Gut as viral reservoir: lessons from gut viromes, HIV and COVID-19

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BACKGROUND: VIRUSES IN THE HEALTHY GUT

The gut microbiota is composed of bacteria, fungi and viruses.[1–3] While the presence and composition of the bacteriome have been extensively studied in recent years, the 'dark matter' of microbiomes including viruses (viral microbiome or virome) remains incompletely understood.[4] Analysis of microbiotic communities in the gut showed that bacteria rapidly colonise the gut after birth, followed by the appearance of prophages by 1 month which are harboured by these bacteria. Subsequently, a gut virome dominated by bacteriophages develops and human viruses can be detected 4 months after birth.[5 6] In adults, recent studies have analysed the gut virome in healthy individuals by using metagenomic approaches.[7 8] For instance, a metagenome analysis in 1986 individuals identified 33 242 unique viral populations, demonstrating that the human gut contains a vast array of bacteriophages and eukaryotic RNA and DNA viruses with a predominance of bacteriophages. Specifically, several groups of prokaryotic crAss-like, Caudovirales and Microviridae bacteriophages and eukaryotic adenoviruses and herpesviruses were characterised as stable colonisers of the human gut.

Further studies described the existence of individual-specific persistent personal viromes. These viromes displayed marked interindividual heterogeneity with age-dependent changes and were critically dependent on host factors such as geography and ethnicity-distinct diets.[9 10] Moreover, the duration of urban residence was associated with the presence of multiple bacteriophages, including *Lactobacillus* and *Lactococcus* phages.[9] Although the functions of most viruses in the gut viromes have not been fully characterised, it has been suggested that viromes play a crucial role in shaping the gut microbiome and intestinal health under homeostatic conditions.

This concept was underlined by experimental studies in murine dextran sulfate sodium (DSS) colitis.[11 12] Treatment of DSS colitis with a cocktail of antiviral nucleoside analogues led to augmented mucosal inflammation, while administration of agonists for Toll-like receptor 3 (TLR3: sensor for double-stranded RNA) or TLR7 (sensor for single-stranded RNA) suppressed colitis activity. Triggering of TLR3 and TLR7 responses was associated with production of interferon-β by mucosal, plasmacytoid dendritic cells. Collectively, these results indicated that recognition of resident luminal viruses regulates protective mucosal immunity during experimental colitis.[11] This protective role of the gut virome in the intestinal microbiota is reminiscent of the well-established protective role of commensal bacteria in intestinal inflammation.[12]

ALTERATIONS OF THE GUT VIROME IN INTESTINAL INFLAMMATORY AND NEOPLASTIC DISORDERS

In spite of the relative stability of personal gut viromes, marked changes may occur in patients with chronic disorders of the intestine. A recent study in human UC used deep metagenomic sequencing in 91 patients and 76 healthy controls.[13] An expansion of mucosal viruses was noted that correlated with the presence of inflammation. In particular, Caudovirales, *Escherichia* and Enterobacteria phages were more frequently detected in patients with UC as compared with controls. Functions of bacteriophages with relationship to host bacteria fitness and pathogenicity were increased in UC mucosa, suggesting that structural and functional changes in the gut virome may play an important role in disease pathogenesis.[13] A second metagenomic study compared the gut virome using colonic/ileal tissues in treatment-naive UC with Crohn's disease (CD) and controls[14] and found an increase of Hepadnaviridae in UC, while Hepeviridae were more frequently detected in CD as compared with controls. In contrast, Virgaviridae and Polydnaviridae were reduced in CD and UC, respectively. Finally, two additional studies reported expansion of Caudovirales or Adenoviridae and Herpesviridae in both CD and UC, respectively.[15 16]

There is evidence that enteric viruses can modulate disease activity in IBD. For instance, rotavirus infections have been shown to induce epithelial damage and proinflammatory intestinal immune responses through increased secretion of cytokines such as interferon-β and thus may augment gut inflammation in IBD.[17] Moreover, reduction of mucosal blood type antigens in non-secretor patients carrying polymorphisms of the α1,2-fucosyltransferase (FUT2) and FUT3 genes has been linked to altered host–microbiome interactions in IBD.[18–20] Specifically, the FUT2 W143X mutation may cause asymptomatic norovirus infection and favour the development of ileitis in CD.[21]

