Rotavirus Infection Enhances Lipopolysaccharide-Induced Intussusception in a Mouse Model

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Unexpected reports of intussusception after vaccination with the live tetravalent rotavirus vaccine Rota-Shield resulted in voluntary withdrawal of the vaccine. Intussusception, a condition in which the intestine acutely invaginates upon itself, is the most common cause of intestinal obstruction in children. We report here the development of a mouse model to study rotavirus-induced intussusception. In this model, both homologous murine and heterologous simian rotavirus strains significantly enhanced the rate of lipopolysaccharide (LPS)-induced intussusception, and this enhancement was replication dependent, requiring rotavirus doses of greater than one 50% infectious dose. Rotavirus-induced intussusceptions did not have observable lymphoid lead points, despite the induction of intestinal lymphoid hyperplasia after rotavirus infection. Intussusceptions are also postulated to result from altered intestinal motility, but rotavirus infection had no effect on gastrointestinal transit. LPS-induced intussusception is associated with the induction of inflammatory mediators, and intussusception rates can be modified by inflammatory antagonists. We show that rotavirus infection significantly enhanced serum tumor necrosis factor alpha and gamma interferon cytokine levels after LPS treatment compared to uninfected mice. Together, these data suggest that rotavirus infection sensitized mice to the inflammatory effects of subsequent LPS treatment to enhance intussusception rates.

Intussusception is the most common cause of intestinal obstruction in children 3 months to 3 years of age, with a peak incidence between 5 and 9 months of age (6, 75). The most frequent, but not exclusive, site of intussusception in children is the ileum, particularly at the ileocecal valve. Intussusception rates vary based on geographical location and other risk factors, including sex, diet, environment, developmental factors, and concurrent viral, bacterial, or parasitic infections (6, 11, 70, 71, 87). In developed countries, the incidence of intussusception is 0.5 to 4.3 cases per 1,000 live births (6).

A suspected etiologic role of infectious agents in intussusceptions is based on detection of bacterial, viral, or parasitic infections in children with intussusception. Bacteria isolated from cases of intussusception include Yersinia enterocolitica and Y. pseudotuberculosis, Clostridium difficile, Mycobacterium avium, Aeromonas spp., Salmonella enterica serovar Typhimurium, Escherichia coli, Shigella spp., and Pasteurella pseudotuberculosis (also known as Malassez and Vignal bacillus) (12, 39, 45, 48, 75, 86, 90). However, direct experimental evidence of a causal role of infectious agents in intussusception is limited to (i) serovar Typhimurium infection of mice, where colonic intussusceptions are reported in 3% of infected mice (3), and (ii) small intestinal and colonic intussusceptions induced by extraintestinal exposure of mice and rats to lipopolysaccharides (LPS) or endotoxin (42, 64, 94). Endotoxemia has been noted in children with intussusception, although it is not known whether this precedes or is coincident with intussusception (14, 98).

Viral infections have also been documented in children with intussusception. Adenovirus is most frequently clinically associated with intussusception (in 17 to 59% of cases) in children and, unexpectedly, there is a higher association (>50%) with respiratory, not intestinal, strains of adenovirus (4, 10, 29, 33, 49). Other viruses found coincidentally with intussusception in either children or animals include enterovirus, human immunodeficiency virus, equine herpesvirus, Epstein-Barr virus, cytomegalovirus, coronavirus, human herpesvirus 6, human herpesvirus 7, and rotavirus (24, 33, 37, 38, 49, 53, 63, 86). However, there is currently no direct experimental evidence for an etiologic role of any of these viruses in intussusception.

