infection (17), we examined the requirement for this pathway (30) in antibiotic suppression of persistent infection. Ifnar1−/− mice (lacking IFN-α receptor 1), Stat2−/− mice (lacking a key transcription factor that conveys IFN-αR1, IFN-γ, and IFN-λ signals), and If3−/− mice (lacking the transcription factor IFN regulatory factor 3 that induces expression of IFN-αR1 and IFN-λR1) (31, 32) were vulnerable to persistent CR6 infection even in the presence of antibiotics (Fig. 4, B to D; fig. S8, A and B; and fig. S9, A to G). Whereas antibiotic treatment prevented persistent infection of control mice inoculated with 10^5 PFU of MNoV (Fig. 4A and fig. S8A), some mice lacking components of the IFN-λ induction or signaling pathway became persistently infected even at this low dose (Fig. 4D and fig. S8A, P < 0.01). This vulnerability to MNoV infection did not correspond with differential effects of antibiotics on bacterial depletion in these mutant mice, as shown by 16S rDNA sequencing studies and by experiments in which fecal transplantation from antibiotic-treated mutant mice did not support establishment of persistent infection (fig. S10, A to D). Resistance to MNoV infection in antibiotic-treated control mice was not explained by up-regulation of Isg15, IFN-stimulated genes including Ifnβ, and IFN-γ receptor, Ifnlr1, and independent of adaptive immunity. These results indicate that innate immunity and in particular the IFN-λ pathway, is required for the effects of antibiotic treatment on persistent infection. These observations suggest that the bacterial microbiota limits the efficacy of IFN-λ-dependent innate immunity or alters some yet-undefined innate immune pathway that renders viruses susceptible to the effects of IFN-λ. These data indicate that the clinical use of antibiotics in humans may alter the enteric virome (6, 34) and that the effects of antibiotics in the treatment of infectious diseases may not be entirely attributable to their antibacterial properties. Given the contribution of the virome to host physiology (46, 6), these data suggest the importance of considering the effects of trans-kingdom interactions for understanding the pathogenesis of infectious diseases.

**REFERENCES AND NOTES**


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**SUPPLEMENTARY MATERIALS**

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Materials and Methods

Figs. 51 to 511

Table S1

References (35–51)

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**NONOROVIRUS**

**Interferon-λ cures persistent murine murine norovirus infection in the absence of adaptive immunity**

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Norovirus gastroenteritis is a major public health burden worldwide. Although fecal shedding is important for transmission of enteric viruses, little is known about the immune factors that restrict persistent enteric infection. We report here that although the cytokines interferon-α (IFN-α) and IFN-β prevented the systemic spread of murine norovirus (MNoV), only IFN-λ controlled persistent enteric infection. Infection-dependent induction of IFN-λ was governed by the MNoV capsid protein and correlated with diminished enteric persistence. Treatment of established infection with IFN-λ cured mice in a manner requiring nonhematopoietic cell expression of the IFN-λ receptor, Ifnr1, and independent of adaptive immunity. These results suggest the therapeutic potential of IFN-λ for curing virus infections in the gastrointestinal tract.

Human noroviruses (HNoVs) are a leading cause of gastroenteritis worldwide (1, 2). Asymptomatic fecal shedding of HNoVs may be important epidemiologically, as it provides a reservoir between outbreaks (1, 3–9). Some strains of murine norovirus (MNoV) also establish persistent enteric infection, providing a model for analyzing mechanisms of enteric NoV persistence and immunity in a natural host (1, 10, 11). Interferons (IFNs) are critical for control of both murine and human NoV replication (12–18). Interferon-α (IFN-α) and IFN-β (also called type I IFNs and hereafter IFN-αβ), IFN-γ (also called type II IFN), and IFN-λ (also called type

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III IFN or interleukin-28(ß/9) signal through the distinct heterodimeric receptors Ifnar1/Ifnar2, Ifngr1/Ifngr2, and Ifnlr1/Ifnlr2b to regulate gene expression through phosphorylation of Stat proteins (19, 20). Although the roles of IFNs in control of persistent enteric infection have not been elucidated, it is of interest that IFN-ß, but not IFN-α, is important for control of acute rotavirus infection in the intestine of mice (21).

