Intestinal Microbiota Promote Enteric Virus Replication and Systemic Pathogenesis
Sharon K. Kuss, et al.
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Intestinal Microbiota Promote Enteric Virus Replication and Systemic Pathogenesis

Sharon K. Kuss,1 Gavin T. Best,1 Chris A. Etheredge,1 Andrea J. Pruissers,2,3 Johnna M. Frierson,3,4 Lora V. Hooper,1,5,6 Terence S. Dermody,2,3,4 Julie K. Pfeiffer1†

Intestinal bacteria aid host health and limit bacterial pathogen colonization. However, the influence of bacteria on enteric viruses is largely unknown. We depleted the intestinal microbiota of mice with antibiotics before inoculation with poliovirus, an enteric virus. Antibiotic-treated mice were less susceptible to poliovirus disease and supported minimal viral replication in the intestine. Exposure to bacteria or their N-acetylglucosamine–containing surface polysaccharides, including lipopolysaccharide and peptidoglycan, enhanced poliovirus infectivity. We found that poliovirus binds lipopolysaccharide, and exposure of poliovirus to bacteria enhanced host cell association and infection. The pathogenesis of reovirus, an unrelated enteric virus, also was more severe in the presence of intestinal microbes. These results suggest that antibiotic-mediated microbiota depletion diminishes enteric virus infection and that enteric viruses exploit intestinal microbes for replication and transmission.

Enteric viruses encounter up to 10^{14} bacteria in the mammalian intestine (1). It is unclear whether commensal microorganisms affect enteric viruses. Poliovirus is an enteric human pathogen transmitted by the fecal-oral route and serves as a model for enteric virus infections (2). Orally acquired poliovirus undergoes a primary replication cycle in the gastrointestinal tract before dissemination. Poliovirus occasionally disseminates from the intestine to the central nervous system, which results in paralytic poliomyelitis days to weeks after initial infection in the gastrointestinal tract. A key question is whether microbiota influence viral replication in the gastrointestinal tract to augment systemic dissemination. To investigate the effect of intestinal microbiota on poliovirus infection, mice susceptible to...

References and Notes
5. U. Dittmer et al., Immunity 20, 293 (2004).
27. R. Nduati et al., JAMA 283, 1167 (2000).

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Supporting Online Material
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Figs. S1 to S8
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poliovirus were treated with antibiotics to deplete microbes, and viral disease was monitored (fig. S1) (3). Murine poliovirus infection requires expression of the human poliovirus receptor, PVR (4–6). PVR-transgenic mice (PVRtg), however, are not susceptible to oral poliovirus infection unless rendered immunodeficient by interferon-α/β receptor gene inactivation (PVRtg-Ifnar1−/−) (7, 8). PVRtg-Ifnar1−/− mice were untreated or treated orally with four antibiotics before oral inoculation with poliovirus. Antibiotic treatment reduced culturable intestinal bacteria by a millionfold (Fig. 1A). The mortality of untreated mice was twice that of antibiotic-treated mice (Fig. 1B). Reintroduction of fecal bacteria into antibiotic-treated mice enhanced poliovirus disease, which suggested that microbiota promote poliovirus pathogenesis. However, when the intestinal lumen was bypassed by intraperitoneal inoculation of poliovirus, pathogenesis was microbiota-independent (Fig. 1C and fig. S2). Given that orally inoculated poliovirus enters the intestine and encounters the large number of bacteria that reside there, the microbiota-mediated enhancement of poliovirus pathogenesis in orally inoculated mice is likely initiated in the intestine.

To determine whether mice harboring microbiota support more efficient poliovirus replication than mice with depleted microbiota, we quantified viral titers from fecal samples (Fig. 1D and fig. S3A), because poliovirus was undetectable in intestinal tissue (fig. S4), and minimal intestinal pathology was evident (fig. S5). Peak poliovirus titers in feces from antibiotic-treated animals were lower than those from untreated mice, but titers from antibiotic-treated mice were higher at later times. Prolonged shedding from antibiotic-treated mice was due to slower peristalsis, because dye transit also was delayed (fig. S6) (9). We postulated that increased poliovirus titers from antibiotic-treated mice at late times might be due to extended shedding of unreplated inoculum virus. To differentiate between replicate and inoculum virus, we first quantified fecal shedding of poliovirus from nonpermissive mice lacking PVR and observed elevated late titers in antibiotic-treated mice, which suggested that total viral titers in feces and replication are not linked (fig. S3B).

