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***Basic Study***

**Neonatal rhesus monkeys as animal model for rotavirus infection**

Yin N *et al*. Monkey model of rotavirus infection

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**Abstract**

***AIM***

To establish a Rotavirus (RV)-included diarrhea model using RV SA11 in neonatal rhesus monkeys for the study of the pathogenic and immune mechanisms of RV infection and evaluation of candidate vaccine.

***METHODS***

Neonatal rhesus monkeys with an average age of 15-20 d and an average weight of 500 g ± 150 g received intragastric administration of varying doses of SA11 RV ( 107 PFU/mL, 106 PFU/mL, or 105 PFU/mL, 10 mL/animal) to determine whether the SA11 strain can effectively infect these animals by observing their clinical symptoms, fecal shedding of virus antigen by ELISA, distribution of RV antigen in the organs by immunofluorescence, variations of viral RNA load in the organs by qRT-PCR, histopathological changes in the small intestine by HE stain, and apoptosis of small intestinal epithelial cells by TUNEL.

***RESULTS***

RV monkey model showed typical clinical diarrhea symptoms in the 108 PFU SA11 group, where we observed diarrhea 1-4 d post infection (dpi) and viral antigen shed in the feces from 1-7 dpi. RV was found in jejunum epithelial cells. We observed a viral load of approximately 5.85 × 103 copies per 100 mg in the jejunum at 2 dpi, which was increased to 1.09 × 105 copies per 100 mg at 3 dpi. A relatively high viral load was also seen in the mesenteric lymph node at 2 dpi and 3 dpi. The following histopathological changes were observed in the small intestine following intragastric administration of SA11 RV: Vacuoles, edema, and atrophy. Apoptosis in the jejunal villus epithelium was also detectable at 3 dpi.

***CONCLUSION***

Our results indicate that we successfully established a RV SA11 strain diarrhea model in neonatal rhesus monkeys. Future studies will elucidate the pathogenesis mechanisms of RV infection, and we will use the model to evaluate the protection of the candidate vaccine.

**Key words:** Rotavirus; Neonatal rhesus monkey; Animal model; Infection; Diarrhea

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**Core tip:** Rotavirus (RV) is one of the main pathogens responsible for severe diarrhea in children under 5 years of age. Vaccine-induced immunity is an effective way to block RV disease. Nonhuman primates are the animals most closely related to humans and have advantages over non-primates as an animal model of RV diarrhea, so development of nonhuman primates animal model of RV infection is needed to ensure the effectiveness and safety of these vaccines. Our current study has indicated that RV SA11 can lead to obvious diarrhea and pathological changes in the intestines of neonatal rhesus monkeys. The RV infection model we established was useful for us to further investigate the RV infection mechanism and the associated immune mechanisms in human infants and evaluate the cross protection of potential HRV vaccine candidates.

Yin N, Yang FM, Qiao HT, Zhou Y, Duan SQ, Lin XC, Wu JY, Xie YP, He ZL, Sun MS, Li HJ. Neonatal rhesus monkeys as animal model for rotavirus infection. *World J Gastroenterol* 2018; In press

**INTRODUCTION**

As the primary cause of severe acute gastroenteritis in infants and young children, rotavirus (RV) is one of the most important causes of [pathogenicity](http://www.baidu.com/link?url=i34xIu2VybK038eOk8irN7TacYCWOR_pWWVjsu5UrAE7-t0xWSgmIwIbrGJC3oVzkGDa_CKIY8HAwdWlJdzWPF5UFug0-Utu1sBPBqqHvqe) worldwide[1]. RV infection can result in vomiting, fever, severe dehydration, diarrhea, and even death. Over 200000 infants and young children die each year due to RV infection worldwide, and low-income countries are disproportionately affected[2].