To define the role of IFNs in MNoV enteric persistence, we measured levels of the persistent MNoV strain CR6 in different tissues and in feces after oral inoculation of control mice and mice deficient in Ifnar1, Ifngr1, Ifnlr1, or Stat1 (Fig. 1) (also see supplementary materials and methods). As expected, Ifnar1 and Stat1 were important for limiting replication in the spleen and mesenteric lymph node (MLN) (12, 13, 16, 17), whereas Stat1 rather than Ifnar1 controlled replication of the colonic cells (Fig. 1A), suggesting that IFN-ß responses did not explain Stat1-dependent control of replication in the intestine. Consistent with this, comparison of the requirement for each IFN receptor in control of fecal shedding revealed that only Stat1 and Ifnlr1 limited levels of fecal shedding of MNoV (Fig. 1B). Furthermore, we observed increased fecal shedding compared with controls in Ifnrl1−/− but not ifnar1−/− mice over 35 days of infection (Fig. 1C).

To define the basis for the intestine-specific role of IFN-ß in control of enteric persistence, we inoculated mice with the persistent MNoV strain CR6 or the nonpersistent MNoV strain CW3 and compared viral replication and induction of IFN-ß and IFN-α in Peyer’s patches, MLNs, and the colon. As expected, CW3 replicated preferentially in MLN, CR6 and CW3 replicated equivalently in Peyer’s patches, and CR6 replicated preferentially in colon (fig. S1) (11, 22). CW3 induced both IFN-ß and IFN-α (Fig. 2, A and B) in MLN and Peyer’s patches. In contrast, CR6 did not induce detectable IFN-ß or IFN-α mRNA in any organ, despite the high level of replication in the intestine (Fig. 2, A and B). Both strains induced equivalent amounts of IFN-ß from bone marrow–derived dendritic cells (BMDCs) in vitro (fig. S2). The capacity of strain CW3 to infect systemic organs maps to the protruding domain of the viral capsid protein (11, 22), whereas a single coding change (Asp94→Glu94, hereafter D94E) in the NS1-2 protein confers the capacity for enteric persistence upon CW3 (11, 23). In chimeric viruses, the presence of the entire CW3 capsid gene or the protruding domain of the CW3 capsid gene correlated with IFN-ß and IFN-α induction (fig. S3, A to F). Furthermore, in CW3-derived viruses carrying the NS1-2 D94E mutation that confers persistence (CW3D94E), the presence of the CR6 capsid lessened IFN-ß and IFN-α induction in MLNs despite similar levels of viral replication (Fig. 2C). This phenotype allowed us to use a chimeric virus to test the hypothesis that IFN-ß responses are required for prevention of persistence. The CW3D94E strain is capable of efficiently establishing enteric persistence only at low doses (fig. S4). When control mice were inoculated with a high dose of CW3D94E, many mice failed to establish persistence (fig. S4 and Fig. 2D).

This failure of CW3D94E to persist was rescued either by the CR6 capsid protein, which is associated with diminished IFN-ß and IFN-α responses (fig. S4), or by infection of Ifnar1−/− mice (Fig. 2D). Persistence of parental CW3, lacking intestinal tropism conferred by the D94E mutation, was not rescued in Ifnar1−/− mice (fig. S5). These data indicate that induction of IFN-ß interferes with the establishment of enteric MNoV persistence.

These findings suggest a primary role for IFN-ß in control of enteric MNoV persistence. Consistent with this, intraperitoneal treatment of mice with IFN-ß 1 day after oral inoculation with CR6 prevented persistent enteric MNoV infection (Fig. 3A). When cured mice were rechallenged with CR6 2 weeks later, persistent infection was established (Fig. 3B), indicating that IFN-ß acted by stimulating innate immunity rather than promoting adaptive immunity. These data also indicate that therapeutic levels of IFN-ß wane in animals within 2 weeks of administration.

