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Microbiota as a potentially-modifiable factor influencing COVID-19

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Impacts of respiratory tract viruses have long been appreciated to highly heterogeneous both between and within various populations. The SARS-CoV-2 pandemic, which is the first time that a pathogen's spread across the globe has been extensively monitored by direct detection of the pathogen itself rather just than the morbidity left in its wake, indicates such heterogeneity is not limited to outcomes of infections but whether infection of a particular host occurs at all. This suggests an important role for yet to be discovered environmental (i.e. non-genetic) factors that influence whether an exposure to the virus initiates a productive infection and, moreover, the severity of disease that results. This article discusses the emerging hypothesis that the composition of a host's commensal microbial communities, that is, its 'microbiome', may be one such determinant that influences outcomes following encounters with respiratory viral pathogens in general and SARS-CoV-2 in particular. Specifically, we will review the rationales and evidence that supports this hypothesis and, moreover, speculate as to possible approaches to manipulate microbiota to ameliorate disease induced by respiratory viral pathogens.

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Introduction: microbiota as a determinant of health and disease

External surfaces of vertebrates are colonized by a diverse heterogenous community of bacteria, their phages, fungi, archea, and viruses, archaea, collectively referred to as microbiota, while their collective genes are termed microbiome. Microbiota are acquired early in life but can be modified by a variety of factors including diet and anti-microbial substances. The great breadth of the microbiome's protein-coding capacity, which is many times that of its host, suggests broad potential to influence host phenotypes, including outcomes following encounters with viral pathogens. For example, one can readily imagine select bacteria, or their products, disrupting viral infection by altering host receptors or their viral ligands, as well as influencing subsequent events in viral uptake and replication. Furthermore, the overarching role of microbiota in regulating immune system development and function presents a myriad of possible means by which present or recent exposures to select microbiota may influence outcomes of viral infection. These notions, combined with the fact that the microbiome is presently a topic of broad interest amongst scientists and the health-conscious lay public, make it tempting to speculate that one's microbiota composition influences susceptibility to SARS/Cov-2. This article discusses the plausibility of this hypothesis, largely based on findings of other viruses and the limited evidence that address it from studies of SARS/Cov-2.

Microbiota impacts viral epithelial interactions in the intestinal tract

The intestinal tract is the site of densest bacterial colonization and, moreover, provides best characterized examples of bacteria impacting viral infection in the tissue they inhabit. For example, multiple studies observed that complete absence of gut microbiota, achieved by use of antibiotics or germ-free mice, impedes infection by several enteric viruses including rotavirus, reovirus, and poliovirus [1,2]. Such viral dependence on microbiota for maximal infection appears to reflect, at least in part, a role for bacterial products, including LPS and peptidoglycan facilitating viral-cell binding. Furthermore, binding of viruses by bacteria can serve as a nucleation site, effectively increasing number of virions that enter a cell [3]. Yet, while complete lack of a microbiota impedes infection, it nonetheless occurs in reduced and/or delayed amounts. In contrast, some specific microbiotas can completely protect mice against infection by rotavirus (RV), which remains amongst the world's most deadly pathogens. Specifically, mice harboring high levels of segmented filamentous bacteria (SFB), which can occur in immune-deficient mice or, transiently, in wild-type mice exposed to SFB, are highly resistant to RV infection [4••]. *In vitro* studies suggests that ability of SFB to impede RV infection may, in part, reflect that some surface components of SFB can interfere with viral entry. Yet, *in vivo* studies indicate that SFB may largely impede by RV infection by increasing proliferation/turnover of

epithelial cells which reduces presence of the highly differentiated villus epithelial cells that rotavirus preferentially infects and, moreover, increases anoikis, the process by which intestinal epithelial cells are extruded into the lumen as they destroy their content likely including any infecting viruses [4••,5•]. The extent to which other bacteria can influence rotavirus infection remains under investigation as does the extent to which any bacteria might influence rotavirus infection in humans. Nonetheless, these unequivocal results in tractable models support the proof of concept that microbiota composition can dramatically alter viral-bacterial interactions and, consequently infectious outcomes, at least in the intestine.

