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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 prior to 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 prior to dissemination. Poliovirus occasionally disseminates from the intestine to the central nervous system, resulting 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, which aids systemic dissemination.

To investigate the effect of intestinal microbiota on poliovirus infection, mice susceptible to poliovirus were treated with antibiotics to deplete microbes, and viral disease was monitored

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Summary Intestinal microbiota enhance poliovirus and reovirus pathogenesis in mice.

(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 prior to oral inoculation with poliovirus. Antibiotic treatment reduced culturable intestinal bacteria by a million-fold (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, suggesting that microbiota promote poliovirus pathogenesis. However, when the intestinal lumen was bypassed by intraperitoneal inoculation of poliovirus, pathogenesis was microbiota-independent (Fig. 1C, 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, 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, since 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, suggesting 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-*Ifnar*-1/- and PVRtg mice harboring microbiota supported efficient intestinal poliovirus replication, whereas antibiotic-treated mice did not (Fig. 1E,1F). 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 *Ifnar1*+/+ and *Ifnar1*-/- mice, suggesting 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 extra-intestinal 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 found that antibiotics do not directly affect poliovirus since 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 microbiota (fig. S8). After several weeks, fecal bacteria were insensitive to antibiotics *in vitro* (Fig. 2B). The multi-antibiotic resistant strain was identified as *Ochrobactrum intermedium* a Gram-negative aerobe, by 16S rDNA sequencing

of fecal-derived subclones (fig. S9). Poliovirus replicated and was pathogenic in antibiotic-treated mice harboring *Ochrobactrum intermedium* (Fig. 2C,D). Furthermore, poliovirus mixed with antibiotics prior to oral inoculation of mice replicated and was pathogenic (Fig. 2C,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. 3A, 3B). Furthermore, analysis of intestines revealed severe reovirus-induced pathology, with enlarged Peyer's patches in untreated but not antibiotic-treated mice (Fig. 3C, 3D). 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, or germ-free mice with poliovirus, harvested lumenal contents from the lower small intestine at two hours post-infection, 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°C or 42°C and viable virus was quantified by plaque assay. Poliovirus incubated in PBS, feces from antibiotic-treated mice, or germ-free feces lost viability (Fig. 4B,4C). 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, Ochrobactrum intermedium*) or Gram-positive (*Bacillus cereus, Enterococcus faecalis*) bacteria had significantly increased viability (Fig. 4D). Exposure to *B. cereus* increased poliovirus infectivity over 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. 4C, E, 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-acetylglucosamine (GlcNAc)-containing polysaccharides demonstrated activity (e.g. chitin, 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 >20-fold lower than 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, aiding infection. Poliovirus incubated with *B. cereus* displayed two-fold higher HeLa cell adherence compared to controls (Fig. 4H). Overall, poliovirus infectivity was enhanced in the presence of intestinal microbiota *in vitro* and *in vivo*, likely contributing 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*), suggesting 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 microbiota promote enteric virus infections may reveal new antiviral strategies. Our results suggest that poliovirus binds specific microbe-associated surface polysaccharides, enhancing 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and Notes

1. Garrett WS, Gordon JI, Glimcher LH. Cell. 2010; 140:859. [PubMed: 20303876]
2. Pallansch, MA.; Roos, RP. Virology. Fields, BN., et al., editors. Lippincott Williams & Wilkins; Philadelphia: 2001. p. 723-775.
3. Materials and methods are available as supporting material on Science online.
4. Ren RB, et al. Cell. 1990; 63:353. [PubMed: 2170026]
5. Koike S, et al. Proc Natl Acad Sci U S A. 1991; 88:951. [PubMed: 1846972]
6. Ida-Hosonuma M, et al. Arch Virol. 2003; 148:29. [PubMed: 12536294]
7. Ida-Hosonuma M, et al. J Virol. 2005; 79:4460. [PubMed: 15767446]
8. Ohka S, et al. J Virol. 2007; 81:7902. [PubMed: 17507470]
9. Abrams GD, Bishop JE. Proc Soc Exp Biol Med. 1967; 126:301. [PubMed: 6066182]
10. Kuss SK, Etheredge CA, Pfeiffer JK. PLoS Pathogens. 2008; 4
11. Tyler, KL. Fields virology. Knipe, DM.; Howley, PM., editors. Vol. 1. Lippincott Williams & Wilkins; Philadelphia: 2001. p. 1729-1945.
12. Barton ES, et al. J Clin Invest. 2003; 111:1823. [PubMed: 12813018]
13. Podolsky DK. J Biol Chem. 1985; 260:8262. [PubMed: 4008490]
14. Hayes KS, et al. Science. 2010; 328:1391. [PubMed: 20538949]
15. Pfeiffer JK, Kirkegaard K. PLoS Pathog. 2005; 1:e11. [PubMed: 16220146]
16. Virgin HW, et al. J Virol. 1988; 62:4594. [PubMed: 2460637]
17. Cash HL, et al. Science. 2006; 313:1126. [PubMed: 16931762]
18. Rakoff-Nahoum S, et al. Cell. 118:229. 204. [PubMed: 15260992]
19. Brandenburg B, et al. PLoS Biol. 2007; 5:e183. [PubMed: 17622193]