Fig. 1. Systemic and intestinal persistence of MNoV are controlled by IFN-α and IFN-ß, respectively. Mice were orally inoculated with 10⁶ plaque-forming units (PFUs) of MNoV strain CR6, and genome copies in the indicated tissue (A) or feces (B and C) were determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR). Genome copies were compared between control, Stat1−/−, and Ihlar1−/− mice at day 21 in tissues (A); between control, Stat1−/−, Ifngr1−/−, Ihlar1−/−, Ifnar1−/−, Ihlar1−/− × Ifngr1−/− mice at day 14 in feces (B); and between control, Ihlar1−/−, and Ifnar1−/− over time in feces (C). Data shown are pooled from at least two independent experiments, with each point representing an individual animal in (A) and (B). Points in (C) represent at least four animals pooled from two to four independent experiments. Dashed lines represent limit of detection. Statistical significance was determined by one-way (A and B) or two-way (C) analysis of variance (ANOVA). n.s., not significant (P > 0.05); *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001. Error bars in (C) denote SD.
**Fig. 2. Induction of IFN-λ prevents enteric NoV persistence.** (A and B) Control mice were orally inoculated with $10^6$ PFUs of either MNoV CW3 or CR6, and total RNA was isolated from MLN, Peyer’s patches, or colon at the indicated times. Relative copy numbers for IFN-β transcripts (A) and IFN-λ transcripts (B) were quantified by qRT-PCR. (C) MNoV genomes, IFN-β transcripts, and IFN-λ transcripts in MLNs 48 hours after inoculation were compared between mice infected with CW3D94E containing either the CW3 or the CR6 capsid gene. (D) MNoV genomes in feces from control or Ifnlr1$^{-/-}$ mice were measured at day 21 after inoculation with $10^6$ PFUs of CW3D94E. Data in (A) to (C) are pooled from three independent experiments for a total of three to four mice per time point. Data points in (D) are individual mice pooled from three independent experiments. Dashed lines represent limit of detection. Statistical significance was determined by two-way ANOVA (A and B) or Mann-Whitney test (C and D). n.s. indicates $P > 0.05$; *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$. Error bars in (A) and (B) denote SD.

**Fig. 3. IFN-λ treatment prevents and cures persistent enteric MNoV infection.** (A to C) Feces were collected at the indicated day after oral inoculation, and MNoV genomes were quantified by qRT-PCR. (A) Mice were injected with 25 μg of IFN-λ or phosphate-buffered saline (PBS) intraperitoneally 1 day after oral inoculation with $10^6$ PFUs of CR6. (B) The IFN-λ–treated mice from (A) were rechallenged with $10^6$ PFUs of CR6 at day 14 after initial infection. (C) Persistent infection with CR6 was established in control, Ifnar1$^{-/-}$, or Ifnlr1$^{-/-}$ mice, followed by intraperitoneal injection of 25 μg of IFN-λ on days 21, 23, and 25. Data shown are pooled from two (A) or three (B and C) independent experiments for a total of four to eight mice per time point. Dashed lines represent limit of detection. Statistical significance was determined by two-way ANOVA. n.s. indicates $P > 0.05$; ***$P \leq 0.001$, ****$P \leq 0.0001$. Error bars in (A) and (B) denote SD and in (C) denote SEM.
To determine if therapeutic IFN-λ was effective against established persistent infection, we treated mice with IFN-λ at 21, 23, and 25 days after oral inoculation with CR6, a time at which stable enteric persistence is established (28). Bone marrow chimeras were generated using control and Ifnar1−/− donor and recipient mice as indicated and 8 to 10 weeks later were orally inoculated with 10⁶ PFUs of CR6. (B) MNoV shedding in feces was quantified on day 14. (C) A single 25-μg dose of IFN-λ or PBS was administered intraperitoneally 21 days after inoculation, and MNoV shedding was quantified on day 23. Data in (B) and (C) are pooled from two independent experiments and shown as individual mice. (D) Control or Rag1−/− mice orally inoculated with CR6 were treated 21 days later with a single 25-μg dose of IFN-λ. Shedding was monitored for 35 days postinjection. Dashed lines in (B) to (D) represent limit of detection. Statistical significance was determined by one-way (B) or two-way (A, C, D) ANOVA. n.s. indicates P > 0.05; *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001. Error bars in (A) denote SEM and in (D) denote SD.

IFN-λ acts on nonhematopoietic cells to control enteric persistence. Experiments in reciprocal bone marrow chimeras revealed that the control of fecal MNoV levels (Fig. 4B) and the capacity for IFN-λ to cure enteric persistence (Fig. 4C) mapped to the nonhematopoietic rather than radiation-sensitive hematopoietic cells. These data were consistent with an IFnar1-independent effect of IFN-λ on enteric persistence through stimulation of immunity via signaling in radiation-insensitive cells.