Rotavirus infects the small intestine and is a leading cause of severe dehydrating diarrhea in young children (19, 85, 97). Rotavirus infections occur frequently in children younger than 1 year of age, who are at highest risk for intussusception (6, 11, 16, 34, 36, 59). Three case series studies implicated rotavirus infections in 3.9, or 37% of children with intussusception (37, 53, 63, 86). However, recent epidemiological studies of intussusception in the United States failed to support an etiologic role for natural rotavirus infection in intussusception, and intussusception cases do not peak temporally coincident with the large number of winter rotavirus cases in temperate climates (15, 53, 69, 74, 76, 91). The lack of case controls in the case series studies and the limited ability of ecological studies to define strong associations for infrequent events confounds the
interpretation of the role of rotavirus in intussusception (31, 57, 76, 82, 83). Therefore, whether natural rotavirus infection plays an etiologic role in intussusception is still controversial. However, a transient intussusception was noted in one child with rotavirus diarrhea participating in an ultrasound study. This study also found increased bowel wall thickness and lymphadenitis in rotavirus-infected children compared to control children, suggesting that these changes could contribute to intussusception (77).

In 1998, a live attenuated, tetravalent, human rotavirus reassortant vaccine, RotaShield, based on rhesus rotavirus (RRV), was licensed in the United States, and approximately 1.5 million doses of RotaShield vaccine were administered to young children. The vaccine was withdrawn from the market a year later after reports of 112 cases of intussusception in vaccinees; 32 children required surgery, and one child died (13, 55, 65, 100). Intussusception was temporally related to RotaShield vaccination, occurring most frequently 3 to 7 days after the first dose of vaccine, with an ~25-fold increased risk compared to unvaccinated children (40, 55–57). Although there was a clear temporal association of RotaShield vaccination with intussusception, the causal role of RotaShield in intussusception in vaccinated children is still debated. RRV was detected by reverse transcription-PCR in the tissues of seven of eight of the RotaShield-vaccinated children who developed intussusception, but this study failed to identify a pathogenic mechanism of RotaShield-associated intussusception (44). Due to lack of overwhelming evidence linking rotavirus infections and intussusception, it has been argued that the vaccine was simply a trigger in children predisposed to the development of intussusception (79, 84). Age at first RotaShield vaccination was a risk factor for intussusception, since all cases occurred in children >60 days old and most frequently in children between 3 and 6 months of age (55, 79, 84), an age when intussusceptions peak in children (11, 16, 34, 36, 59). The RotaShield vaccine experience supports a possible association of rotavirus infection with intussusception and raises the possibility that rotavirus infection may contribute to intussusception in children in which other risk factors are present and necessary for development of intussusception. The three main theories proposed to explain why the RRV-based vaccine may have triggered intussusception in RotaShield-vaccinated children are (i) intussusception was uniquely due to infection with the RRV strain, (ii) the large bolus dose of the vaccine initiated an unusual response that induced intussusception, and (iii) rotavirus replication contributes to intussusception (66).

To investigate a possible causative role of rotavirus in intussusception, we established a mouse model of intussusception based on the adult mouse rotavirus model. This infection-only (i.e., no diarrheal disease is observed) model was used to determine whether the rate or nature of intussusceptions were altered by viral or host factors hypothesized to be responsible for the RotaShield- or rotavirus-associated intussusceptions and intussusception in general. Because intussusceptions associated with rotavirus infections in children and animals appear to be rare, we hypothesized that cofactors would be required to induce intussusception, and we tested LPS. LPS, an outer membrane component of gram-negative bacteria, administered intraperitoneally (10 to 12 mg/kg) to adult mice and rats induces a transient intestinal intussusception in 20 to 40% of animals at 6 to 9 h after LPS administration (42, 64, 94). At 15 h after LPS administration, intussusceptions are not present, although the mortality rate in LPS-treated mice is ~5% due to the effects of endotoxic shock (64). The development of LPS-induced intussusception is associated with alterations in gastrointestinal transit and the induction of inflammatory mediators, including tumor necrosis factor alpha (TNF-α), platelet activation factor, nitric oxide, and hemoxigenase, but not lymphoid hyperplasia or a recognizable anatomical lead point (42, 64, 73, 89, 94).

We show here that rotavirus infection combined with LPS treatment of adult mice increased the rates of intussusception compared to rotavirus infection or LPS treatment alone, and we further demonstrate that rotavirus-associated intussusception does not appear to be mediated by global changes in gastrointestinal transit or hyperplasia of intestinal lymph nodes or Peyer’s patches. Rotavirus infection exacerbated the early inflammatory responses to LPS and increased the rate of intussusception in mice, supporting a possible inflammatory mechanism for rotavirus-enhanced intussusception.