Gut microbiota has broad impacts on adaptive immunity

Beyond the specific examples above, gut microbiota also has a major influence over immune system development and regulation such that germ-free mice are largely devoid of mucosal lymphoid tissue. More physiological examples of the microbiota influence on immune regulation can be seen in the stark differences in immune cell populations in mice from different sources, which can be normalized by cohousing or fecal transplant [6•,7]. SFB provides a striking example of the ability of microbiota to impact the immune system in that its presence in the intestine is sufficient to result in Th17 cells in mice that would otherwise lack this important lymphocyte subtype [8]. While there is not much basis to speculate on how the presence of Th17 cells will impact viral infection *per se*, expansion of lung resident Th17 cells associates with severe lung inflammation in SARS/Cov-2 patients thus suggesting this bacteria, and perhaps others like it, may impact severity of Covid-19 irrespective of viral loads [9]. In this context, it is important to note that impacts of gut bacteria on immune cell populations are not gut-restricted but rather dictate T-cell populations in blood and distal tissues, including the lung [10] and, consequently impact diseases seemingly unrelated to the gut such as arthritis [11]. While the specific mechanisms by which SFB and other bacteria impact T-cell populations are not well defined at the molecular level, they are thought to generally reflect the ability of bacteria to activate innate immune signaling by direct adherence to epithelial cells and/or by producing products that activate innate immunity.

Another means by which gut bacteria can modulate adaptive immunity, and consequently potentially impact outcomes of viral infection, is by impacting levels of regulatory T-cells, which can suppress immune cell activation and thus dampen inflammation. A broad variety, but not all, gut bacteria, can promote development of such T-reg cells and the extent to which they do so is impacted by diet. Specifically, development of T-regs is driven by the major products of fermentation of dietary fiber, namely short-chain fatty acids (SCFA) [12]. Thus, we envision that interaction of diet and microbiota will play a role in helping avoid immune-mediated pathology in response to viral infections.

Impacts of microbiota in respiratory tract viral infections

The notion that microbiota might impact COVID-19 stems in large part from trying to understand the heterogeneity in outcomes following exposure to SARS/CoV-2. Specifically, there are many instances of closely related individuals (i.e. similar genetics) being subjected to seemingly identical exposures to SARS/Cov-2 but only some persons ever test positive for the virus and, of those, some have minimal diseases while others becoming severely ill. That microbiota composition can often differ markedly within immediate families [13], combined with the increasingly appreciation that microbiome can broadly impact host phenotype suggests the possibility that it might play a role in such heterogeneity. Indeed, gut microbiota can influence many of the metabolic phenotypes (i.e. obesity, diabetes, and hypertension), known to associate with severe disease. Moreover, given the broadly held view that many of the severe consequences of SARS/CoV-2 infection are driven by the host immune response suggest the microbiota's influence on the immune system might contribute to disease severity. Additionally, many respiratory viral infections are associated with gastrointestinal symptoms and, in the case of SARS/Cov-2 such symptoms associate with severe disease outcomes [14•]. SARS/Cov-2 can be readily detected in feces in infected persons and replicates efficiently in enterocytes [15] suggest the possibility for gut microbiota in impacting SARS/Cov-2.

The upper airways, where respiratory viruses initially infect their hosts, are also relatively densely colonized with microbiota, which might influence viral infection. Culture-independent analysis of the oral and nasopharyngeal microbiota indicate the upper airway microbiota is quite distinct from the gut [16], which is not overly surprising given the stark differences in these environments in numerous factors such as oxygen and nutrients. Furthermore, the extent of inter-personal differences in microbiota composition (i.e. β-diversity) is greater in the upper airway than in the gut [16] potentially allowing it to account for a greater portion of heterogeneity of outcomes following SARS/Cov-2 exposures. Thus, one can imagine SARS/Cov-2 infection in the respiratory tract might be influenced by local bacteria, immune responses they influence, or immune responses originating in other tissues such as the gut.