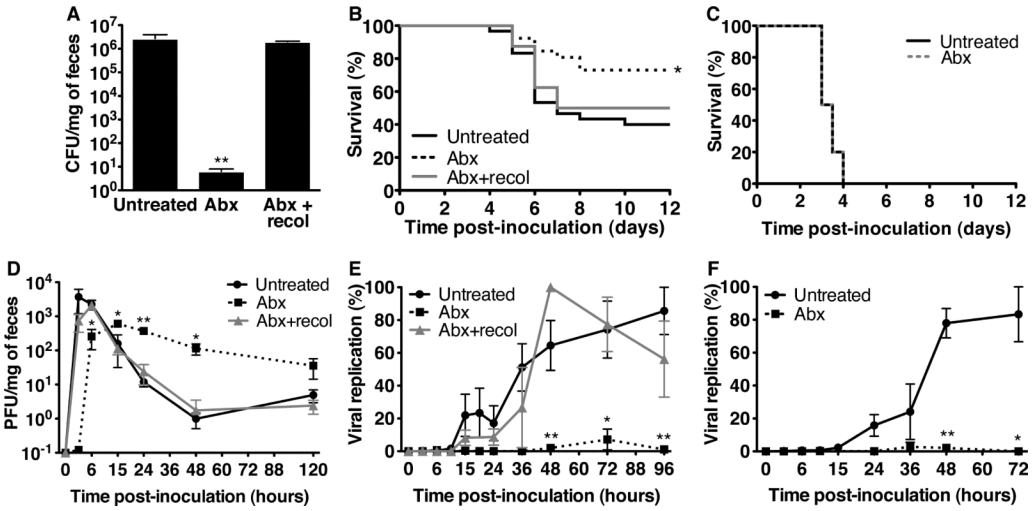

Fig. 1.

Poliovirus pathogenesis, shedding, and replication in microbiota-depleted mice. (**A**) Bacterial loads in feces. PVRtg-*Ifnar1*-/- mice (n=4-7) were untreated, antibiotic-treated (Abx) for 10 days, or antibiotic-treated for 8 days and recolonized for 2 days with fecal bacteria (Abx+recol). Feces were plated and grown anaerobically, yielding colony-forming units (CFU) per milligram of feces. (**B**) Survival of PVRtg-*Ifnar1*-/- mice orally inoculated with poliovirus (untreated: n=30, Abx: n=26, Abx+recol: n=8). **p*=0.012, Log-rank test. (**C**) Survival of PVRtg-*Ifnar1*-/- mice intraperitoneally inoculated with poliovirus (n=10 mice each). (**D**) Poliovirus shedding from PVRtg-*Ifnar1*-/- mice. Mice were orally inoculated with poliovirus, feces were collected (n=2-26 per interval), and poliovirus was isolated and quantified by plaque assay, yielding plaque-forming units (PFU) per milligram of feces. (**E**,**F**) Poliovirus replication in intestinal tracts of PVRtg-*Ifnar1*-/- (**E**) or PVRtg (**F**) mice orally inoculated with light-sensitive poliovirus (n=3-9 mice per interval). Feces were harvested, and virus was quantified +/- light exposure to determine percent replication. Symbols represent mean + SEM, **p*<0.05, ***p*<0.01, Student's t-test. N=2-6 for all experiments.