There are currently no specific drugs for the treatment of diarrhea caused by RV infection[3]; therefore, the development of safe and effective vaccines to control RV infection is particularly important[4-7]. Furthermore, an effective animal model of RV infection is needed to ensure the effectiveness and safety of these vaccines. Nonetheless, some progress has been made in the development of animal RV models, and have included gnotobiotic piglets, calves, lambs, suckling mice, and rabbits. While RV infection in calves and lambs results in mild clinical disease[8-10], gnotobiotic piglets are more susceptible to RV infection, since their immune system more closely resembles that of a human infant, and the period of susceptibility is very long[11-13]. However, breeding conditions for gnotobiotic piglets are quite strict and their cost is prohibitively high, restricting the study of RV infection in these animals. Suckling mice and rabbits have also been used to study RV infection[14-18] and they have strong reproductive abilities and are easy to maintain; however, these animal models have a distant evolutionary relationship with humans, which can limit the ability of data obtained from these animal models to improve the understanding of the pathogenesis of RV infection in humans. Therefore, developing models using non-human primates, the species more closely related to humans, is necessary[19-23].

The SA11 strain is a simian RV strain, obtained from an asymptomatic vervet monkey *in vitro*[24]. As described before, the SA11 strain can infect not only non-human primates, such as chimpanzee, macaque (cynomolgus monkey, rhesus monkey)[20,21], but also other non-primates, such as mice[14]. [Petschow *et al*[25]](https://www.ncbi.nlm.nih.gov/pubmed/?term=Petschow%20BW%5BAuthor%5D&cauthor=true&cauthor_uid=1326241) inoculated 5 newborn cynomolgus monkeys with the simian RV strain SA11, and detected SA11 in feces of 3 monkeys for up to 2 d after inoculation. In this study, we infected the neonatal rhesus monkeys with RV SA11 through oral gavage to establish an RV diarrhea model. Our data indicated that 108 PFU SA11 can infect intestinal villous epithelial cells in neonatal rhesus monkeys, and result in obvious pathological changes in the small intestine as well as clinical symptoms including diarrhea. Together, these findings indicate that the neonatal rhesus monkey could be used as an animal model for RV infection, providing a powerful tool for further study of the pathogenesis of RV and the associated immune mechanisms in human infants and evaluation of RV vaccines.

MATERIALS AND METHODS

***Ethics statement and experimental animals***

The experimental animals in this study were provided by the Primate Experimental Center of the Institute of Medical Biology, Chinese Academy of Medical Sciences. A total of 12 healthy neonatal rhesus monkeys with an average age of 15-20 d and an average weight of 500 g ± 150 g were randomly divided into three experimental groups and a control group, each with three monkeys. The experimental animal procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the Institute of Medical Biology, Chinese Academy of Medical Sciences. A neutralizing antibody test was conducted to confirm that the monkeys did not have antibodies against RV SA11 prior to the study. All of the animals were housed in a separate incubator one week before the initiation of the experiment.

***Neutralization test***

The activated RVs were adjusted 1000 PFU/100 μL in serum-free MEM. The serum samples were diluted from 1:10 to 1:1280 in 100 μL serum-free MEM. The diluted RVs and serum samples were mixed with each and incubated at 37 ºC for 1 h. The mixtures were transferred to the 96-well plates covered with a confluent monolayer of MA104 cell and were cultured at 37 °C for 5 d. The cultures were completely transferred to the wells of the ELISA plate coated with a goat anti-RV polyclonal antibody (Millipore, AB1129) and blocked with 3% (w/v) BSA (Biosharp, BS043D). The cultures were inoculated at 37 °C for 1 h. A rabbit anti-RV polyclonal antibody (prepared by the Department of Molecular Biology, Institute of Medical Biology, Chinese Academy of Medical Science and Peking Union Medical College) conjugated with horseradish peroxidase (HRP) was used to detect RV antigen at dilution of 1:2000 (v/v) in PBS at 37 °C for 1 h. All ELISA were developed using TMB (TIANGEN, PA107-01) to generate a colorimetric reaction and terminated by 2 mol/L H2SO4. The absorbance was read on a universal microplate reader (El × 800, Bio-Tek, United States) at 450 nm and 630 nm. A serum specimen was determined to be positive if the OD value was less than or equal to the average OD value of the negative control × 2. The neutralization titers were defined as the highest dilution.