The myriad of ways microbiota might possibly impacts SARS/Cov-2 infection has made this topic the subject of numerous review and opinion articles, including this one. The published data that directly supports this view

is fairly modest at present consisting of 2 studies, from the same research group, that associated differences in gut microbiota composition with severity of disease following SARS/Cov-2 infection. One study compared microbiomes of COVID-19 patients, during hospitalization and after discharge, to SARS/Cov-2-negative control subjects [17]. They observed that SARS/Cov-2 infection was associated with enrichment of Bacteroidetes and reduced abundance of Actinobacteria, which was driven in part by reductions in Bifidobacterium species. SARS/Cov-2 infection also associated with reduced levels of *Fecalbacterium prausnitzii*. In accord with the notion that both Bifido and *F. prausnitzii* are associated with health and have ability to dampen inflammation in model systems suggest their absence might promote inflammation COVID-19 but might also be consequence of SARS/Cov-2-induced inflammation. To avoid this caveat, the group's other study compared microbiomes of SARS/Cov-2-negative pneumonia patients to hospitalized COVID-19 subjects. They observed SARS/Cov-2 infection associated with reduced abundance of presumed beneficial bacteria including *Fecalbacterium*, *Eubactrium*, and *Lachnospiracceae* species and enrichment of pathobionts in the *Clostridium* and *Actinomyces* phyla again suggesting that, even when controlling for inflammation, SARS/CoV-2 infection associates with altered microbiota composition [18•]. That both studies are from the same research group and used cohorts from the same region suggest that the specific taxa that drove disease associations, may not prove generalizable to other regions/cohorts. However, we speculate that the broad conclusions of these studies, namely that severe COVID-19 associates with reduced abundance in beneficial bacteria and enrichment of pathobionts, will prove broadly applicable although, again, cause and effect are difficult to disentangle. There are not yet published peer reviewed studies on lung microbiome on SARS/Cov-2 infection but one publicly available study reports elevated presence of *Klebsiella oxytoca* that would be generally characterized as dysbiosis [19]. Given that even mild gut inflammation can have a marked impact on gut microbiota, often increasing levels of γ-Proteobacteria, and that such changes can promote inflammation, it seems likely that, at least in part, this observation may be a consequence of, and amplifier, of disease induced by SARS/Cov-2 infection. Whether microbiota composition may actually influence SARS/Cov-2 infection itself is far more speculative but given potential of microbiota to impact viruses and cells they infect, we submit the possibilities are worth considering.

The general notion that microbiota impacts respiratory viral infection is supported by several studies associating microbiome composition with infectious outcomes [20–23]. Yet, while such association are generally convincing with the study population, at present, such studies have not revealed a clear pattern of differences across multiple cohorts. For example, one study noted species richness (i.e. α-diversity), which is often associated with health, associates with more severe influenza infections [24] while others identified cluster of specific taxa or bacterial networks that associate with more severe disease in RSV infection [25,26]. Such observations are not mutually exclusive and may all prove reproducible for those pathogens or the cohorts studied but their apparent discrepancies highlight that this field of research is still in its infancy. Another line of evidence relates to use of antibiotics, typically administered systemically, which have been observed to enhance the severity of influenza infection and increase the risk of secondary bacterial infections [27,28]. However, use of topical antibiotics to specifically deplete nasal microbiota reduced influenza transmissibility [29]. Studies elucidating mechanisms by which microbiota alter respiratory viral infection remain scarce but nonetheless suggest that mechanisms will vary markedly based on specific bacteria and virus. For example, analogous to studies in the gut, some lung bacteria can directly stabilize viral structures *ex vivo* thereby promoting infection [29]. Yet, other bacteria may directly impede viral infection in that *Bacillus subtilis*, namely Surfactin, a cyclic lipopeptide can reduce CoV infectivity *in vitro* [30]. Hence, at present, we can simply conclude that there are a myriad of potential means by which microbiota may influence SARS/Cov-2 infection and its consequences but much more work is needed to assess the extent to which such mechanisms play significant roles in disease.

Strategies for manipulating microbiota to prevent and treat COVID-19

The potential of microbiota to impact COVID-19 suggest potential strategies to manipulate the gut microbiome, including probiotics, prebiotics, fecal microbiota transplantation, or direct use of bacterial components to possibly treat, prevent, or at least lower the severity of COVI-19 diseases.