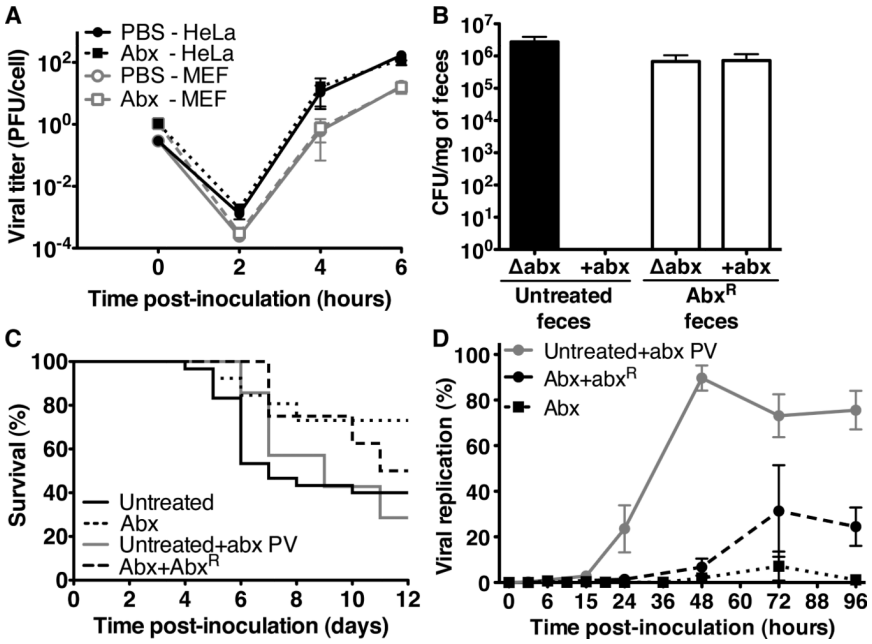

Fig. 2.

The effects of antibiotic treatment on poliovirus replication and pathogenesis. (**A**) Poliovirus replication kinetics in MEFs and HeLa cells +/- antibiotics. (**B**) Fecal bacterial loads from untreated or antibiotic-treated mice harboring antibiotic-resistant (abxR) bacteria. Feces were plated on rich medium +/- four antibiotics. (**C**) Survival of PVRtg-*Ifnar1*-/- mice orally inoculated with poliovirus pre-mixed with four antibiotics (Untreated+abx PV, n=9) or poliovirus alone in antibiotic-treated mice harboring AbxR bacteria (Abx+abxR, n=8). (Results from untreated and antibiotic-treated mice are from Fig. 1B.) (**D**) Replication of light-sensitive poliovirus in untreated mice receiving poliovirus+antibiotics inoculum and antibiotic-treated mice harboring abxR bacteria in comparison to antibiotic-treated mice. (Results from antibiotic-treated mice are from Fig. 1E.) Each symbol represents mean + SEM. A and B, N=2-5 experiments, C and D are from a representative experiment.

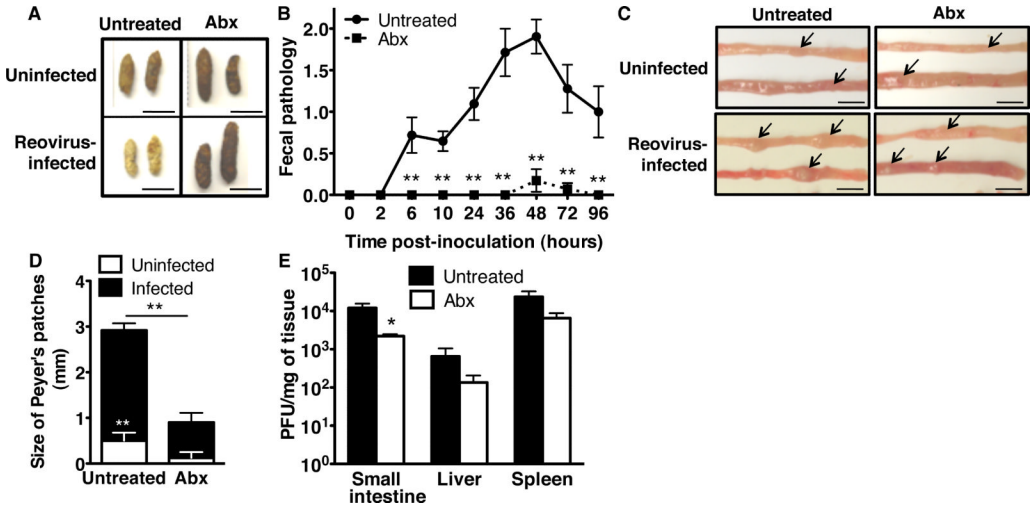

Fig. 3.

