Intestinal Microbiota Promote Enteric Virus Replication and Systemic Pathogenesis

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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 1014 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 microbe biota on poliovirus infection, mice susceptible to

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Supporting Online Material
www.sciencemag.org/cgi/content/full/334/6053/245/DC1
Materials and Methods
Figs. S1 to S8
Table S1
References (29–40)
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CD14KO, but not TLR2KO, mice (Fig. 4B and table S1). Thus, our results support a model (fig. S7) whereby LPS-induced signaling drives a viral “subversion” pathway via IL-10 production that promotes viral transmission to successive generations.

Detailed analysis of the actual viral load in subsequent generations of infected IL-16KO and IL-6KO mice revealed that it was reduced gradually and that it took different numbers of passages for various families to completely eliminate the virus (table S1 and fig. S8). Thus, it appears that, in early generations, a high viral load can compensate for the loss of the TLR4-dependent subversion pathway and overpower the adaptive immune response. However, the virus is eventually lost in each infected mouse pedigree, as the virus load is reduced with each subsequent passage (fig. S8).

Commensal microbiota are required for many homeostatic functions of the intestinal mucosa and other barrier tissues. Microbiota control tissue repair (16), induction of tolerance to self [including tolerance to itself (17) and to autoantigens of the host (18)], and oral tolerance of adults to ingested antigens (19, 20). At present, it is unknown whether neonatal oral tolerance is also dependent on microbiota or whether MMTV induces neonatal oral tolerance to itself by a unique mechanism available to retroviruses. Commensals interact with various pathogens and protect the host against infection with pathogenic and opportunistic bacteria (21), protozoa (22), and fungi (23) or facilitate infection with helminthes (24). However, the role of commensal bacteria in viral transmission and/or pathogenesis is only beginning to unravel. It is highly likely that bacterial microbiota can play both protective (25) and abetting roles (present report) in their interactions with viruses. The lack of knowledge in this area makes it important to expand such investigations to other systems. It is not yet clear whether other viruses take advantage of bacterial products, such as LPS, to achieve successful transmission. Retroviruses transmitted through mucosal surfaces may also use similar strategies.

In humans, the highest risk of human immunodeficiency virus (HIV) transmission occurs across mucosal surfaces among individuals who practice receptive anal intercourse (26), and risk is also high in infants breastfed by HIV-infected mothers (27, 28). This study sheds light on the previously unknown role of commensal microbiota in retroviral pathogenesis and suggests new approaches to the prevention of mucosal transmission, viral-specific vaccination, and therapies.
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 unreplicated inoculum virus. To differentiate between replicated 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 viruses 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...
antibiotic-treated PVRTg-Ifnar1−/− mice on day 4 post infection or from untreated mice. Arrows indicate Peyers patches. (D) Quantification of Peyers patch sizes from (C). (F) Poliovirus replication kinetics in primary MEFs and HeLa cells. (G) Poliovirus infectivity after exposure to intestinal microbiota in vivo. We tested whether poliovirus infectivity was altered mediated by microbiota-induced host 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 poliovirus replication kinetics were identical in the presence and absence of antibiotics in HeLa cells and PVRTg mouse embryo fibroblasts (MEFs) (Fig. 2A). We next assayed poliovirus replication and pathogenesis in antibiotic-treated mice harboring antibiotic-resistant bacteria. For these experiments, we treated PVRTg-Ifnar1−/− mice with antibiotics to select antibiotic-resistant microorganism (Fig. S8). After several weeks, fecal bacteria were insensitive to antibiotics in vitro (Fig. 2B). The strain resistant to multiple antibiotics was identified as Ochrobactrum intermedium, a Gram-negative aerobe, by 16S ribosomal DNA sequencing of fecal-derived subclones (fig. S9). Poliovirus replicated and was pathogenic in antibiotic-treated mice harboring O. intermedium (Fig. 2, C and D). Finally, poliovirus mixed with antibiotics before oral inoculation of mice replicated and was pathogenic (Fig. 2, C and D). Therefore, diminished poliovirus replication and pathogenesis in antibiotic-treated mice is not due to direct antiviral effects of antibiotics.

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 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 Peyers 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–host interactions, 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
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. intermediate) 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 (13). 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 B. cereus exposure produced the largest increase in poliovirus yield, we tested whether exposure to B. cereus enhanced radiolabeled poliovirus binding to HeLa cells, which would aid infection. Poliovirus incubated with B. cereus displayed adherence to HeLa cells 2 times that of controls (Fig. 4H). Overall, poliovirus infectivity was enhanced in the presence of intestinal microbiota in vitro and in vivo, which likely contributed to the enhanced replication and pathogenesis in microbiota-harboring mice.

Despite the well-known beneficial effects of intestinal microbes, we discovered that they augment enteric virus pathogenesis by enhancing viral replication. Intestinal microbes also induce egg hatching of an intestinal nematode in mice (14), which suggests that diverse pathogens exploit intestinal microbes for propagation. Our work implies that antibiotic-mediated microbiota depletion can have antiviral effects, although we do not advocate the use of antibiotics to prevent viral disease. However, understanding how microbe promote enteric virus infections may reveal new antiviral strategies. Our results suggest that poliovirus binds specific microbe–associated surface polysaccharides, which enhances viral thermostability and attachment to host cells. Contrary to the known benefits of intestinal microbiota to the host (1), enteric viruses may have evolved to use intestinal microbes as a trigger for replication at a site optimal for transmission.

**References and Notes**

3. Materials and methods are available as supporting material on Science online.

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**MED12, the Mediator Complex Subunit 12 Gene, Is Mutated at High Frequency in Uterine Leiomyomas**

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Uterine leiomyomas, or fibroids, are benign tumors that occur in 60% of women by the age of 45 years 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.

**References and Notes**

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