***Cells and virus***

MA104 cells were maintained in MEM supplemented with 10% fetal bovine serum (Gibco, 16000-044) and grown to a confluent monolayer in roller bottles (850 cm2) (CORNING, 430849). The RV strain used in this study was standard strain SA11 (G3P[2]) that was originally isolated from a monkey. Prior to infection, SA11 was activated with 20 μg/mL trypsin (Gibco, 15090-046) at 37 °C for 45 min. Infected cultures were harvested by freezing at -20 °C and thawing at room temperature, and followed by centrifugation at 8873 g for 20 min. The harvested virus titer reached 107/mL plaque forming units (PFU)[26].

***Inoculation of neonatal rhesus monkeys with SA11***

The neonatal rhesus monkeys were inoculated with varying doses of RV SA11 (108/107/106 PFU/monkey) or 10 mL medium without serum *via* oral gavage (Supplementary Table 1). After the infection, each neonatal rhesus monkey was housed alone in the incubator.

***Fecal samples collection and processing***

From 0 to 14 d post infection (dpi), the infected neonatal rhesus monkeys were monitored once daily for clinical signs, such as mental status, weight, body temperature, and hair, and diarrhea situation. The fecal samples were collected in a fecal collector by gently pressing the abdomen of the neonatal rhesus monkeys every day. According to the color, hardness, and quantity of the feces, diarrhea was scored from 1 to 4 points[11,15], and > 2 points were considered to be indicative of diarrhea. The fecal sample was suspended in 10% (s/v) cold PBS, followed by centrifugation at 8873 g for 20 min, collected the supernatant and stored at -80 °C for the subsequent study. At 2, 3 dpi, one monkey that was infected with 108 PFU SA11 or medium without serum were killed by electric shock with anesthesia. Two aliquots of each tissue sample, including the heart, liver, spleen, lung, kidney, intestine (duodenum, jejunum and ileum), and brain, were harvested. One sample was stored in liquid nitrogen and the other was fixed in 10% formalin.

***Detection of RV antigen shedding in feces by ELISA***

The wells of the ELISA plate were coated with a goat anti-RV polyclonal antibody (Millipore, AB1129) diluted at 1:1000 (v/v) in carbonate-bicarbonate buffer overnight at 4 °C. Then, the plates were blocked with 3% (w/v) BSA (Biosharp, BS043D) in PBS at 37 °C for 2 h. The fecal samples (100 µL/well) were serially twofold diluted (dilution range from 1:2 to 1:4096) and incubated at 37 °C for 1 h. After washing, a rabbit anti-RV polyclonal antibody conjugated with HRP was used to detect RV antigen at dilution of 1:2000 (v/v) in PBS at 37 °C for 1 h. All of the ELISA plates were developed using TMB (TIANGEN, PA107-01) to generate a colorimetric reaction and terminated by 2 mol/L H2SO4. A sample was determined to be positive if the OD value was greater than or equal to the average OD value of the negative control × 2.

***Histopathological and immunofluorometric assays***

The small intestine was fixed in 10% (v/v) formalin in PBS, then dehydrated in 70% (v/v) graded ethanol series, and embedded in paraffin before being sectioned at the 4.0 μm thickness for further hematoxylin and eosin staining. Histopathological observation of the small intestine was performed by light microscopy. For immunofluorescence microscopy, tissue sections were deparaffinized in xylene and hydrated through graded ethanol, followed by adding 0.03% (v/v) hydrogen peroxide solution. Antigen retrieval was performed with 0.01 mol/L sodium citrate (pH 6.0). The glass slides were blocked with normal goat serum (BOSTER, SA1006) in 1:10 dilution for 20 min. Next, the glass slides were incubated with goat anti-RV polyclonal antibody (prepared by the Department of Molecular Biology, Institute of Medical Biology, Chinese Academy of Medical Science and Peking Union Medical College) in 1:500 dilution at 37 °C for 90 min. The glass slides were incubated with FITC-labeled rabbit anti-goat IgG antibody (Jackson ImmunoResearch, 705-095-147) in 1:1000 dilution at 37 °C for 60 min. After incubation, the glass slides detected under a fluorescence microscope.