Reovirus pathogenesis in microbiota-depleted mice. (**A**) PVRtg-*Ifnar1*-/- mice were either 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 untreated 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-E, n=4-9 untreated mice, n=2-9 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=5mm. A and C, representative of 3-5 experiments; N=2-4 for B, D, and E.

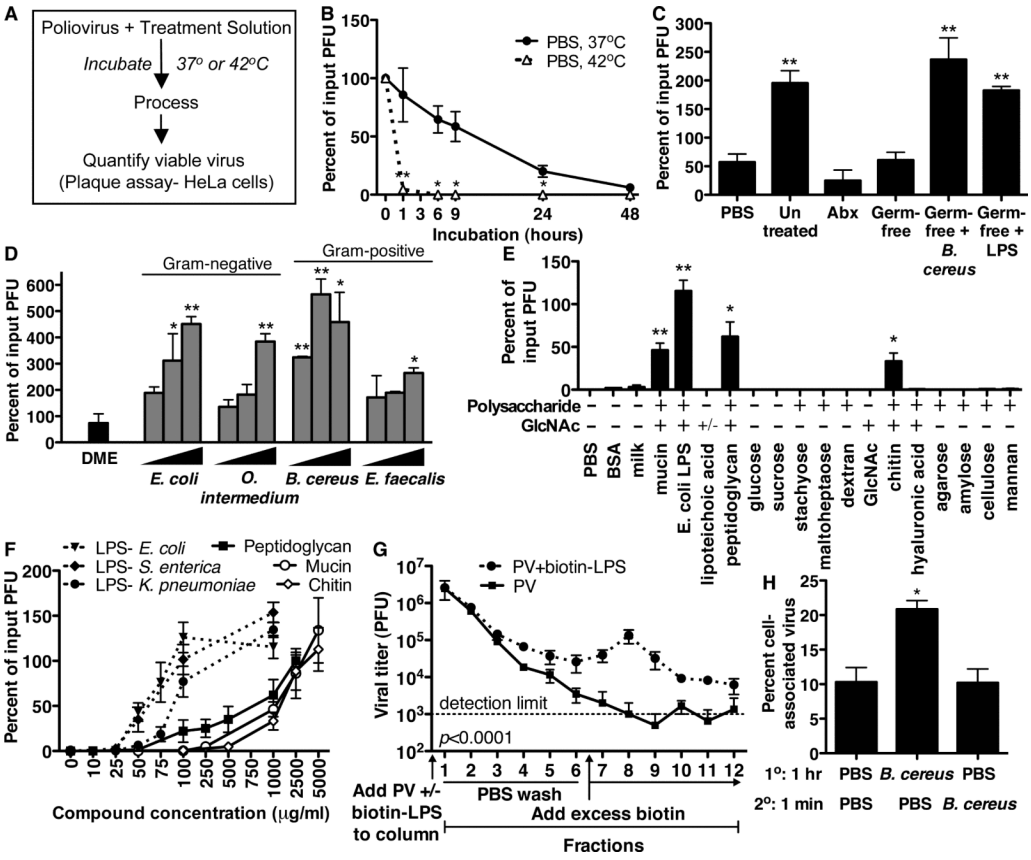

Figure 4.

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 following exposure to PBS, feces, or feces supplemented with *Bacillus cereus* or lipopolysaccharide (LPS) (6 hours/37°C). (**D**) Poliovirus infectivity after exposure to medium (DME) or bacterial strains (10^7, 10^8, or 10^9 CFU) (6 hours/37°C). (**E**) Poliovirus infectivity after incubation with compounds (1 mg/ml) (6 hours/42°C). (**F**) Poliovirus infectivity after incubation with various concentrations of compounds (6 hours/42°C). (**G**) Poliovirus binding to LPS. Poliovirus was incubated +/- biotinylated LPS for 1 hour at 37°C. A monomeric avidin column was loaded with samples and washed with PBS to collect fractions 1-6. Excess biotin was added to elute (fractions 7-12). Poliovirus was quantified yielding PFU per fraction, $p<0.0001$, 2-way ANOVA. (**H**) Binding of radiolabeled poliovirus to HeLa cells. ^{35}S-labeled poliovirus was incubated with PBS or 10^8 CFU *B. cereus* for 1 hour at 37°C. An equal volume of PBS or *B. cereus* was added followed by immediate incubation with HeLa cells. After washing, cell-associated radioactivity was quantified. For all experiments, N=2-8 and bars and symbols denote mean + SEM, *$p<0.05$, **$p<0.01$, Student's t-test.