been insightful since the gastrointestinal tract is closely similar to that of the human infant. A study using gnotobiotic piglets provided evidence that virulent human RV Wa strain can cause transient viremia and upper respiratory tract infection in addition to gastrointestinal infection [4]. More recent studies on RV pathogenicity using colostrums-deprived piglets and calves have showed viremia and extra-intestinal dissemination of the virus [32–34]. Viral RNA using SYBR Green real-time PCR was detected in the sera of calves at 1 dpi and peaked at 3 dpi, which gradually decrease until 14 dpi [32, 33]. The extra-intestinal spread of bovine reassortant RVs in piglets and calves were seen in the mesenteric lymph node, lungs, liver, choroid plexus, and cerebrospinal fluid by using real-time PCR and immunofluorescence assay [32, 33]. Infection of homologous porcine strains G9[P7] and G9P [23] also caused viremia and extra-intestinal spread in colostrums-deprived piglets [34]. Studies using colostrums-deprived piglets and calves to investigate the pathogenesis of RV also showed excretion of this virus in the nasal cavity and can be correlated to the detection of RV in the lungs and other part of the respiratory tract [2, 32]. These results reinforced that cell-free RV have the potential to cross the gut barrier from the luminal side after the destruction of enterocytes in the villi, and migrated into the circulatory system. This assumption can be supported since RV antigen or RNA can be detected in extra-intestinal organs like mesenteric lymph node, liver, lung, pancreas, spleen, and choroid plexus of experimental animals. In addition, extra-intestinal histopathological changes associated with RV infection were observed in calves and piglets [32–34]. These include marked lymphoid cell depletion in

the cortex of the mesenteric lymph node, interstitial pneumonia due to the infiltration of macrophages, lymphocytes, and neutrophils into the alveolar interstitium and hyperplasia of type II pneumocytes, multiple scattered necrotic hepatocytes, and epithelial degeneration and necrosis in choroid plexus [32–34]. In these cases, RV infection may cause these manifestations, leading to a more serious systemic disease. Nonetheless, these reports also imply that extra-intestinal dissemination of RV is not solely dependent on the strain. Since there are limited samples or tissues to conduct studies for examining the tropism and extra intestinal spread in humans, animal models are valuable tools to understand the course of RV infection which can help in combating this notorious disease. In future studies, the extra-intestinal manifestations in naturally infected animals with RV need to be addressed to strengthen the evidences of systemic occurrence of RV.

Involvement of rotavirus gene segments in the extra-intestinal spread

The question whether which rotaviral gene segment can influence the extra-intestinal spread still remains to be fully understood. Several studies had mentioned that serotypes might play a role in the extra-intestinal spread [1, 9, 51]. In a study that high rate of antigenemia was observed in children, reverse-transcriptase-PCR was performed to determine the P and G genotypes of the RV [9]. Based on this result, analysis of variance among the serum antigenemia showed no significant association between the type of virus and antigenemia [9]. Similarly, rates of antigenemia did not vary significantly between those infected with different serotypes in Indian children [51]. A more recent study also suggested that there was no significant difference of genotypes between Bangladeshi children with or without antigenemia [1]. In contrast, children infected with G1 strains more often had antigenemia (85 %) than did those infected with other G serotypes (48 %) [53]. Using a neonatal mouse model of RV infection, NSP3 was shown to be a key determinant of extra-intestinal spread [43]. In addition, another study described that neonatal mice infected with RRV and reassortant R7 were able to cross the peripheral tissues and the spread phenotype is primarily determined by NSP3 and VP6 [44]. In one study in a mouse model, VP7 may function in collaboration with VP4 and NSP1 to enhance RV infection in biliary tissues, but it does not independently determine RV infectivity in vivo [20]. Presently, there are still no concrete evidences of which rotaviral genes are involved in the extra-intestinal spread of RV. More studies are needed to identify the gene segments that play key roles in the extra-intestinal manifestation of RV infection.

Concluding remarks

Alongside with the evidences presented, RV has the potential to cause extra-intestinal spread in young animals and children. Furthermore, detection of RV RNA, antigens, and infectious virion in the sera strongly suggest that RV migrate to the extra-intestinal organs and tissues through the blood or lymphatic vascular systems. In conjunction with this, cell-associated or cell-free viremia is also manifested during RV infection. In cell-associated viremia condition, RV might infect immune cells particularly antigen presenting cells (macrophages and dendritic cells) and/or B lymphocytes, and moves through other organs via the lymphatic and blood vascular systems (Fig. 1). Although this may present a big possibility, it still remains to be explored. In addition, with the knowledge that RV cause extra-intestinal dissemination, does it have significant consequences on infected patients or animals? Or the presense of RV RNA or virion in the human samples were results of contamination from stool samples? Despite reported cases that associate RV gastroenteritis with other related systemic diseases, it is still a vague concept on how the virus can induce such conditions.

As mentioned, different strains of RV can induce extra-intestinal transmission. Does the severity of extra-intestinal virus dissemination vary in different strains? Moreover, viremia was also noted in patient without diarrhea. Does this mean that RV can be in a latent form and certain RV can not induce any significant gastrointestinal manifestation? Since human samples are difficult to obtain, animal models present a critical role in verifying the extra-intestinal spread of RV. It will also contribute in assessing the systematic infection in conjunction with the extra-intestinal spread of RV. The extra-intestinal manifestations on naturally RV infected animals should also be addressed to further broaden the knowledge of RV infection. The results that will be obtained from subsequent animal experiments for extra-intestinal spread of RV will help in the development of better vaccines and proper management of infected animals and children. In the coming years, with extensive studies on the extra-intestinal dissemination, emerging questions will soon be clarified.

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