**MATERIALS AND METHODS**

**Mice.** Specific-pathogen-free female outbred CD-1 or inbred BALB/c mice were obtained from Charles River Laboratories (Portage, MI) and were 6 to 8 weeks of age at the time these studies were undertaken. Mice were divided randomly into treatment groups, housed in microisolator cages, and provided with autoclaved water and laboratory autoclaveable rodent diet 5010 (Purina Mills, Inc., St. Louis, MO) ad libitum. For rotavirus infections, the mice were moved to a physically separated animal facility and housed under BSL-2 conditions.

**Viruses.** A murine ECwt (P[17], G3) rotavirus stock was produced, and the 50% infectious doses (ID50) was determined in mice as described previously (68). The EDIMwt (P[17], G3) virus stock used was kindly characterized and supplied by Richard Ward (96). Simian rotavirus RRV (P[3]), G3 (88), and SA11 clone 3 (P3B[2], G3) [22] tissue culture-adapted rotaviruses were cultivated in fetal rhesus monkey kidney (MA104) cells in the presence of trypsin as previously described (23). RRV triple-layered particles, composed of the viral genome and all rotavirus structural proteins, were purified by using isopycnic CsCl followed by sucrose gradient centrifugation (18). To compare the activity of an equivalent dose of replication-competent and inactivated virus, a portion of a single purified RRV triple-layered particle preparation was inactivated by using UV exposure and 4’-aminomethyltrioxsalin hydrochloride treatment as previously described (18). Virus inactivation was confirmed by a single blind passage of virus in MA104 cells, followed by a fluorescent-focus assay. Rotavirus titers were determined by using standard plaque assay or by fluorescent-focus assay (18).

**Oral inoculations.** Mice were orally inoculated with 0.1 ml of phosphate-buffered saline (PBS), tissue culture-adapted rotavirus stocks (doses per mouse: for RRV, 1.2 x 10⁷, 1.2 x 10⁶, 1.2 x 10⁵, or 1.2 x 10⁴ PFU; for SA11, 1.3 x 10⁷, 1.3 x 10⁶, 1.3 x 10⁵, or 1.3 x 10⁴ PFU), homologous ECwt (dose per mouse: 10⁷ ID50 or EDIMwt, rotavirus (dose per mouse: 10⁶ ID50 as indicated. The ID50 in CD-1 mice were 10⁶.5 PFU for RRV and ≥10⁷ PFU for SA11. The ID50 of all rotavirus strains were determined based on detection of virus excretion in stool and/or development of rotavirus-specific antibody responses after oral inoculation with serial decreasing doses of rotavirus (data not shown).

**LPS treatment.** As previously described (42, 64), mice were injected intraperitoneally with E. coli LPS at 12 mg/kg (~240 mg/mouse; Sigma, St. Louis, MO). Intussusception was assessed visually by laparotomy following cervical dislocation without anesthesia at 6 or 15 h after LPS injection. The choices for the dose of LPS and timing of the intussusception assessment were those previously reported to induce maximal effects (42). No other LPS doses or timings were tested in the rotavirus model because the initial experiments worked and large numbers of mice would have been needed to test alternative protocols. Based on an LPS-induced intussusception rate in CD-1 mice of 3 to 10% (mean 7%) and a rate of intussusception in mice given both ECwt rotavirus and LPS (RV/LPS) of 15 to 19% that was always significantly greater than the LPS rate, it was determined statistically that the appropriate sample size per group to detect an approximately two- to threefold difference in rate between LPS and RV/LPS treatment groups was n ≥ 60. The statistical analyses also indicated that the time
that the TNF-α (Biosource, Belgium) and IFN-γ levels were determined by enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, and Endogen, Woburn, MA, respectively) according to the manufacturer’s directions, except that the TNF-α coating antibody was used at 1.6 μg/ml. Fecal rotavirus antigen shedding and total rotavirus-specific antibody titers were assessed in individual mice by using established ELISAs (68).

