**COMMENTS**

**Gut viruses firm the “Great Wall”**

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**Editor’s note**

A commentary on “Commensal viruses maintain intestinal intraepithelial lymphocytes via noncanonical RIG-I signaling”.

Emerging evidences shows that gut viruses are involved in many physiological processes and are detrimental to human health. A recent study published in *Nature Immunology* describes a novel function of intestinal commensal viruses, maintaining the intestinal intraepithelial lymphocytes (IELs) numbers in the intestine to firm the “great wall” to protect the host against the pathogen invasion (Fig. 1).

There are $10^{13}$ bacteria and $10^{14}$ viruses living in the gut. Numerous studies investigated the composition of commensal bacteria, and their physiological and pathological functions. However, little is known for the role of commensal viruses in the intestine because of limit tools and lack of attention. Recently, emerging evidences shows commensal viruses also play important roles in the intestine. In 2003, about 1200 types of viruses in gut were first detected by shotgun method. Subsequently, metagenomics studies have revealed a bunch of viral components in the microbiome. As the consequence of the sequencing studies, we now know the intestinal virome is composed of eukaryotic viruses, prokaryotic viruses and endogenous retroviruses.

The commensal viruses in the gut is associated with human health and diseases. They benefit the host while in certain circumstances they are opportunistically pathogenic. Kernbauer et al highlighted the beneficial function of commensal viruses showing they contribute to the development of intestinal epithelial cells. They found murine norovirus (MNV) protects antibiotics-treated mice from DSS-induced enteritis dependent on IFNAR1 and MNV infects tuft cells, which contributes to type 2 immune responses. Furthermore, they demonstrate that MNV provides a striking IL-22-dependent protection against early-life lethal infection by *Citrobacter rodentium*. Because norovirus RNA is detected in up to 16% of healthy humans, it is necessary to explore asymptomatic enteric viral infections and the potential effect on human health. Researchers focused on composition and function of intestinal virome and tried to explore the relationship between intestinal virus and diseases such as inflammatory bowel disease (IBD) and type I diabetes. In addition they also explored the immunology, structure, and pathogenesis of enterovirus like Norovirus.
IBDs, including Crohn’s disease (CD) and ulcerative colitis (UC), are chronic diseases with persistent intestinal inflammation\textsuperscript{10}. Disease-specific changes in enteric virome occur in both CD and UC\textsuperscript{8}. Compared with control, there is a higher virus diversity and phage-related abundance (mainly Caudoviridae and Microviridae in IBD fecal viromes) in feces or biopsy tissues of newly diagnosed IBD/CD patients. Epstein-Barr virus (EBV) and human cytomegalovirus (HCMV) infection usually occur in childhood, and healthy people show no symptoms after the infection. However, these viruses may live with the host for lifelong, and they were considered as the risk factor of the IBD\textsuperscript{11}.

More and more basic and clinical studies have provided evidences that support the close relationship between intestinal commensal viruses and the immune system. However, how these viruses are sensed by innate recognition receptor Rig-I, triggering the activation of IRF1/IL-15 pathway to maintain intestinal intraepithelial lymphocyte (IELs) numbers in the intestine\textsuperscript{1}.

First of all, Liu et al. used metagenomics sequencing to confirm the presence of large numbers of phage and eukaryotic viruses in the gut of specific pathogen-free (SPF) mice. Using an antiviral cocktails (AVC) to deplete the intestinal commensal viruses, they found that the number of IELs especially CD8αβ+ TCRβ+ and CD8αα + TCRβ+ cells in the AVC-treated group was significantly reduced. However, there was no significant change in the number and proportion of immune cells in other organs such as spleen or liver in AVC treated mice. The above results indicate that intestinal commensal viruses are important for maintaining IELs homeostasis.

Subsequently, to explore how the intestinal commensal virus is perceived by the body and regulates the IELs homeostasis, the researchers used a variety of natural immune recognition receptors and their adaptors-deficient mice to analyze the proportion and number of intestinal IELs subsets. They found that the deficiency of intracellular RNA sensor Rig-I (Ddx58\textsuperscript{-/-}) and its downstream adaptor protein MAVS (Mavs\textsuperscript{-/-}) result in significantly reduced IELs in the intestine of the mice, which exactly phenocopy AVC treated mice, suggesting
that intestinal commensal virus maintains the IELs homeostasis by activating the RIG-I signaling. Furthermore, the researchers used bone marrow chimeric experiments and conditional knock out mice to verify that the RIG-I signal in lamina propria antigen-presenting cells (APCs) maintained IELs homeostasis.