***Viral RNA Extraction and qRT-PCR assay***

Viral RNA was isolated from fresh tissue of experimental animals by Trizol (Ambion, 15596026). 1 mL Trizol was added to 50-100 mg of fresh tissue and the sample was grinded on the ice with an electric grinder. The homogenized sample was incubated for 5-10 min at room temperature. 0.2 mL of chloroform was added to the homogenized samples. The tube was shaked vigorously by hands for 15 seconds and incubated for 15 min at room temperature. Next the tube was centrifuged at 12000 rpm for 15 min at 4 °C and the aqueous phase was transfered to a clean tube. 0.5 mL isopropyl alcohol was added to the tubes. Then the tube was incubated for 10 min at room temperature and centrifuged at 12000 rpm for 10 min at 4 °C. The supernatant was removed and the RNA was washed with 1mL 75% ethanol and the tube was centrifuged at 12000 rpm for 5 min at 4 °C. Then RNA was washed again and dryed for 5-10 min at room temperature. Finally, RNA was completely dissolved in 30 µL RNase-free water and stored at -70 °C.

At the same time, viral RNA was isolated from venous blood samples using the QIAamp Viral RNA Mini Kit, according to the manufacture’s protocol (Qiagen, 52904).

RT-PCR assays were performed using the TransScript II Probe One-Step qRT-PCR SuperMix (TRANS, AQ321-01) in the Real-Time System (CFX96, BIO-RAD, United States). The reaction system included 5 μL RNA template, forward primer at 20 nm, reverse primer at 20 nm, FAM-labeled probe at 20 nm, and E-mix in a 20 μL total reaction volume. The sequences for the primers were as follows: forward primer, 5’-GTTGTCATCTATGCATAACCCTC-3’; reverse primer, 5’-ACATAACGCCCCTATAGCCA-3’; FAM-labeledProbe, 5’-ATGAGCACAATAGTTAAAAGCTAACACTGTCAA-3’. The following protocol was used for all of the RT-PCR: 5 min at 50 °C; 30 s at 94 °C; followed by 40 cycles of 94 °C for 5 s and 60 °C for 30 s. A standard reference curve was obtained by measurement of standard virus RNA. According to the standard reference curve, the viral load was quantified in each sample.

***Detection of apoptosis in small intestine epithelial cells***

We detected apoptosis in small intestine of the infected neonatal rhesus monkeys using the TUNEL Bright Green Apoptosis Detection Kit (Vazyme, A112-03), according to the manufacturer’s protocol. The analysis of apoptosis was performed by a fluorescence microscopy.

RESULTS

***Clinical symptoms in SA11-infected neonatal rhesus monkeys***

Neonatal rhesus monkeys received intragastric administration of 108/107/106 PFU SA11 or medium without serum. Only the 108 PFU group showed significantly characteristic symptoms, and all of the neonatal rhesus monkeys in that group showed obvious clinical symptoms including depression, dull hair color, lethargy, weakened activity, and diarrhea between 1 d and 3 d post infection (dpi). On day 1, monkeys infected with 108 PFU SA11 developed diarrhea that was flocculent or watery (Table 1). On day 2 and 3, significant fecal pollution was observed around the anus, and diarrhea symptoms were the most serious. On day 4, obvious symptom relief was observed, physiological characteristics begun to improve, diarrhea stopped gradually, and animals recovered to normal excreta. In the 107 PFU group, only one monkey developed severe diarrhea on 1 dpi. None of the neonatal rhesus monkeys infected with 106 PFU or medium without serum developed obvious clinical symptoms of RV infection, and other physiological characteristics, including body temperature and body weight, did not change significantly from 0-4 dpi in the experimental group compared to the control group (Supplementary Figure 1).