**Data analysis.** Data are presented as means ± the standard deviation or the standard error of the mean, as indicated. Statistical comparisons of rates of intussusception were analyzed by a two-sided Fisher exact test. Statistical comparisons of cell numbers and geometric mean center scores were analyzed by analysis of variance using SigmaStat for Windows (version 2.03). Differences in cytokine levels were determined by using a two-sided Student t test. Statistical significance was set at P ≤ 0.05.

**RESULTS**

**Rotavirus contributes to the formation of intussusception.** To test whether rotavirus infection could increase rates of LPS-induced intussusception, outbred adult female CD-1 mice were inoculated orally with PBS or murine EC wt rotavirus and, at 3 days postinoculation (dpi), mice were injected intraperitoneally with LPS. Intussusceptions were noted in both PBS/EC wt groups at 6 h after LPS injection. All intussusceptions, irrespective of the treatment groups, were identical in gross appearance (Fig. 1A and B), location (primarily in the jejunum or ileum), length (0.5 to 2.4 cm), and histopathological findings (i.e., acute intussusception with no notable inflammatory or structural changes, Fig. 1C and D).

Intussusception rates were significantly elevated in mice given either 10^5 or 10^6 ID50 of EC wt rotavirus and LPS (EC wt/ LPS) compared to those given LPS treatment alone (P = 0.0076 and P = 0.0005, respectively; Fig. 2A). Oral inoculation with PBS or 10^5 ID50 of EC wt, followed by PBS administered intraperitoneally at 3 dpi (PBS/PBS and PBS/EC wt, respectively) failed to induce intussusception (data not shown and Fig. 2A). A second murine rotavirus strain, EDIM wt (EDIM/ LPS) compared to those given LPS treatment alone (P = 0.0076 and P = 0.0005, respectively; Fig. 2A). Oral inoculation with PBS or 10^5 ID50 of EC wt, followed by PBS administered intraperitoneally at 3 dpi (PBS/PBS and PBS/EC wt, respectively) failed to induce intussusception (data not shown and Fig. 2A). A second murine rotavirus strain, EDIM wt (EDIM/ LPS), also significantly enhanced the intussusception rate compared to PBS/LPS mice (P = 0.0162, Fig. 2B). The rates of LPS

**FIG. 1.** There were no distinct differences in the gross appearance or histology of the intussusceptions in PBS- or rotavirus-inoculated mice. (A and B) Intussusceptions induced in PBS (A)- or EC wt rotavirus (B)-inoculated CD-1 mice treated with LPS. Double intussusceptions (see panel A) occurred at a low rate in both PBS- and rotavirus-inoculated mice. The arrow indicates the direction of the intussusception, and the dotted circle indicates the circumference of a Peyer’s patch. (C and D) Histological examination of the intussusceptions from PBS (C)- or EC wt rotavirus (D)-inoculated mice treated with LPS did not reveal significant inflammatory changes, suggesting the intussusceptions were acute.

The circumference of a Peyer’s patch. (C and D) Histological examination of the intussusceptions from PBS (C)- or EC wt rotavirus (D)-inoculated mice treated with LPS did not reveal significant inflammatory changes, suggesting the intussusceptions were acute.
intussusception in PBS- or murine EDIM wt rotavirus-inoculated (10^4 ID50) mice at 3 dpi with or without LPS administration, as indicated (n = 118 to 120 for each bar). (B) Incidence of intussusception in PBS- or murine EDIM wt rotavirus-inoculated (10^6 ID50) mice (n = 119 to 120 for each bar). (C) Incidence of intussusception in PBS- or ECwt rotavirus-inoculated (10^5 ID50) BALB/c mice treated with LPS for 6 h on 3 dpi (n = 119 to 121 for each bar). * P ≤ 0.05 (determined using the Fisher exact test to compare the RV/LPS and PBS/LPS groups).