Probiotics

At present, there are no experimental data to support probiotics as a therapeutic method for COVID-19. However, bacterial components and metabolites derived from gut microbiota significantly contribute to the gut-lung axis regulating respiratory tract immunity [31–36]. It is plausible to hypothesize that normalization of intestinal dysbiosis with probiotics could serve as one of an approach to treat COVID-19, as this approach had shown effectiveness in treating other viral respiratory tract infections [35,37,38]. Experimental and clinical data have shown that probiotics administration has conferred host protection and has therapeutic effects in viral respiratory tract infections, including RSV, influenza, or rhinovirus [39–42]. The complications of SARS-CoV-2 infection are stemmed from immune dysregulation

involving neutrophilia, lymphocytopenia, unbalanced differentiation between T helper cell subsets, and disproportionate productions of inflammatory cytokines/chemokines [43,44]. Certain probiotic strains in the *Lactobacillus* family can modulate immune responses to protect the host from viral respiratory infections. For instance, orally administration of *Lactobacillus plantarum* to mice confer protection against multiple different influenza virus strains by stimulate Th1-mediated immune response, boost NK cell activities, and promoted IgA-mediated mucosal immunity in GI and respiratory tract [39,45]. Also, clinical data have shown that oral intake of *L. plantarum* results in the activation of cytotoxic $CD8^+$ T cells, increase phagocytic activity of granulocytes, and decrease pro-inflammatory cytokines IFN-γ and TNF-α, the two cytokines that contribute to the creation of cytokines storm during COVID-19 infection, and elevation of anti-inflammatory cytokines IL-4 and IL-10. Severe COVID-19 patients usually require to ventilation machine for breathing assistance [46]. Interesting, several studies demonstrated that administration of *B. subtilis* and *Enterococcus faecalis* together or *Lactobacillus rhamnosus* GG can reduce the occurrence of ventilator-associated pneumonia [47,48].

Furthermore, based on previous experimental data on influenza virus infection, we can hypothesize that probiotics decrease the disease's severity by balancing gut microbiota, which might have valuable outcomes due to its role in the gut-lung axis communication, and vitamin A regulation, which is directly linked with immune responses [49,50].

Prebiotics

Prebiotics are compounds in food that induce the growth or activity of beneficial bacterial in the GI tract. As with probiotics, there is currently a lack of experimental data to back-up prebiotics' benefits during SARS-CoV-2 infection. We speculate that prebiotics can offer various benefits to decrease the disease's severity. Prebiotics may have potential effects on COVID-19 by enhancing probiotics growth and survivability and increasing gut diversity and immunity, especially for an aging population. These prebiotics' benefits are likely derived from promoting the increased expansion of SCFAs producing bacteria populations [51]. SCFAs are well known for regulating the immune response [33,35,52]. During SARS-CoV-2 infection, perhaps due to the immune response overreact to the virus, it produces excessively a large amount of inflammatory cytokine leading to 'cytokine storm.' These cytokines storms promote death of infected cells but they also cause multi-organ malfunction. SCFAs such as butyrate is well documented to reduce inflammatory responses [51]. Conceptually, prebiotic administration to encourage SCFA-producing bacterial populations' growth can be a probable method to decrease COVID-19 complications.

Fecal microbiota transplant

The microbiota from an unhealthy host is distinctively different compared to a healthy host. As discussed above, the gut-lung axis may influence systematic inflammation in COVID-19 patients. The dysbiosis of microbiota could result in abnormal inflammatory cytokines productions and T cell hyperstimulation, which is relevant to COVID-19 [44,53]. In addition to prebiotics and probiotics, fecal microbiota transplant (FMT) is another potential approach that can be investigated for COVID-19 therapeutic purposes. Tocilizumab, a monoclonal antibody that inhibited the IL-6 receptor and is used as a treatment for arthritis and is currently in clinical trials for patients infected with SARS-CoV-2 [54]. Several studies have shown the reduction of IL-6 and other inflammatory cytokines post-FMT [55]. Furthermore, microbiota analysis in IBD patients treated with FMT shows a shift toward bacterial species that can induce anti-inflammatory cytokine IL-10 and stimulate regulatory T-cell expansion [56]. Collectively, these data advocate for the use of FMT as either a treatment or an adjuvant to quell the cytokine storms in severe cases of COVID-19 patients.