***RV shedding in feces of SA11-infected neonatal rhesus monkeys***

RV is transmitted through the fecal-oral route[27]. RV primarily infects human or animal small intestinal villi epithelial cells[28], and is expulsed through feces *in vivo*. The viral shedding observed in the feces reflects the replication and infection level of the virus in the body. We evaluated fecal viral shedding by ELISA in neonatal rhesus monkeys receiving either SA11 or medium without serum Neonatal rhesus monkeys infected with 108 PFU SA11 virus shed antigen beginning on 1 dpi and it lasted for seven days (Figure 1). A small amount of virus antigen was shed in the feces of neonatal rhesus monkeys infected with 107 PFU or 106 PFU SA11 strain on 1 dpi, and no virus antigen was detected in the control group (Figure 1).

***Distribution of RV antigen in the jejunum of SA11-infected neonatal rhesus monkeys***

The distribution of RV in the jejunum was detected by immunofluorescence to confirm that the RV infects villus epithelial cells in the small intestine. RV antigen was detected in the jejunum epithelial cells in the 108 PFU SA11-infected neonatal rhesus monkeys at 3 dpi. No RV antigen was detected in the jejunum epithelial cells in the control group (Figure 2).

***Viral load variations in the tissues of SA11-infected neonatal rhesus monkeys***

The viral load variations in various organs of the monkeys infected by 108 PFU SA11 were detected by qRT-PCR at 2 and 3 dpi to understand the transmission and distribution of RV across the various organs. We observed a viral load of approximately 5.85 × 103copies per 100 mg in the jejunum at 2 dpi, which was increased to 1.09 × 105 copies per 100 mg at 3 dpi (Figure 3). A relatively high viral load of 9.9 × 106 copies per 100 mg was seen in the mesenteric lymph node at 2 dpi, but was decreased to 2.42 × 106 copies per 100 mg at 3 dpi (Figure 3). A viral load of 1.02 × 105 copies per 100 mg in the kidney was detected at 2 dpi, but no viral load was detected in the kidney at 3 dpi (Figure 3). No virus was detected in the heart, liver, spleen, lung, or brain (Supplementary Table 2). We also collected blood samples at 12 hpi, 24 hpi, 48 hpi, and 72 hpi, and detected no viral load.

***Histopathological changes in the small intestine of SA11-infected neonatal Rhesus monkeys***

We examined the small intestine of the infected neonatal rhesus monkeys at 2 dpi and 3 dpi to confirm whether RV infection caused histopathological changes, and found inflation and swelling in the small intestines of some infected animals. We collected the tissues (duodenum, jejunum, and ileum) and examined the small intestine by HE staining. We found obvious pathological changes in the small intestinal tissues in the RV SA11 group compared to negative controls, including vacuoles, edema, atrophy, and breakage of the small intestinal villus cells, as well as an absence of obvious inflammatory cell infiltration (Figure 4).

***Apoptosis of jejunal epithelial cells increases during SA11 infection***

We used the TUNEL method to detect apoptosis of jejunal epithelial cells after SA11 infection. Apoptotic cells were not observed in the mock infection group (Figure 4). In infected animals, apoptosis of jejunal epithelial cells was not increased at 2 dpi (Figure 4), but at 3 dpi, apoptosis of the upper jejunal villus epithelial cells increased significantly, with apoptotic cells arranged in clusters along the villi top and some apoptotic cells detached from the jejunal villi (Figure 4).

DISCUSSION

A RV diarrhea model is crucial for the study of the pathogenic and immune protection mechanisms of RV infection and vaccine development. Various models have been used to study RV infection; however, these animal models are evolutionarily distant from humans and cannot simulate the process of RV infection in human infants. Therefore, a nonhuman primate RV diarrhea model is needed for the study of human RV infection.