FIG. 2. Rotavirus is a cofactor for the induction of intussusception. CD-1 mice were orally inoculated with PBS (■) or rotavirus (■) prior to LPS treatment. At 6 h after LPS treatment, laparotomy was performed, and intestines were examined visually for intussusception. (A) Incidence of intussusception in PBS or murine ECwt rotavirus-inoculated (10^2 or 10^3 ID50) mice at 3 dpi with or without LPS administration, as indicated (n = 118 to 120 for each bar). (B) Incidence of intussusception in PBS- or murine EDIM wt, rotavirus-inoculated (10^6 ID50) mice (n = 119 to 120 for each bar). (C) Incidence of intussusception in PBS- or ECwt, rotavirus-inoculated (10^5 ID50) BALB/c mice treated with LPS for 6 h on 3 dpi (n = 119 to 121 for each bar). * P ≤ 0.05 (determined using the Fisher exact test to compare the RV/LPS and PBS/LPS groups).

(PBS/LPS)-induced intussusception in CD-1 mice varied among individual experiments (range, 3 to 10%; Fig. 2A and B).

In contrast to the 3 to 10% rate of LPS-induced intussusception we observed in CD-1 mice, the reported rate of intussusception in BALB/c mice is ~25% (42, 64), suggesting that genetics may be an important risk factor for intussusception in mice. We assessed whether host genetics impacted the rate of intestinal intussusceptions by determining the intussusception rates of inbred BALB/c under the same environmental conditions as the CD-1 outbred mice (Fig. 2C). We found the rate of intussusception in PBS/LPS BALB/c mice to be 28% (33 of 119), similar to the previous reports. Therefore, host genetics is an important risk factor for LPS-induced intussusception in mice. Analogous to our results in CD-1 mice, the rates of intussusception in the BALB/c mice were consistently, but not significantly, elevated in ECwt/LPS mice (11/31, 11/31, and 22/61; total = 44/123 [35.7%]) compared to PBS/LPS mice [10/30, 8/30, and 17/60; total = 35/120 (29.2%); P = 0.22]. Because the rotavirus enhancement of the LPS-induced intussusception rates in BALB/c mice was <2-fold, larger group sizes will be needed to determine whether rotavirus significantly enhances intussusception rates in BALB/c mice.

Heterologous rotaviruses induce intussusception, and induction of intussusception is dependent on rotavirus dose and replication. The RotaShield vaccine was a tetravalent live virus vaccine composed of RRV and three human RRV reassortant rotaviruses. To test whether RRV in conjunction with LPS would increase the rate of intussusception in mice, CD-1 mice were orally inoculated with RRV and treated with PBS, as described for the murine rotaviruses. The rate of intussusception was significantly elevated in mice infected with 10^7 PFU (~10 ID50) of RRV compared to PBS/LPS mice (15% versus 4.2%; P = 0.003, Fig. 3A). A second heterologous (G3) simian rotavirus, SA11, also enhanced the rate of intussusception when administered at the same high dose (P = 0.042, Fig. 3B). To determine whether heterologous rotavirus-induced intussusception was dose dependent, mice were inoculated with decreasing doses of simian RRV or SA11. The minimum doses required to significantly increase the rate of intussusception were 10^6 and 10^7 PFU/mouse for simian RRV and SA11, respectively (Fig. 3A and B). These doses were approximately equivalent to the ID50 of each virus strain, 10^3 and ~10^7 PFU, respectively, in the CD-1 mice.

The finding that the viral ID50 and the virus doses required for the induction of intussusception were similar suggested that the induction of intussusception by rotavirus was replication dependent. This hypothesis was tested directly by orally inoculating mice with purified live (10^7 PFU) or inactivated RRV at a dose equivalent to 10^7 PFU (Fig. 3C). Live, but not inactivated, RRV in combination with LPS treatment significantly enhanced the rate of intussusception compared to PBS/LPS mice. Therefore, enhancement of intussusception by heterologous RRV was dependent on live, replication-competent virus.

Concurrent rotavirus infection and LPS treatment induced transient intussusceptions. LPS induces transient intussusception in the BALB/c mouse model where intussusception rates decrease from ~25% at 6 h to 0% at 15 h after LPS injection (42). To determine whether the combined RV/LPS treatment would extend the duration or increase the severity of intussusceptions compared to LPS administration alone, CD-1 mice were inoculated with PBS or ECwt rotavirus and injected with LPS at 3 dpi, and laparotomy was performed 15 h later. There was no difference in the intussusception rate at 15 h between the PBS/LPS (6.7% [8/120]) and RV/LPS (7.5% [9/119]) groups (P = 0.79). All intussusceptions observed at 15 hpi were grossly similar to each other and to those seen at 6 hpi.