The authors next tried to figure out the downstream pathway how Rig-I signaling in APC such as DCs maintains the IELs in the gut. The authors first excluded the participation of type I interferon (IFN-I) as Ifnar1−/− mice didn’t show the IEL loss phenotype. Instead, the authors found a much lower level of IL-15 in APCs from Ddx58−/− mice and AVC treated mice, which might be responsible for the IELs loss phenotype. Next, the authors found IRF-1, which control IL-15 production and is also activated for the IELs loss phenotype. Next, the authors found Mavs−/− mice and AVC treated mice, which might be responsible for the IELs loss phenotype. Moreover, some enteroviruses may benefit host via immune-regulating signals18, indicating possibilities to design enteric viruses with desirable characteristics to treat human infection, inflammation or cancer conditions, e.g. current attempts at oncolytic virus treatment of tumor19. In summary, identification and characterization of the beneficial or pathogenic enteric viruses may improve our understanding of the role of microbiota on human health and diseases, and may lead to novel diagnosis and therapies.

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Conflict of interest

The authors declare no conflicts of interest.

References

1. Liu L, Gong T, Tao W, et al. Commensal viruses maintain intestinal intraepithelial lymphocytes via noncanonical RIG-I signaling. Nat Immunol 2019;20:1681–91. doi: 10.1038/s41590-019-0513-z.
2. Breitbart M, Hewson I, Felts B, et al. Metagenomic analyses of an uncultured viral community from human feces. J Bacteriol 2003;185:6220–3. doi:10.1128/jb.185.20.6220-6223.2003.
3. Dinsdale EA, Edwards RA, Hall D, et al. Functional metagenomic profiling of nine biomes. Nature 2008;452:629–32.
4. Kernbauer E, Ding Y, Cadwell K. An enteric virus can replace the beneficial function of commensal bacteria. Nature 2014;516:94–8. doi: 10.1038/nature13960.
5. Wilen CB, Lee S, Hishe LL et al. Tropism for tuft cells determines immune promotion of norovirus pathogenesis. Science 2018;360:204–8. doi: 10.1126/science.aar5799.
6. Neill JA, Matsuzawa-Ishimoto Y, Kernbauer-Hözl E, et al. IFN-I and IL-22 mediate protective effects of intestinal viral infection. Nat Microbiol 2019;4:1737-49. doi: 10.1038/s41564-019-0470-1.
7. Zhao G, Vatanen T, Droit L, et al. Intestinal virome changes precede autoimmunity in type 1 diabetes-susceptible
children. Proc Natl Acad Sci U S A 2017;114:E6166–75. doi: 10.1073/pnas.1706359114.
8. Norman JM, Handley SA, Baldridge MT, et al. Disease-specific alterations in the enteric virome in inflammatory bowel disease. Cell 2015;160:447–60. doi: 10.1016/j.cell.2015.01.002.
9. Orchard RC, Wilen CB, Donch JC, et al. Discovery of a proteinaceous cellular receptor for a norovirus. Science 2016;353:933–6. doi: 10.1126/science.aaf1220.
10. Becker C, Neurath MF, Wirtz S. The intestinal microbiota in inflammatory bowel disease. ILAR J 2015;56:192–204. doi: 10.1093/ilar/ilv030.
11. Lopes S, Andrade P, Conde S, et al. Looking into enteric virome in patients with IBD: Defining guilty or innocence? Inflamm Bowel Dis 2017;23:278–84.
12. Ma H, Tao W, Zhu S. T lymphocytes in the intestinal mucosa: Defense and tolerance. Cell Mol Immunol 2019;16:216–24. doi: 10.1038/s41423-019-0208-2.
13. Thaiss CA, Levy M, Korem T, et al. Microbiota diurnal rhythmicity programs host transcriptome oscillations. Cell 2016;167:1495–510.
14. Zhang B, Chassaing B, Shi Z, et al. Prevention and cure of rotavirus infection via TLR5/NLRC4-mediated production of IL-22 and IL-18. Science 2014;346:861–5. doi: 10.1126/science.1256999.
15. Nice TJ, Baldridge MT, McCune BT, et al. Interferon-lambda cures persistent murine norovirus infection in the absence of adaptive immunity. Science 2015;347:269–73. doi: 10.1126/science.1258100.
16. Torres-Barceló C. The disparate effects of bacteriophages on antibiotic-resistant bacteria. Emerg Microbes Infect 2018;7:168. doi: 10.1038/s41426-018-0169-z.
17. Duan Y, Llorente C, Lang S, et al. Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. Nature 2019;575:505–11. doi: 10.1038/s41586-019-1742-x.
18. Virgin HW, Wherry EJ, Ahmed R. Redefining chronic viral infection. Cell 2009;138:30–50. doi: 10.1016/j.cell.2009.06.036.
19. Kaufman HL, Kohlhapp FJ, Zloza A. Oncolytic viruses: A new class of immunotherapy drugs. Nat Rev Drug Discov 2015;14:642–62. doi: 10.1038/nrd4663.