RV infection is age-restricted[17]. We inoculated rhesus monkeys of various ages (15-20 d, 60 d, 120 d, and 1 year) with the same dose of SA11 strain in previous studies. Monkeys with an average age of 15-20 d were more sensitive to the SA11 strain and presented obvious clinical symptoms.

The SA11 is one of classic RV that several study have used it to model infection of newborn animals. They showed that 5 × 106 PFU infection of newborn mice and 108 PFU infection of newborn rats could induce obvious diarrhea[29,30]. In our present study, we used different doses of SA11 virus to infect neonatal rhesus monkeys based on our calculation of its DD50 (107.47 PFU/kg), and observed the most obvious symptoms in animals receiving 108 PFU (3.38DD50) of the virus. This dose was consistent with that used in newborn mice study, after equal conversion of body weight / metering. The clinical symptoms of RV-infected human infants include fever, vomiting, and diarrhea, with the clinical symptoms lasting for 7-14 d[31]. We observed a similar time course in the neonatal rhesus monkeys infected with SA11. Compared to the clinical symptoms observed in RV-infected infants, neonatal rhesus monkeys infected with SA11 suffered from diarrhea only. This symptom developed at 1 dpi, and the most serious diarrhea was observed from 2-3 dpi, which was alleviated and improved from 4 dpi. Viral shedding in the feces occurred from 1-7 dpi, and was the highest at 1 to 3 dpi. RV can cause weight loss in suckling mice during the early stages of infection[32]; however, the weight of the neonatal rhesus monkeys infected with SA11 was not significantly lower than the negative control group in this study. RV infection in human infants can also cause serious dehydration and even death in the absence of aggressive hydration. However, no monkeys died during these experiments, which may be related to the sensitivity of the virus strain or the immune state of the monkey. RV infection can cause viremia and results in lesions to the liver, gallbladder, respiratory system, nervous system, and urinary system[33].The occurrence of viremia depends on the virus strain and the immune state of the host[34,35]. RV escape the gastrointestinal tract through the blood and lymphatic system. Previous reports have detected RV in the blood of some children infected with RV in the clinic[36-38]; but no RV was found in the blood following infection in the current study. Furthermore, we determined the viral load of RV in the organs of the infected monkeys at 2 and 3 dpi. Our data showed that a viral RNA load of 1.02 × 105 copies per 100 mg in the kidney was detected at 2 dpi and a relatively high viral load was seen in the mesenteric lymph node at 2 dpi. So we speculate that RV SA11 was capable of escaping from the intestine and transmitted to the kidney *via* the mesenteric lymph nodes. A mechanism of the extra-intestinal spread of RV was discussed in a report of a neonatal mouse model of RV[39]. RRV and R7 RV can spread from the intestine to the terminal ileum, mesenteric lymph nodes, and to the peripheral tissues. Previous studies suggested that the transmission capacity of RV in the neonatal mouse was related to the NSP3 and VP6 regions[40], and whether they facilitate RV transmission in the monkeys remains to be examined.

Diarrhea is one of the most typical symptoms of RV infection. Early studies have shown that RV infection leads to shortened intestinal villi and loss of epithelial cells at the top of the villus[28]. In the current study, a large amount of vacuolization, cell edema, and intestinal villus atrophy, and various degrees of breakage occurred in the villus cells of neonatal rhesus monkeys infected with SA11. Apoptosis of small intestinal epithelial cells is also a cause of diarrhea[41], and apoptosis has been observed in RV-infected HT-29 cells[42-45]. We analyzed the apoptosis of small intestinal epithelial cells after infection, and observed apoptosis in the apical layer of the intestinal villi epithelial cells.

In conclusion, our results indicate that we successfully established a RV SA11 strain diarrhea model in neonatal rhesus monkeys. The RV infection model we established was useful for us to further investigate the RV infection mechanism and evaluate the cross protection of potential HRV vaccine candidates[29,46].