Hyperplastic lymph nodes are not mechanical lead points of intussusception. Hyperplastic lymph nodes are often observed in intussusception and can be anatomical or mechanical lead points for intussusception (30, 49, 70). Rotavirus-infected infants exhibit lymphadenopathy and an increase in distal ileum wall thickness compared to healthy infants that might contrib-
Lymphoid hyperplasia was not detected in mice inoculated with either RotaShield or WC3-PV (Rota Teq) (52). However, we have demonstrated that both homologous and heterologous rotavirus infection of mice cause significant lymphoid hyperplasia of Peyer’s patches and mesenteric lymph nodes between 2 and 4 dpi (7, 9; also data not shown). To determine whether hyperplastic lymph nodes were involved in rotavirus-associated intussusceptions, we examined a large number of intussusceptions for the involvement of hyperplastic lymph nodes. Only 1 of 287 RV/LPS intussusceptions and no LPS-associated intussusceptions involved a Peyer’s patch as a possible anatomical lead point. Lymphoid hyperplasia induced by rotavirus was not enhanced by LPS treatment, nor was it induced by LPS (42; also data not shown). Our data indicate that hyperplastic lymph nodes were not mechanical lead points for the intussusceptions induced by LPS or RV/LPS in our model, which is similar to findings in RotaShield-vaccinated children with intussusception (44, 55).

Rotavirus infection does not alter gastrointestinal transit in adult mice. Alteration of intestinal motility is postulated to be a mechanism of intussusception in children and the LPS mouse model (42, 87). Rotavirus infection affects gastrointestinal motility in children, delaying gastric emptying and decreasing total intestinal transit (2, 47). We determined whether gastrointestinal motility was altered in rotavirus-infected mice. Gastrointestinal transit was compared between uninfected (PBS or PBS/LPS) and ECwt rotavirus-infected (RV or RV/LPS) adult CD-1 or BALB/c mice after the oral administration of FITC-dextran (50). In both strains of mice, gastrointestinal transit in PBS/LPS mice was significantly decreased compared to PBS mice (Fig. 4), a finding similar to published data in BALB/c mice (42). Gastrointestinal transit was not consistently altered in RV-infected mice compared to PBS mice, and RV/LPS treatment did not synergistically alter gastrointestinal transit compared to PBS/LPS mice (Fig. 4). Therefore, rotavirus did not alter gastrointestinal transit in adult mice in the presence or absence of LPS. This suggests that rotavirus does not contribute to intussusception by altering intestinal motility and that gastrointestinal transit is not a predictive measure of changes in motility resulting in intussusception due to rotavirus infection.

Rotavirus infection sensitizes mice to the effects of LPS. TNF-α, platelet activation factor, and nitric oxide are all individually identified as mediators of LPS-induced intussusception (64, 73, 89, 94). Besides intussusception, treatment of mice with LPS can induce inflammation, toxic shock, and death (27). We noted that at 15 hpi, the mortality rate in RV/LPS mice was significantly increased compared to PBS mice (Fig. 5A). Sensitization of mice to LPS (2.5 to 10 mg/kg)-induced mortality by vesicular stomatitis virus and lymphocytic choriomeningitis virus is attributed to increases in IFN-α/β and IFN-γ levels, which subsequently enhance TNF-α production (21, 60, 61). Therefore, we determined whether rotavirus infection enhanced the serum TNF-α or IFN-γ cytokine response to LPS. Neither TNF-α nor IFN-γ were detected in the sera of RV-infected mice (data not shown). Both serum TNF-α and IFN-γ levels were significantly elevated in RV/LPS mice compared to LPS mice between 1 to 6 h after LPS administration (Fig. 5B and C). The enhanced cytokine responses to LPS in virus-
infected mice may be a mechanism by which rotavirus infection enhanced LPS-induced mortality and intussusception rates.