We then quantified viral replication in PVR mice using light-sensitive poliovirus. Poliovirus propagated in the presence of neutral red dye is sensitive to light-induced inactivation by RNA cross-linking but loses light sensitivity upon replication in the dark inside mice, facilitating assessment of replication (10). We orally inoculated untreated or antibiotic-treated mice with light-sensitive poliovirus and collected feces in the dark. Fecal virions were light-exposed or unexposed and quantified to determine replication status (fig. S7). PVrtg-Ifnar1−/− and PVRtg mice harboring microbiota supported efficient intestinal poliovirus replication, whereas antibiotic-treated mice did not (Fig. 1, E and F). Therefore, total fecal titers do not reflect viral replication, a fact only revealed by using light-sensitive viruses. Moreover, poliovirus intestinal replication was equivalent in Ifnar1M−/− and Ifnar1−/− mice, which suggested that intestinal replication was IFNAR-independent. Because poliovirus infection was lethal for a fraction of antibiotic-treated mice (Fig. 1B), it is possible that either minimal viral replication was sufficient for lethality or inoculum virus breached the epithelium and replicated in extraintestinal sites, occasionally initiating disease. Collectively, these results indicate that the microbiota enhance gastrointestinal poliovirus replication.

We gathered several lines of evidence suggesting that diminished poliovirus replication and disease in antibiotic-treated mice is due to microbiota depletion rather than direct effects of antibiotic treatment. We first tested whether antibiotics directly affect poliovirus and found that

![Fig.1](https://www.sciencemag.org/content/334/6056/250/F1.large.jpg)
Binding of radiolabeled poliovirus to HeLa cells. 35S-labeled poliovirus was incubated with PBS or 10^8 CFU B. cereus for 1 hour at 37°C. An equal volume of PBS or incubation with HeLa cells. After washing, cell-associated radioactivity was quantified. For all, poliovirus was quantified yielding PFU per fraction, samples and washed with PBS to collect fractions 1 to 6. Excess biotin was added to elute (fractions 7 to 12).

Fig. 3. Reovirus pathogenesis in microbiota-depleted mice. (A) PVRtg-ifnar1−/− mice were uninfected, untreated (n = 5), or antibiotic-treated (n = 5) or infected perorally with reovirus, untreated (n = 13) or antibiotic-treated (n = 15). Feces were collected 24 hours post inoculation. (B) Fecal pathology (table S1). (C) Upper (top) and lower (bottom) small intestines were harvested from uninfected and antibiotic-treated PVRtg-ifnar1−/− mice on day 4 post infection or from uninfected mice. Arrows indicate Peyer’s patches. (D) Quantification of Peyer’s patch sizes [from (C)] from uninfected and infected mice. (E) Reovirus titers from day 4 post infection PVRtg-ifnar1−/− mouse tissues. Plaque assays were performed using murine L929 cells, yielding PFU per milligram of tissue. For (B) to (E), n = 4 to 9 untreated mice, n = 2 to 9 antibiotic-treated mice. Each symbol or bar denotes the mean + SEM. *P < 0.05, **P < 0.01, Student’s t test. Scale bars in (A) and (C), 5 mm. (A) and (C), representative of 3 to 5 experiments; n = 2 to 4 experiments for (B), (D), and (E).