**ARTICLE HIGHLIGHTS**

***Research background***

Rotavirus (RV) is one of the main pathogens responsible for severe diarrhea in children under 5 years of age. There are currently no specific drugs for the treatment of diarrhea caused by RV infection. Therefore, the development of safe and effective vaccines to control RV infection is particularly important. Furthermore, an effective animal model of RV infection is needed to ensure the effectiveness and safety of these vaccines.

***Research motivation***

Nonhuman primates are the animals most closely related to humans and have advantages over non-primates as an animal model of RV diarrhea, so development of nonhuman primates animal model of RV infection is needed to ensure the effectiveness and safety of RV candidate vaccines.

***Research objectives***

To establish a monkey model of RV Infection.

***Research methods***

Neonatal rhesus monkeys with an average age of 15-20 d and an average weight of 500 g ± 150 g received intragastric administration of varying doses of SA11 RV to determine whether the SA11 strain can effectively infect these animals by observing their clinical symptoms, fecal shedding of virus antigen by ELISA, distribution of RV antigen in the organs by immunofluorescence, variations of viral RNA load in the organs by qRT-PCR, histopathological changes in the small intestine by HE stain, and apoptosis of small intestinal epithelial cells by TUNEL.

***Research results***

RV monkey model showed typical clinical diarrhea symptoms in the 108 PFU SA11 group, where we observed diarrhea 1-4 d post infection (dpi) and viral antigen shed in the feces from 1-7 dpi. RV was found in jejunum epithelial cells. We observed a viral load of approximately 5.85 × 103 copies per 100 mg in the jejunum at 2 dpi, which was increased to 1.09 × 105 copies per 100 mg at 3 dpi. A relatively high viral load was also seen in the mesenteric lymph node at 2 dpi and 3 dpi. The following histopathological changes were observed in the small intestine following intragastric administration of SA11 RV: Vacuoles, edema, and atrophy. Apoptosis in the jejunal villus epithelium was also detectable at 3 dpi.

***Research conclusions***

We successfully established a RV SA11 strain diarrhea model in neonatal rhesus monkeys.

***Research perspectives***

The monkey model of RV infection was useful for us to further investigate the RV infection mechanism and evaluate the protection of potential HRV vaccine candidates.

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**Figure 1** **Rotavirus antigen-shedding in feces of neonatal rhesus monkeys inoculated with SA11 or medium without serum from 0 dpi to 14 dpi.** A: 10 mL of 108 PFU of SA11; B: 10 mL of 107 PFU of SA11; C: 10 mL of 106 PFU of SA11; D: 10 mL of medium without serum. PFU: Plaque forming unit.





**Figure 2 Immunofluorescence of rotavirus antigen in the jejunum of neonatal rhesus monkeys inoculated with SA11 or medium without serum.** A-C: The jejunum of neonatal rhesus monkeys inoculated with medium without serum at 3 dpi; D-F: Jejunum of neonatal rhesus monkeys inoculated with 108 PFU SA11/monkey at 3 dpi. The glass slides were incubated with goat anti-rotavirus (RV) polyclonal antibody and then incubated with rabbit anti-goat IgG antibody labeled with FITC (green). Cell nuclei are shown with DAPI staining (blue). White arrows indicate representative RV-positive cells. Magnification × 20. Bar: 50 μm.



**Figure 3** **Comparison of viral load in different organ of neonatal rhesus monkeys inoculated with SA11 or medium without serum.** A: Viral load in jejunum of neonatal rhesus monkeys inoculated with SA11 or medium without serum at 2 dpi and 3 dpi; B: Viral load in kidney of neonatal rhesus monkeys inoculated with SA11 or medium without serum at 2 dpi and 3 dpi; C: Viral load in MLN of neonatal rhesus monkeys inoculated with SA11 or medium without serum at 2 dpi and 3 dpi. Data are expressed as the mean ± SD, *n* = 3, b*P* < 0.01.



