Precision Clinical Medicine, 2(4), 2019, 209–212

doi: 10.1093/pcmedi/pbz027
Advance Access Publication Date: 2 December 2019
Comments

COMMENTS

Gut viruses firm the "Great Wall"

Anmin Wang[1] and Shu Zhu[1,2,3,*]

[1]Hefei National Laboratory for Physical Sciences at Microscale, CAS Key Laboratory of Innate Immunity and Chronic Disease, School of Basic Medical Sciences, Division of Life Science and Medicine, University of Science and Technology of China, Hefei 230027, China; [2]School of Data Science, University of Science and Technology of China, Hefei 230026, China; [3]CAS Centre for Excellence in Cell and Molecular Biology, University of Science and Technology of China, Hefei 230026, China

*Correspondence: Shu Zhu, zhushu@ustc.edu.cn

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 [1] (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[2]. Subsequently, metagenomics studies have revealed a bunch of viral components in the microbiome[3]. 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[4]. They found murine norovirus (MNV) protects antibiotics-treated mice from DSS-induced enteritis dependent on IFNAR1[3] and MNV infects tuft cells, which contributes to type 2 immune responses[5]. Furthermore, they demonstrate that MNV provides a striking IL-22-dependent protection against early-life lethal infection by Citrobacter rodentium[6]. 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[7,8]. In addition they also explored the immunology, structure, and pathogenesis of enterovirus like Norovirus[9].