Bacterial components and metabolites. Another approach for harnessing microbiota for COVID-19 prevention/treatment is to use bacterial components such as flagellin. Flagellin is recognized by toll-like receptor 5 (TLR5), which is expressed by epithelial cells and select mucosal leukocytes, and the NLRC4 inflammasome, which is broadly expressed. Activation of TLR5 broadly reprograms cell gene expression enabling the host to broadly withstand a variety of insults including exposure to chemicals, radiation, and pathogenic microbes [57] thus suggesting that some of the beneficial immune stimulatory aspects of a commensal microbiota might be therapeutically mimicked by administration of purified flagellin. Activation of the NLRC4 inflammasome results in secretion of IL-1β and IL-18, and death of some virus-infected cells [5•]. However, while flagellin administration does not cause the severe adverse impacts induced by some TLR agonists such as LPS, its broad systemic induction of cytokines may, nonetheless, prove problematic. Hence, a more specific treatment opinion might be to directly administer the specific cytokines, namely IL-22 and IL-18 that mediate flagellin's antiviral effects. That expression of ACE2 receptor, the central mediator of SARS-CoV-2 entry, is preferentially expressed on highly differentiated epithelial cells [15], whose levels are reduced by IL-22, supports this notion. Thus, we speculate that flagellin and or cytokines it induces might have potential to prevent/treat COVID-19 [58].

Perspective

The commensal microbiota is a plausible determinant of outcomes following exposure to a broad array of pathogens, including SARS/CoV-2. Possible microbiota

influences on such infections might reflect direct bacterial-viral interactions, impacts of bacteria on virus-infected cells, bacterial influences on the immune system, or be a consequence of microbiota's influences on health and metabolism in general. At present, evidence to support an important role of microbiota in influencing Covid-19 is largely based on general proof of concepts from study of other viruses in animal models or human association data that does not hint at mechanism nor does it rule out the possibility that such associations are a consequence of SARS/Cov-2 infection rather than a determinant. Nonetheless, we speculate the microbiota holds promise to better understand the heterogeneity of the Covid-19 pandemic and may suggest novel treatment strategies.

Conflict of interest statement

Nothing declared.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Kuss SK, Best GT, Etheredge CA, Pruijssers AJ, Frierson JM, Hooper LV, Dermody TS, Pfeiffer JK: **Intestinal microbiota promote enteric virus replication and systemic pathogenesis**. *Science* 2011, **334**:249-252.

2. Karst SM: **The influence of commensal bacteria on infection with enteric viruses**. *Nat Rev Microbiol* 2016, **14**:197-204.

3. Erickson AK, Jesudhasan PR, Mayer MJ, Narbad A, Winter SE, Pfeiffer JK: **Bacteria facilitate enteric virus co-infection of mammalian cells and promote genetic recombination**. *Cell Host Microbe* 2018, **23**:77-88 e5.

4. •• Shi Z, Zou J, Zhang Z, Zhao X, Noriega J, Zhang B, Zhao C, Ingle H, Bittinger K, Mattei LM *et al.*: **Segmented filamentous bacteria prevent and cure rotavirus infection**. *Cell* 2019, **179**:644-658 e13.

Demonstrates potential for a single bacteria in a complex microbiota to prevent viral infection independent of interferon.

5. • Zhang Z, Zou J, Shi Z, Zhang B, Etienne-Mesmin L, Wang Y, Shi X, Shao F, Chassaing B, Gewirtz AT: **IL-22-induced cell extrusion and IL-18-induced cell death prevent and cure rotavirus infection**. *Sci Immunol* 2020, **5**.

Demonstrates a potential mechanisms whereby microbial products can prevent viral infection.

6. • Beura LK, Hamilton SE, Bi K, Schenkel JM, Odumade OA, Casey KA, Thompson EA, Fraser KA, Rosato PC, Filali-Mouhim A *et al.*: **Normalizing the environment recapitulates adult human immune traits in laboratory mice**. *Nature* 2016, **532**:512-516.

Striking demonstration of how microbial environment can impact immune development.

7. Ivanov II, Frutos Rde L, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, Finlay BB, Littman DR: **Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine**. *Cell Host Microbe* 2008, **4**:337-349.

8. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV *et al.*: **Induction of intestinal Th17 cells by segmented filamentous bacteria**. *Cell* 2009, **139**:485-498.

9. Zhao Y, Kilian C, Turner JE, Bosurgi L, Roedl K, Bartsch P, Gnirck AC, Cortesi F, Schultheiss C, Hellmig M *et al.*: **Clonal expansion and activation of tissue-resident memory-like Th17 cells expressing GM-CSF in the lungs of severe COVID-19 patients**. *Sci Immunol* 2021, **6**.

10. Bradley CP, Teng F, Felix KM, Sano T, Naskar D, Block KE, Huang H, Knox KS, Littman DR, Wu HJ: **Segmented filamentous bacteria provoke lung autoimmunity by inducing gut-lung axis Th17 cells expressing dual TCRs**. *Cell Host Microbe* 2017, **22**:697-704 e4.

11. Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, Umesaki Y, Littman DR, Benoist C, Mathis D: **Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells**. *Immunity* 2010, **32**:815-827.

12. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly YM, Glickman JN, Garrett WS: **The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis**. *Science* 2013, **341**:569-573.

13. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP *et al.*: **A core gut microbiome in obese and lean twins**. *Nature* 2009, **457**:480-484.

14. • Pan L, Mu M, Yang P, Sun Y, Wang R, Yan J, Li P, Hu B, Wang J, Hu C *et al.*: **Clinical characteristics of COVID-19 patients with digestive symptoms in Hubei, China: a descriptive, cross-sectional, multicenter study**. *Am J Gastroenterol* 2020, **115**:766-773.

Study connects intestine with respiratory infection.

15. Zang R, Gomez Castro MF, McCune BT, Zeng Q, Rothlauf PW, Sonnek NM, Liu Z, Brulois KF, Wang X, Greenberg HB *et al.*: **TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes**. *Sci Immunol* 2020, **5**.

16. Human Microbiome Project C: **Structure, function and diversity of the healthy human microbiome**. *Nature* 2012, **486**:207-214.

17. Yeoh YK, Zuo T, Lui GC, Zhang F, Liu Q, Li AY, Chung AC, Cheung CP, Tso EY, Fung KS *et al.*: **Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19**. *Gut* 2021, **70**:698-706.

18. • Zuo T, Zhang F, Lui GCY, Yeoh YK, Li AYL, Zhan H, Wan Y, Chung ACK, Cheung CP, Chen N *et al.*: **Alterations in gut microbiota of patients with COVID-19 during time of hospitalization**. *Gastroenterology* 2020, **159**:944-955 e8.

Supports concept that microbiota might impact SARS/CoV-2.

19. Han Y, Zhilong J, Shi J, Wang W, He K: **The active lung microbiota landscape of COVID-19 patients**. *medRxiv* 2020 http://dx.doi.org/10.1101/2020.08.20.20144014.

20. Budden KF, Gellatly SL, Wood DL, Cooper MA, Morrison M, Hugenholtz P, Hansbro PM: **Emerging pathogenic links between microbiota and the gut-lung axis**. *Nat Rev Microbiol* 2017, **15**:55-63.

21. Enaud R, Prevel R, Ciarlo E, Beaufils F, Wieers G, Guery B, Delhaes L: **The gut-lung axis in health and respiratory diseases: a place for inter-organ and inter-kingdom crosstalks**. *Front Cell Infect Microbiol* 2020, **10**:9.

22. Groves HT, Cuthbertson L, James P, Moffatt MF, Cox MJ, Tregoning JS: **Respiratory disease following viral lung infection alters the murine gut microbiota**. *Front Immunol* 2018, **9**:182.

23. Groves HT, Higham SL, Moffatt MF, Cox MJ, Tregoning JS: **Respiratory viral infection alters the gut microbiota by inducing inappetence**. *mBio* 2020, **11**.

24. Lee KH, Foxman B, Kuan G, Lopez R, Shedden K, Ng S, Balmaseda A, Gordon A: **The respiratory microbiota: associations with influenza symptomatology and viral shedding**. *Ann Epidemiol* 2019, **37**:51-56 e6.

25. Grier A, Gill AL, Kessler HA, Corbett A, Bandyopadhyay S, Java J, Holden-Wiltse J, Falsey AR, Topham DJ, Mariani TJ *et al.*: **Temporal dysbiosis of infant nasal microbiota relative to respiratory syncytial virus infection**. *J Infect Dis* 2020 http://dx.doi.org/10.1093/infdis/jiaa577.

26. Schippa S, Frassanito A, Marazzato M, Nenna R, Petrarca L, Neroni B, Bonfiglio G, Guerrieri F, Frasca F, Oliveto G *et al.*: **Nasal microbiota in RSV bronchiolitis**. *Microorganisms* 2020, **8**.

27. Sencio V, Barthelemy A, Tavares LP, Machado MG, Soulard D, Cuinat C, Queiroz-Junior CM, Noordine ML, Salome-Desnoulez S, Deryuter L *et al.*: **Gut dysbiosis during influenza**

contributes to pulmonary pneumococcal superinfection through altered short-chain fatty acid production. *Cell Rep* 2020, **30**:2934-2947 e6.