**Figure 4** **Histopathological changes and apoptosis of small intestine of neonatal rhesus monkeys infected with SA11 or medium without serum.** A: duodenum of neonatal rhesus monkeys infected with medium without serum at 3 dpi; B: Jejunum of neonatal rhesus monkeys infected with medium without serum at 3 dpi; C: Ileum of neonatal rhesus monkeys infected with medium without serum at 3 dpi; D: Duodenum of neonatal rhesus monkeys infected with 108 PFU SA11 at 3 dpi; E: Jejunum of neonatal rhesus monkeys infected with 108 PFU SA11 at 3 dpi; F: Ileum of neonatal rhesus monkeys infected with 108 PFU SA11 at 3 dpi; histopathological changes in the small intestinal tissues including vacuoles (white arrow), edema, atrophy and breakage of the small intestinal villus cells (black arrow); G-J: Apoptosis of jejunal epithelial cells during SA11 infection detected by TUNEL; G: Inoculated with medium without serum at 2 dpi; H: Inoculated with medium without serum at 3 dpi; I: Inoculated with 108 PFU SA11 at 2 dpi; J: Inoculated with 108 PFU SA11 at 3 dpi; the numbers of the jejunal villus epithelial apoptosis cells increased significantly at 3 dpi. Magnification × 20. Bar: 50 μm.

**Table1 Diarrhea score of the neonatal rhesus monkeys from 1 d post infection to 4 d post infection**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Dose (PFU)** | **Monkey ID** | **Diarrhea score1** | **Mean diarrhea score2** | **Percentage with diarrhea (%)3** |
| **1 dpi** | **2 dpi** | **3 dpi** | **4 dpi** | **1 dpi** | **2 dpi** | **3 dpi** | **4 dpi** | **1 dpi** | **2 dpi** | **3 dpi** | **4 dpi** |
| 108 | 17020 | 4 | 4 | 3 | 2 |  |  |  |  |  |  |  |  |
| 17011 | 3 | 4 | 4 | - | 3.67 | 4 | 3.5 | - | 100 | 100 | 100 | - |
| 17015 | 4 | 4 | - | - |  |  |  |  |  |  |  |  |
| 107 | 17018 | 2 | 0 | 0 | 1 |  |  |  |  |  |  |  |  |
| 17003 | 2 | 0 | 0 | 1 | 2.67 | 0.67 | 0.33 | 0.67 | 100 | 33.3 | 0 | 0 |
| 17001 | 4 | 2 | 1 | 1 |  |  |  |  |  |  |  |  |
| 106 | 17008 | 0 | 2 | 0 | 1 |  |  |  |  |  |  |  |  |
| 17012 | 0 | 1 | 2 | 0 | 0 | 1.33 | 1 | 0.33 | 0 | 33.3 | 33.3 | 0 |
| 17016 | 0 | 1 | 1 | 0 |  |  |  |  |  |  |  |  |
| 0 | 17006 | 0 | 0 | 0 | 0 |  |  |  |  |  |  |  |  |
| 17002 | 0 | 0 | 0 | - | 0 | 0 | 0 | - | 0 | 0 | 0 | - |
| 17004 | 1 | 0 | - | - |  |  |  |  |  |  |  |  |

10 (failure to collect feces by gently pressing the abdomen of the neonatal rhesus monkeys, we collected feces from the bottom of the incubator); 1 was considered no diarrhea (brown hard feces); 2 was considered common diarrhea(soft feces); 3 was considered severe diarrhea(loose feces); 4 was considered very severe diarrhea (watery feces); 2Mean diarrhea score: The sum of all diarrhea or not-diarrhea scores/*n* (*n* = the number of total samples); 3Percentage with diarrhea (%): The number of diarrhea monkeys/the total number of monkeys in this group; -: The monkey was sacrificed for histologicalanalysis.