Received: 26 November 2019; **Accepted:** 27 November 2019

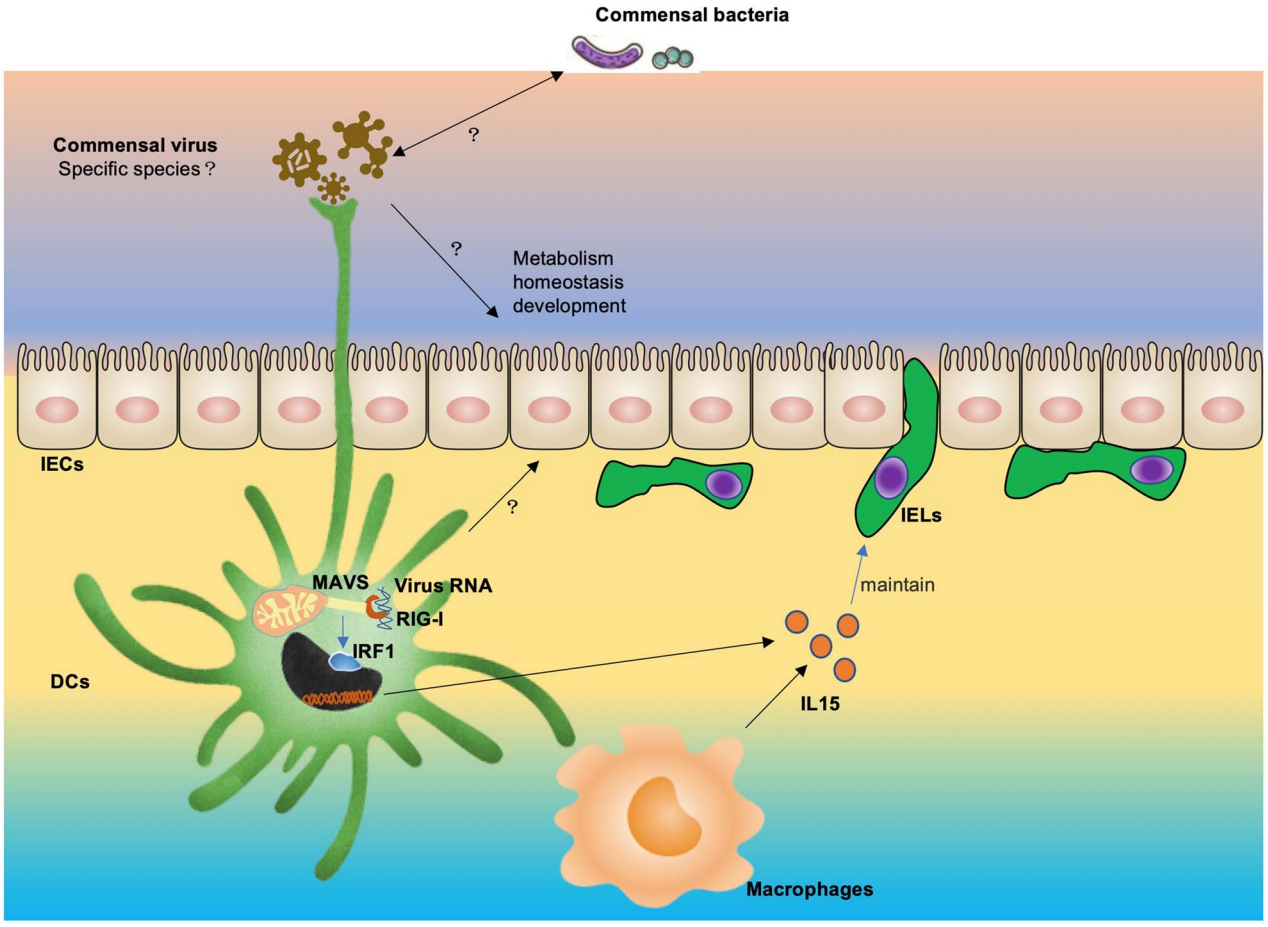

Figure 1. Intestinal commensal viruses help to firm the "Great Wall" against pathogens by maintaining the intestinal intraepithelial lymphocytes (IELs) numbers in the intestine.

IBDs, including Crohn's disease (CD) and ulcerative colitis (UC), are chronic diseases with persistent intestinal inflammation[10]. Disease-specific changes in enteric virome occur in both CD and UC[8]. Compared with control, there is a higher virus diversity and phage-related abundance (mainly *Caudoviridaes* and *Microviridae* in IBD fecal virions) 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[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 immune system in the gut as well as the immune outcome of the viral sensing in the gut is still unknown. In a recent study published in *Nature Immunology,* Liu *et al.* demonstrated that under physiological conditions, intestinal commensal 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[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 CD8$\alpha\beta$ + TCRβ+ and CD8$\alpha\alpha$ + 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*$^{-/-}$) and its downstream adaptor protein MAVS (*Mavs*$^{-/-}$) 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 by RIG-I-MAVS, can maintain IELs numbers. *Irf1*$^{-/-}$mice have the same IELs loss phenotype with *Ddx58*$^{-/-}$ mice, and administration of IL-15 rescue the IEL loss phenotype in these mice, showing the importance of commensal virus/Rig-I/Mavs/IRF1/IL-15 signaling cascade in IEL maintenance.

Since IELs as well as intestinal epithelial cells (IECs) act as the first line of defense against the numerous microbes in the gut lumen[12], the authors tested that whether the maintenance of IEL homeostasis by commensal viruses contribute to intestinal tissue damage and inflammation. *Ddx58*$^{-/-}$ mice as well as virus-depleted mice showed more severe colitis during DSS treatment. AAV-mediated IL-15 delivery rescue the severer colitis phenotype of AVC treated mice and *Mavs*$^{-/-}$ mice, suggesting that Rig-I sensing of commensal viruses is important for the barrier function of intestine.

Recent years witnessed fast growing researches on enteroviruses, including pathogenic viruses, commensal viruses and opportunistically pathogenic viruses. Sequencing, culturing, and examination of the precise role of them in health and disease will help provide novel diagnosis markers or therapies. However, there are still some major challenges in this field.

First, there is still lack of tools to study intestinal virome composition and function. A more thorough examination of the virome with more advanced viral particle (both DNA and RNA viruses) isolation and sequencing methods as well as the establishment of the virus reference database is essential. More specific depletion method of different types of the viruses, as well as the advanced culture methods is also needed to study their precise role in health and diseases.

Second, the commensal viruses are not the only player in the intestine. Besides our current studies on immune-virome crosstalk in intestine, the thorough evaluation of the interaction between virome and other members of the intestinal microbiota, such as bacteria and fungi, is needed to understand the complexity of transkingdom interaction among virome, other microbes, and host.

Third, like bacteria in the microbiota[13], the composition of the intestinal virome is dynamic. How it changes over time is essential to evaluate its impact on human health. Will daily diets and circadian rhythm affect the virome composition or function? Will it change along the year or the whole life? More studies are needed to address these questions.

Finally, intestinal virome research needs an application-reorientation. With the accumulation of the knowledge of transkingdom interaction among virome, other microbes, and host, the targeted therapies became more realistic. For example, treatment with bacterial flagellin or IFNλ cure the rotavirus and MNV infection[14,15]. Also, bacteria phages are used to treat antibiotic resistant bacterial infection or alcoholic liver disease[16,17]. Moreover, some enteroviruses may benefit host via immune-regulating signals[18], 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 tumor[19]. 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.

Acknowledgements

This work is supported by grant from the National Key R&D Program of China (grant No. 2018YFA0508000); the National Natural Science Foundation of China (grants No. 81822021, 91842105, 31770990, 81788101); the Strategic Priority Research Program of the Chinese Academy of Sciences (grant No. XDB29030101).

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, Hsieh LL *et al*. Tropism for tuft cells determines immune promotion of norovirus pathogenesis. *Science* 2018;**360**:204–8. doi: 10.1126/science.aar3799.
6. Neil JA, Matsuzawa-Ishimoto Y, Kernbauer-Hölzl 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 I 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, Doench JG, *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.