**DISCUSSION**

Although natural rotavirus infections have been associated with intussusception in earlier phenotypic, observational studies (37, 38, 63), recent epidemiological studies fail to support a major etiologic role for rotavirus in intussusception (15–17, 34, 62, 76, 91). Although controversial, the temporal association of the live attenuated rotavirus vaccine, RotaShield, in particular the first dose, with LPS administration, suggested a role for rotavirus in the induction of intussusception (5, 54–57, 79, 84). Here, we provide direct experimental evidence in a mouse model that rotavirus contributes to the induction of LPS-induced intussusception. Rotavirus enhancement of intussusception rates occurred with two wild-type murine and two simian rotavirus strains, one of which was the parental strain for the RotaShield vaccine strains. Viral replication was essential for the enhancement of intussusception, and a concurrent rotavirus infection enhanced the inflammatory cytokine response induced by LPS. The data obtained from this model lend support to the hypothesis that rotavirus infection plays an etiologic role in intussusception.

The failure of rotavirus infection without LPS administra-
tion to induce intussusception in mice suggests that either rotavirus infection alone induced intussusceptions at a low rate that was not detectable in our studies, at different times than we examined, or that it was important in, but not sufficient to cause, intussusception. If the latter is true, it may explain why rotavirus infections are not strongly associated with intussusception and why there is not a peak of intussusception cases coincident with seasonal peaks of rotavirus cases (15–17, 34, 53, 62, 63, 72, 76, 91). Whether exposure to LPS is a risk factor for intussusception in children with or without rotavirus infection is not known. Endotoxia is noted in patients with obstructing intussusception, but whether it is the result of translocation of bacteria across the intestine damaged by the intussusception or it is the initiating event leading to intussusception and the level of endotoxemia at which this might occur is not known (14, 98). Mice are highly resistant to the effects of LPS compared to humans or other animal species (e.g., the lethal dose in humans [1 to 2 µg] greatly exceeds a uniformly lethal dose in mice [~500 µg]) (20, 26). Therefore, the LPS dose required to induce intussusception in mice, which can also induce endotoxic shock, likely is not predictive of and greatly exceeds the dose that might induce intussusception in humans. To our knowledge, endotoxia has not been examined in rotavirus-infected or RotaShield-vaccinated children with intussusception. Concurrent bacterial and rotavirus infections have been documented, and rotavirus can enhance the invasiveness or disease severity associated with Yersinia enterocolitica and Yersinia pseudotuberculosis, Escherichia coli, Salmonella species, and Cryptosporidium species (28, 32, 41, 43, 51, 67, 99), most of which are also associated with intussusception in children (39, 49, 75, 86). Our model of rotavirus infection suggests that the concurrent presence of rotavirus and a bacterial product results in a synergistic effect on the induction of intussusception. This finding suggests the need for future studies to determine whether concurrent rotavirus and bacterial infections contribute to intussusception in children.

Genetics are thought to play a role in the development of intussusception in children (6, 11, 81). Based on the differences we observed in the LPS-induced intussusception rates of CD-1 (7%) and BALB/c (28%) mice, there is clearly a genetic disposition to development of LPS-induced intussusception in mice. Rotavirus consistently enhanced the rate of intussusception above the background rate with LPS in both BALB/c and CD-1 strains of mice, but confirmation of a significant enhancement in BALB/c mice will require larger group sizes. Therefore, whether host genetics is a risk factor for rotavirus-associated intussusception in mice remains to be determined. Our investigations were limited to two strains of mice and other strains of mice may demonstrate greater or lesser sensitivity to rotavirus enhancement of intussusception. Future studies in additional strains of mice will be necessary to elucidate potential genetic factors that play a role in the link between rotavirus and intussusception.