Fig. 4. Effects of bacteria and polysaccharides on poliovirus. (A) Strategy for in vitro poliovirus infectivity experiments. (B) Poliovirus recovered after incubation in PBS. (C) Poliovirus infectivity after exposure to PBS, feces, or feces supplemented with B. cereus or LPS (6 hours at 37°C). (D) Poliovirus infectivity after exposure to medium (DME) or bacterial strains (10^7, 10^8, or 10^9 CFU) (6 hours at 37°C). (E) Poliovirus infectivity after incubation with compounds (1 mg/ml) (6 hours at 42°C). (F) Poliovirus binding to LPS. Poliovirus was incubated with or without biotinylated LPS for 1 hour at 37°C. A monomeric avidin column was loaded with various concentrations of compounds (6 hours at 42°C). (G) Poliovirus infectivity after incubation with bacteria within the host, we examined the specificity of the microbiota effects using reovirus, an enteric virus that infects most mammals (11). Although immunocompetent adult mice do not display overt reovirus disease symptoms, immunocompromised adult mice develop nonfatal disease after oral inoculation with reovirus strain T3SA+. We orally inoculated untreated or antibiotic-treated immunocompromised PVRtg-ifnar1−/− mice with reovirus. Feces from untreated mice were yellow, oily, and hardened, typical of biliary obstruction from T3SA+ reovirus replication and damage (12), whereas feces from antibiotic-treated mice appeared normal (Fig. 3, A and B). Furthermore, analysis of intestines revealed severe reovirus-induced pathology, with enlarged Peyer’s patches in untreated, but not antibiotic-treated, mice (Fig. 3, C and D). Reovirus titers in intestines from untreated mice were significantly higher than those from antibiotic-treated mice (Fig. 3E). These results suggest that intestinal microbes promote reovirus disease and, therefore, may promote infection with other enteric viruses. Because all enteric viruses encounter intestinal bacteria within the host, we examined the specificity of the microbiota effects using reovirus, an enteric virus that infects most mammals (11). Although immunocompetent adult mice do not display overt reovirus disease symptoms, immunocompromised adult mice developed nonfatal disease after oral inoculation with reovirus strain T3SA+. We orally inoculated untreated or antibiotic-treated immunocompromised PVRtg-ifnar1−/− mice with reovirus. Feces from untreated mice were yellow, oily, and hardened, typical of biliary obstruction from T3SA+ reovirus replication and damage (12), whereas feces from antibiotic-treated mice appeared normal (Fig. 3, A and B). Furthermore, analysis of intestines revealed severe reovirus-induced pathology, with enlarged Peyer’s patches in untreated, but not antibiotic-treated, mice (Fig. 3, C and D). Reovirus titers in intestines from untreated mice were significantly higher than those from antibiotic-treated mice (Fig. 3E). These results suggest that intestinal microbes promote reovirus disease and, therefore, may promote infection with other enteric viruses.

The microbiota-dependent enhancement of poliovirus replication and pathogenesis could be mediated by microbiota-induced host effects, viral effects, or both. To discriminate between these possibilities, we investigated whether intestinal microbes alter poliovirus infectivity. First, we tested whether poliovirus infectivity was altered by exposure to intestinal microbiota in vivo. We orally inoculated untreated, antibiotic-treated, and germ-free mice with poliovirus; harvested luminal contents from the lower small intestine 2 hours postinfection; and quantified infectivity of isolated poliovirus in primary MEFs and HeLa cells. The infectivity in MEFs of poliovirus isolated from untreated mice was twice that of tissue culture-derived virus and antibiotic-treated and germ-free intestinal virus (fig. S10). Second, we developed an ex vivo–in vitro assay to examine
MED12, the Mediator Complex Subunit 12 Gene, Is Mutated at High Frequency in Uterine Leiomyomas

Netta Mäkinen,1* Miika Mehine,1* Jaana Tolvanen,3 Eevi Kaasinen,1 Yilong Li,1 Heli J. Lehtonen,1 Massimiliano Gentile,2 Jian Yan,3 Martin Enge,3 Minna Taipale,1,3 Mervi Aavikko,5 Riku Katainen,3 Elina Virolainen,4 Tom Böhlöing,4,5 Taru A. Koski,1 Virpi Launonen,1 Jari Sjöberg,6 Jussi Taipale,1,3 Pia Vahteristo,1 Lauri A. Aaltonen1†

Uterine leiomyomas, or fibroids, are benign tumors that affect millions of women worldwide and that cause considerable morbidity. To study the genetic basis of this tumor type, we examined 18 uterine leiomyomas derived from 17 different patients by exome sequencing and identified tumor-specific mutations in the mediator complex subunit 12 (MED12) gene in 10. Through analysis of 207 additional tumors, we determined that MED12 is altered in 70% (159 of 225) of tumors from a total of 80 patients. The Mediator complex is a 26-subunit transcriptional regulator that bridges DNA regulatory sequences to the RNA polymerase II initiation complex. All mutations resided in exon 2, suggesting that aberrant function of this region of MED12 contributes to tumorigenesis.