28. Bradley KC, Finsterbusch K, Schnepf D, Crotta S, Llorian M, Davidson S, Fuchs SY, Staeheli P, Wack A: **Microbiota-driven tonic interferon signals in lung stromal cells protect from influenza virus infection**. *Cell Rep* 2019, **28**:245-256 e4.

29. Rowe HM, Livingston B, Margolis E, Davis A, Meliopoulos VA, Echlin H, Schultz-Cherry S, Rosch JW: **Respiratory bacteria stabilize and promote airborne transmission of influenza A virus**. *mSystems* 2020, **5**.

30. Johnson BA, Hage A, Kalveram B, Mears M, Plante JA, Rodriguez SE, Ding Z, Luo X, Bente D, Bradrick SS *et al.*: **Peptidoglycan-associated cyclic lipopeptide disrupts viral infectivity**. *J Virol* 2019, **93**.

31. Levy M, Thaiss CA, Elinav E: **Metabolites: messengers between the microbiota and the immune system**. *Genes Dev* 2016, **30**:1589-1597.

32. Rooks MG, Garrett WS: **Gut microbiota, metabolites and host immunity**. *Nat Rev Immunol* 2016, **16**:341-352.

33. Trompette A, Gollwitzer ES, Pattaroni C, Lopez-Mejia IC, Riva E, Pernot J, Ubags N, Fajas L, Nicod LP, Marsland BJ: **Dietary fiber confers protection against flu by shaping Ly6c(-) patrolling monocyte hematopoiesis and CD8(+) T cell metabolism**. *Immunity* 2018, **48**:992-1005 e8.

34. Yang W, Yu T, Huang X, Bilotta AJ, Xu L, Lu Y, Sun J, Pan F, Zhou J, Zhang W *et al.*: **Intestinal microbiota-derived short-chain fatty acids regulation of immune cell IL-22 production and gut immunity**. *Nat Commun* 2020, **11**:4457.

35. Steed AL, Christophi GP, Kaiko GE, Sun L, Goodwin VM, Jain U, Esaulova E, Artyomov MN, Morales DJ, Holtzman MJ *et al.*: **The microbial metabolite desaminotyrosine protects from influenza through type I interferon**. *Science* 2017, **357**:498-502.

36. Bandoro C, Runstadler JA: **Bacterial lipopolysaccharide destabilizes influenza viruses**. *mSphere* 2017, **2**.

37. Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, Murray TS, Iwasaki A: **Microbiota regulates immune defense against respiratory tract influenza A virus infection**. *Proc Natl Acad Sci U S A* 2011, **108**:5354-5359.

38. Eguchi K, Fujitani N, Nakagawa H, Miyazaki T: **Prevention of respiratory syncytial virus infection with probiotic lactic acid bacterium *Lactobacillus gasseri* SBT2055**. *Sci Rep* 2019, **9**:4812.

39. Park MK, Ngo V, Kwon YM, Lee YT, Yoo S, Cho YH, Hong SM, Hwang HS, Ko EJ, Jung YJ *et al.*: ***Lactobacillus plantarum* DK119 as a probiotic confers protection against influenza virus by modulating innate immunity**. *PLoS One* 2013, **8**:e75368.

40. Jung YJ, Lee YT, Ngo VL, Cho YH, Ko EJ, Hong SM, Kim KH, Jang JH, Oh JS, Park MK *et al.*: **Heat-killed *Lactobacillus casei* confers broad protection against influenza A virus primary infection and develops heterosubtypic immunity against future secondary infection**. *Sci Rep* 2017, **7**:17360.

41. Kitazawa H, Villena J: **Modulation of respiratory TLR3-anti-viral response by probiotic microorganisms: lessons learned from *Lactobacillus rhamnosus* CRL1505**. *Front Immunol* 2014, **5**:201.

42. Lehtinen MJ, Hibberd AA, Mannikko S, Yeung N, Kauko T, Forssten S, Lehtoranta L, Lahtinen SJ, Stahl B, Lyra A, Turner RB: **Nasal microbiota clusters associate with inflammatory response, viral load, and symptom severity in experimental rhinovirus challenge**. *Sci Rep* 2018, **8**:11411.