Intussusception in children is postulated to occur as a result of alterations in gastrointestinal motility, lymphoid hyperplasia as lead points, or inflammation (11). We examined whether any of these potential mechanism were associated with the increase in intussusception observed during rotavirus infection in LPS-injected mice. Rotavirus did not contribute to intussusception by synergistically altering intestinal motility or by an association with a hyperplastic Peyer’s patch as a lead point. In contrast, rotavirus infection enhanced TNF-α and IFN-γ levels in the circulation of LPS-treated, rotavirus-infected mice compared to mice treated with LPS alone. Modulation of TNF-α, nitric oxide, and other inflammatory factors significantly alters the rates of LPS-induced intussusception in rodents (64, 73, 89, 94). Therefore, our data suggest that inflammation may be a critical component of the mechanism of rotavirus-associated intussusception. The apparent lack of intussusception in rotavirus-infected mice further suggested that the inflammatory response to rotavirus infection alone may not be sufficient to cause intussusception. In support of this hypothesis, rotavirus infection failed to induce measurable TNF-α or IFN-γ levels in mouse sera. Together, these data support previous conclusions from the LPS model that suggest that the level of inflammatory mediators influences the rate of intussusception in mice. In children with intussusception not related to rotavirus or Rota- Shield vaccination, C-reactive protein (CRP), interleukin-6 (IL-6), and neopterin are significantly elevated in sera, and CRP levels correlate positively with intussusception disease severity, suggesting that more severe inflammatory responses yield more severe intussusceptions (98). The levels of CRP, nitric oxide, IL-6, IFN-α, TNF-α, and IFN-γ in serum are elevated in children with rotavirus-induced diarrhea (35, 46, 78). Whether increased cytokine levels correlate with a risk of intussusception in children vaccinated with RotaShield or naturally infected with rotavirus has not been examined but should be tested.

Enhancement of intussusception rates in the mouse model required rotavirus replication, suggesting that the host response to infection, not simply the presence of the virus, was necessary for intussusception. Consistent with our data, the RotaShield vaccine strains replicate in and are excreted by vaccinated children (95) and were detected by reverse transcription-PCR in seven of eight intestines from RotaShield-vaccinated children (44). Unexpectedly, rotavirus excretion in the stool was not a necessary corollary of intussusception in mice, since not all rotavirus-infected mice with intussusception had detectable levels of fecal antigen (data not shown). This observation raises the possibility that if virus excretion in stools is the only diagnostic criteria, rotavirus-associated intussusception cases may be underdiagnosed and suggests that future epidemiological studies of intussusception should assess multiple markers of rotavirus infection (rotavirus excretion, antigenemia, or seroconversion) or use more sensitive assay methods to detect virus excretion (8).

The data from our model are consistent with the concept that intussusception is a complex disease. Our findings that rotavirus enhances intussusception in an LPS model in mice suggests that rotavirus may sensitize the immune system to other pathogenic components, and rotavirus infection may be one of multiple factors that needs to be present to induce intussusception. Whether other viruses associated with intussusception, including enteradenoviruses, adenoviruses, noroviruses, astroviruses, enteroviruses, contribute to intussusception through a similar sensitization of the immune system should be examined. Although all four rotavirus strains tested in our model enhanced intussusception, we cannot exclude the possibility that some virus strains might pose an increased risk.
for the development of intussusception based on the induction of greater inflammatory cytokine responses. In piglets, virulent human WA rotavirus induced significantly higher inflammatory cytokine responses, including TNF-α, INF-γ, and IL-6, than did attenuated WA human virus (1). It has been postulated that certain strains of rotavirus (serotype G3) may pose a higher risk of intussusception in children (58). Unfortunately, this hypothesis cannot be tested in the mouse model because non-serotype G3 and human viruses do not readily infect mice (25, 96). This constraint also precluded our testing of the two currently licensed vaccines, Rotarix, based on a human G1 virus, and Rota Teq, based on bovine WC3. Although the Rota Teq vaccine does contain a human G3 reassortant virus that might infect mice, our experience with WC3 suggests that the virus titer in the vaccine is unlikely to exceed the ID₅₀ of this strain in mice (data not shown). Neither Rotarix nor Rota Teq, both recently licensed for use, was associated with increased rates of intussusception in children younger than 3 months (80, 92, 93). The risk of intussusception resulting from vaccination with RotaShield was highest in children who received their first vaccine dose at greater than 3 months of age (79, 84). Therefore, it is possible that first vaccine administration of Rotarix or Rota Teq to similar aged children may pose an increased risk of intussusception, possibly due to the presence of other risk factors for intussusception that may occur more commonly in older children.

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REFERENCES
Rotavirus enhances LPS-induced intussusception in mice