Uterine leiomyomas, also called fibroids, are benign tumors that occur in 60% of women by the age of 45 years and that cause symptoms in about half of the cases (J). These symptoms include abdominal pain and discomfort and abnormal bleeding. Uterine leiomyomas are also an important cause of infertility [reviewed in (2, 3)], and they are the most common medical reason for hysterectomy (4). Several recurrent genetic aberrations such as deletions in 7q, trisomy of chromosome 12, and various rearrangements affecting the high mobility group AT-hook 2 (HMGA2) gene mapping to chromosome 12q14 (5–7) have been observed in uterine leiomyomas, but these occur at low frequency. To investigate whether these tumors have high-frequency genetic alterations, we investigated all protein-coding genes by exome sequencing in 18 uterine leiomyomas and the respective normal tissue DNAs. These tumors came from 17 different patients.

The most frequent tumor-specific alterations in the set of 18 tumors affected the MED12 gene on chromosome Xq13.1. MED12 is a subunit of the Mediator complex, which is thought to regulate global, as well as gene-specific, transcription (8). Ten tumors displayed a mutation, and eight

References and Notes
3. Materials and methods are available as supporting online material (J.K.P.). The data reported in the paper are tabulated in some 12q14 (2). Ten tumors displayed a mutation, and eight tumors had MED12 alterations (accession no. BankIt1475845 SEq. 10 JN613288).

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*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: lauri.aaltonen@helsinki.fi

1Department of Medical Genetics, Genome-SCALE Biology Research Program, 00014 University of Helsinki, Helsinki, Finland.
2Center for Science Ltd., 02101 Espoo, Finland.
3Science for Life Laboratory, Department of Biosciences and Nutrition, Karolinska Institutet, 14183 Stockholm, Sweden.
4Department of Pathology, Haartman Institute, 00014 University of Helsinki, Helsinki, Finland.
5Laboratory of Hospital District of Helsinki and Uusimaa, 00029 Helsinki University Central Hospital, Helsinki, Finland.
6Department of Obstetrics and Gynecology, 00029 Helsinki University Central Hospital, Helsinki, Finland.
7These authors contributed equally to this work.
8To whom correspondence should be addressed. E-mail: lauri.aaltonen@helsinki.fi

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poliovirus infectivity (Fig. 4A). Poliovirus was incubated at 37° or 42°C, and viable virus was quantified by plaque assay. Poliovirus incubated in phosphate-buffered saline (PBS), feces from antibiotic-treated mice, and germ-free feces lost viability (Fig. 4, B and C). However, poliovirus incubated in untreated feces or germ-free feces supplemented with bacteria had significantly increased viability (Fig. 4C). Similarly, poliovirus incubated with Gram-negative (Escherichia coli or O. intermedium) or Gram-positive (Bacillus cereus or Enterococcus faecalis) bacteria had significantly increased viability (Fig. 4D). Exposure to B. cereus increased poliovirus infectivity more than 500%. Enhancement of poliovirus infectivity did not require live bacteria (fig. S11). Moreover, poliovirus incubated with certain bacterial surface polysaccharides, including lipopolysaccharide (LPS) and peptidoglycan (PG), had significantly enhanced yield over PBS-treated controls (Fig. 4, C and E, and fig. S12). The enhancement was not due to cellular effects of LPS or PG treatment (fig. S13). We tested a variety of glycans and other compounds, and only N-acetylgalactosamine (GlcNAc)–containing polysaccharides (e.g., chitin) demonstrated activity (Fig. 4E). Mucin, a host protein modified with GlcNAc-containing polysaccharides, also had activity (J3). Of the purified components tested, LPS was the most potent enhancer of poliovirus infectivity, with activity at concentrations 1/20th those of chitin or mucin (Fig. 4F). Using biotinylated LPS and monomeric avidin columns, we found that poliovirus binds LPS (Fig. 4G). Because MED12 is mutated at high frequency

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