Despite the capacity of mice to mount protective adaptive immune responses to MNoV infection (29–30), their susceptibility to reinfection after treatment with IFN-λ (Fig. 3B) suggested that IFN-λ might act preferentially through the innate immune system. However, it is generally thought that in mammals, viral clearance requires adaptive B and T cell immunity. To test whether IFN-λ can clear an established infection in the absence of an adaptive immune response, we inoculated Rag1−/− mice with CR6 and 21 days later treated them with a single dose of IFN-λ. Enteric persistence of CR6 was cured by IFN-λ treatment of both control and Rag1−/− mice (Fig. 4D). All Rag1−/− mice and the majority of control mice remained MNoV-free for 35 days after treatment, well after therapeutic levels of IFN-λ waned (Fig. 3B). The absence of infectious MNoV was confirmed by fecal transplantation from IFN-λ-cured Rag1−/− mice to naïve Rag1−/− or Stat1−/− mice (fig. S7, B and C). Together, these data demonstrate that IFN-λ treatment both prevents and cures established enteric persistence of MNoV in the absence of an adaptive immune response.

Our study establishes the importance of IFN-λ in innate immunity to persistent enteric viral infection. Establishment of persistent enteric infection by certain strains of MNoV was related to failure to induce IFN-λ responses. Whereas MNoV replicates in hematopoietic cells, IFN-λ was found to act on nonhematopoietic cells, suggesting that the mechanism of action is indirect. The efficacy of IFN-λ in the prevention and cure of enteric persistence did not require adaptive immunity. These findings provide a different view of the immune system because it is generally believed that the development of an adaptive immune response is required for clearance of viral infection by antigen-specific targeting. One implication of our findings is that immune therapies may be able to control persistent virus infection regardless of their effects on adaptive immune responses. We propose that this is an example of sterilizing innate immunity wherein viral clearance can be determined by immune responses apart from adaptive immunity. A similar observation of viral clearance by an independent mechanism of a second enteric virus, rotavirus, supports the existence of sterilizing innate immunity (30). Analogously, metazoan organisms lacking adaptive immune systems are probably capable of clearing some pathogens and preventing persistent infection. We speculate that evolutionarily conserved innate immune mechanisms with sterilizing potential exist, perhaps with particular importance in protection against persistent infection at mucosal surfaces such as the intestine.

Fig. 4. IFN-λ durably clears enteric MNoV persistence through effects on radiation-insensitive cells in the absence of adaptive immunity. (A) BMDCs were treated with media, 100 IU/ml IFN-β, or 100 ng/ml IFN-λ for 24 hours, then inoculated with CR6 at a multiplicity of infection of five, and viral titers were determined by plaque assay 12 hours later. Data are pooled from three independent experiments performed in triplicate and normalized to untreated. (B and C) Bone marrow chimeras were generated using control and Ifnar1−/− donor and recipient mice as indicated and 8 to 10 weeks later were orally inoculated with 10⁶ PFUs of CR6. (B) MNoV shedding in feces was quantified on day 14. (C) A single 25-μg dose of IFN-λ or PBS was administered intraperitoneally 21 days after inoculation, and MNoV shedding was quantified on day 23. Data in (B) and (C) are pooled from two independent experiments and shown as individual mice. (D) Control or Rag1−/− mice orally inoculated with CR6 were treated 21 days later with a single 25-μg dose of IFN-λ. Shedding was monitored for 35 days postinjection. Dashed lines in (B) to (D) represent limit of detection. Statistical significance was determined by one-way (B) or two-way (A, C, D) ANOVA. n.s. indicates P > 0.05; *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001. Error bars in (A) denote SEM and in (D) denote SD.
TELOMERS IN CANCER

Alternative lengthening of telomeres renders cancer cells hypersensitive to ATR inhibitors

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Cancer cells rely on telomerase or the alternative lengthening of telomeres (ALT) pathway to overcome replicative mortality. ALT is mediated by recombination and is prevalent in a subset of human cancers, yet whether it can be exploited therapeutically remains unknown. Loss of the chromatin-remodeling protein ATRX associates with ALT in cancers. Here, we show that ATRX loss compromises cell-cycle regulation of the telomeric noncoding RNA TERRA and leads to persistent association of replication protein A (RPA) with telomeres after DNA replication, creating a recombinogenic nucleoprotein structure. Inhibition of the protein kinase ATR, a critical regulator of recombination recruited by RPA, disrupts ALT and triggers chromosome fragmentation and apoptosis in ALT cells. The cell death induced by ATR inhibitors is highly selective for cancer cells that rely on ALT, suggesting that such inhibitors may be useful for treatment of ALT-positive cancers.

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Supplementary materials

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Supplementary Text
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SUPPLEMENTARY MATERIALS

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