43. Leisman DE, Ronner L, Pinotti R, Taylor MD, Sinha P, Calfee CS, Hirayama AV, Mastroiani F, Turtle CJ, Harhay MO *et al.*: **Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison with other inflammatory syndromes**. *Lancet Respir Med* 2020, **8**:1233-1244.

44. Karlsson AC, Humbert M, Buggert M: **The known unknowns of T cell immunity to COVID-19**. *Sci Immunol* 2020, **5**.

45. Kikuchi Y, Kunitoh-Asari A, Hayakawa K, Imai S, Kasuya K, Abe K, Adachi Y, Fukudome S, Takahashi Y, Hachimura S: **Oral administration of *Lactobacillus plantarum* strain AYA enhances IgA secretion and provides survival protection against influenza virus infection in mice**. *PLoS One* 2014, **9**: e86416.

46. Auld S, Caridi-Scheible M, Blum JM, Robichaux CJ, Kraft CS, Jacob JT, Jabaley CS, Carpenter D, Kaplow R, Hernandez AC *et al.*: **ICU and ventilator mortality among critically ill adults with COVID-19**. *Crit Care Med* 2020, **48**:e799-e804.

47. Morrow LE, Kollef MH, Casale TB: **Probiotic prophylaxis of ventilator-associated pneumonia: a blinded, randomized, controlled trial**. *Am J Respir Crit Care Med* 2010, **182**:1058-1064.

48. Zeng J, Wang CT, Zhang FS, Qi F, Wang SF, Ma S, Wu TJ, Tian H, Tian ZT, Zhang SL *et al.*: **Effect of probiotics on the incidence of ventilator-associated pneumonia in critically ill patients: a randomized controlled multicenter trial**. *Intensive Care Med* 2016, **42**:1018-1028.

49. Chong HX, Yusoff NAA, Hor YY, Lew LC, Jaafar MH, Choi SB, Yusoff MSB, Wahid N, Abdullah M, Zakaria N *et al.*: ***Lactobacillus plantarum* DR7 improved upper respiratory tract infections via enhancing immune and inflammatory parameters: a randomized, double-blind, placebo-controlled study**. *J Dairy Sci* 2019, **102**:4783-4797.

50. Rask C, Adlerberth I, Berggren A, Ahren IL, Wold AE: **Differential effect on cell-mediated immunity in human volunteers after intake of different lactobacilli**. *Clin Exp Immunol* 2013, **172**:321-332.

51. Archer DL, Kramer DC: **The use of microbial accessible and fermentable carbohydrates and/or butyrate as supportive treatment for patients with coronavirus SARS-CoV-2 infection**. *Front Med (Lausanne)* 2020, **7**:292.

52. Dang AT, Marsland BJ: **Microbes, metabolites, and the gut-lung axis**. *Mucosal Immunol* 2019, **12**:843-850.

53. Fajgenbaum DC, June CH: **Cytokine storm**. *N Engl J Med* 2020, **383**:2255-2273.

54. Gentile G, Davies R, Manfreda VM, Ul Abideen Z: **Successful treatment of severe COVID-19 pneumonia and hyperinflammatory syndrome with tocilizumab**. *BMJ Case Rep* 2021, **14**.

55. Konturek PC, Koziel J, Dieterich W, Haziri D, Wirtz S, Glowczyk I, Konturek K, Neurath MF, Zopf Y: **Successful therapy of *Clostridium difficile* infection with fecal microbiota transplantation**. *J Physiol Pharmacol* 2016, **67**:859-866.

56. Quraishi MN, Shaheen W, Oo YH, Iqbal TH: **Immunological mechanisms underpinning faecal microbiota transplantation for the treatment of inflammatory bowel disease**. *Clin Exp Immunol* 2020, **199**:24-38.

57. Vijay-Kumar M, Aitken JD, Sanders CJ, Frias A, Sloane VM, Xu J, Neish AS, Rojas M, Gewirtz AT: **Flagellin treatment protects against chemicals, bacteria, viruses, and radiation**. *J Immunol* 2008, **180**:8280-8285.

58. Golonka RM, Saha P, Yeoh BS, Chattopadhyay S, Gewirtz AT, Joe B, Vijay-Kumar M: **Harnessing innate immunity to eliminate SARS-CoV-2 and ameliorate COVID-19 disease**. *Physiol Genomics* 2020, **52**:217-221.