In addition to UC and CD, changes in the virome of faecal samples were noted in IBS. A study in 55 patients with IBS and 51 controls showed lower alpha diversity of viral clusters in the former as compared with the latter patients and abundant bacteriophage clusters (Caudovirales) in IBS. Additional analysis of CRISPR (clustered regularly interspaced short palindromic repeats) spacers indicated that changes in the faecal virome were related in part to the alterations in the bacteriome, suggesting that their cross-sectional relationship may affect IBS pathogenesis.[22] After allogeneic haematopoietic stem cell transplantation (HSCT), a significant increase in the viral sequences of faecal samples was found in a recent study in 44 patients. Moreover, enteric graft-versus-host disease (GVHD) following HSCT was characterised by reduced phage richness and expansion of persistent DNA viruses such as anelloviruses, herpesviruses, papillomaviruses and polyomaviruses. In particular, the presence of picobirnaviruses was predictive of severe enteric GVHD.[23] In addition, after repeated faecal microbiota transplantation (FMT) in severe colitis due to GVHD, the gut virome showed a stable rise in diversity. While the levels of Torque teno viruses decreased after FMTs, an increase in the relative abundance of Caudovirales bacteriophages in faecal samples was observed.[24] Collectively, these findings demonstrated marked alterations of the gut virome in GVHD and GVHD-associated enteritis that may affect the course of the disease.

In addition to intestinal inflammation, it should be noted that viruses have also been implicated in the pathogenesis of neoplastic GI disorders such as colorectal cancer.[25] Several studies supported the concept that the gut virome is involved in cancerogenesis and tumour growth in this disease by viral

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infection of epithelial cells or modulation of the composition of bacterial communities.[25 26] For instance, the large T-antigen of polyomavirus was suggested to drive activation of the β-catenin/Wnt pathway, which favours cell proliferation and apoptosis resistance in colorectal cancer.[27–29] Moreover, the diversity of the gut bacteriophage community was significantly increased in patients with colorectal cancer as compared with control patients without cancer,[30] and certain bacteriophages (Caudovirales, *Inovirus*, *Orthobunyavirus*) were associated with dysbiosis as potential driver of cancer development.[30 31] Finally, virus-based therapeutic concepts (engineered oncolytic viruses and bacteriophages) have been suggested as potential future therapy for colorectal cancer.[25]

THE GUT AS RESERVOIR FOR HIV AND SARS-COV-2

The mucosal surface of the gut plays a key role during infection with HIV.[32 33] In fact, the gut contains a large number of infected cells, even in individuals receiving antiretroviral therapy (ART). This process is facilitated by the increased susceptibility of mucosal CD4+ T cells for HIV infection due to greater C-C chemokine receptor (CCR)-5 expression and local T cell activation.[34 35] On infection, there is a rapid loss of mucosal barrier function with translocation of microbial products[36] and loss of mucosal CD4+ T cells.[37] These changes favour local and systemic inflammation in HIV-infected individuals. Probiotic treatment of HIV-infected patients has been shown to suppress microbial translocation and serum inflammatory markers in ART-naive patients,[38 39] highlighting a leaky barrier as key component of HIV-induced gut inflammation. Moreover, it has been shown that the mucosa, as the largest lymphoid organ of the body, is an important reservoir for HIV-infected cells. Consistently, studies reported that HIV-DNA levels in CD4+ T cells were on average 5–6 times higher in the ileum compared with blood in patients on ART,[40] and 2-fold to 12-fold higher in the duodenum, ileum, right colon and rectum as compared with peripheral blood.[41] Based on these findings it was estimated that the gut harbours 83%–95% of all HIV-infected cells in the body and thus provides a key reservoir for disease persistence. Local, infected CD13+ myeloid cells and CD4+ T cells may reach regional lymph nodes and the bloodstream via immune cell trafficking, thereby favouring viral dissemination throughout the body.[35 42 43] This concept has important implications for clinical therapy. In fact, ART does not cure HIV infection due to the persistence of HIV reservoirs in long-lived memory CD4 T cells present in the blood, lymph nodes and intestinal tract.[44]

The COVID-19 pandemic is caused by SARS-CoV-2. Infection of more than 144 million cases has been reported and has led to 3 million confirmed deaths.[45] As intestinal epithelial cells express high amounts of the SARS-CoV-2 receptor ACE2 and transmembrane protease serine subtype 2 (TMPRSS2), a cellular protease important for viral entry, GI infection is frequently seen in COVID-19 and GI symptoms have been reported in 30%–70% of patients.[46–48] Remarkably, GI infection results in limited or absent signs of local inflammation as well as low mortality in COVID-19, suggesting a potential role of the GI tract in attenuating SARS-CoV-2 infection.[49] Stool analyses confirmed the presence of SARS-CoV-2 genomic and subgenomic RNA in affected patients,[50] but isolation of the virus from stool samples has rarely been successful,[51] indicating that GI infection may be self-limiting. However, local infection of intestinal epithelial cells in COVID-19 may affect the gut virome. In patients with COVID-19, a significantly reduced viral Shannon diversity was noted as compared with the gut viromes of healthy controls.[52] Specifically, viromes were composed of DNA/RNA viruses (mainly Herelleviridae and Virgaviridae families) as well as bacteriophages (Caudovirales, crAss-like phage, Inoviridae, Microviridae, Myoviridae, Podoviridae and Siphoviridae families).[52] A recent metagenomic study profiled the faecal RNA and DNA viromes of patients with COVID-19 and found that the faecal virome in SARS-CoV-2 infection harboured more stress-associated, inflammation-associated and virulence-associated gene-encoding capacities, including those pertaining to bacteriophage integration, DNA repair, and metabolism and virulence associated with their bacterial host. Several gut viruses also correlated with COVID-19 disease severity, suggesting that the gut virome may calibrate host immunity and regulate severity to SARS-CoV-2 infection.[53]

Cellular and humoral immunity mediated by T and B cells plays a key role in COVID-19.[54–57] In particular, B cell-derived antibodies against the spike protein and its receptor-binding domain are relevant in this context, as they prevent virus binding to epithelial cells.[54 55] Moreover, an expansion of T follicular helper cells is indicative of a matured humoral immune response allowing memory B cells to prevent possible reinfection.[54] However, the reasons for persistent antigen-specific memory responses in COVID-19 remained mysterious, despite the above findings, suggesting the presence of reservoirs for viral proteins that might trigger prolonged immune responses. Interestingly, there is a high rate of positive PCR findings in stool even weeks or months after respiratory samples became negative, indicating persistence of mucosal SARS-CoV-2 mRNA in patients with COVID-19.[58] This could be due to infection of intestinal epithelial cells, as SARS-CoV-2 could infect and
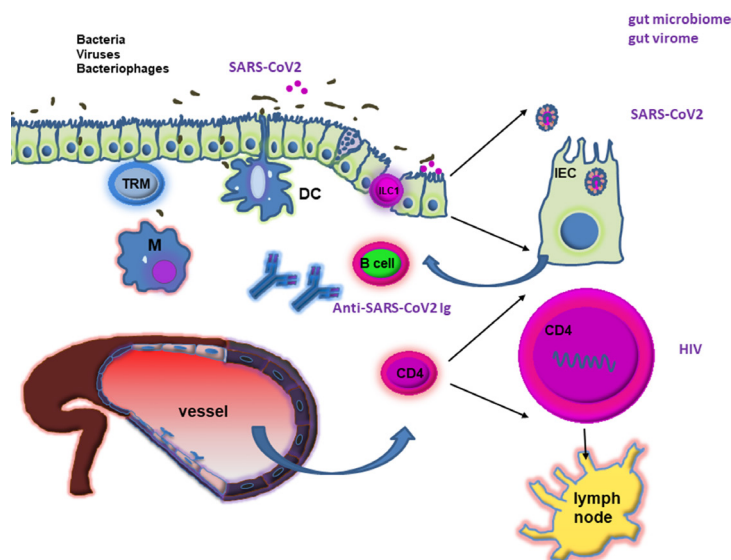

Figure 1 The gut virome and microbiome are displayed in the lumen. SARS-CoV-2 may infect intestinal epithelial cells, leading to persistent activation of memory B and T cell responses. Moreover, in HIV-infected patients, the gut serves as a reservoir for infected cells, which may reach the bloodstream via lymph nodes and immune cell trafficking. DC, dendritic cell; IEC, intestinal epithelial cell; ILC, innate lymphoic cell; M, macrophage; TRM, tissue resident memory T cell.

productively replicate in human intestinal tissues ex vivo with subsequent release of infectious virus particles, suggesting that the GI tract serves as a potential route of virus dissemination within an infected host.[59] Consistently, a recent study showed that SARS-CoV-2 nucleoprotein (N) is present in intestinal epithelial cells of approximately 35% of patients with COVID-19 even several weeks or months after initial diagnosis, indicating antigen persistence in the intestine after resolution of clinical illness.[60] Given the high turnover of intestinal epithelial cells persistence of the viral antigen is also suggestive of continuous virus replication. As even small amounts of persistent viral antigen have been suggested to fuel antibody evolution, the observation on persistent N protein expression over prolonged periods of time is consistent with the relative persistence of SARS-CoV-2 IgA antibodies and continued antibody evolution, as well as persistent polyfunctional SARS-CoV-2 antigen-specific B and T cell memory responses.[61] These immune responses in turn are likely to support a rapid and effective adaptive immune response to the virus on re-exposure and thus provide a critical cornerstone in immune protection against COVID-19. However, one cannot exclude the possibility that long-term shedding of viruses might contribute to long-term COVID-19 and we feel that this point requires further investigation.

IMPLICATIONS AND CONCLUSION

Recent studies have started to explore the composition and function of the gut virome, which provides a rich reservoir for bacteriophages and viruses. These studies have highlighted the concept that bacteriophages and viruses play a seminal role in shaping the bacterial microbiome and are important in controlling gut homeostasis.[7 8] Moreover, transfer of bacteriophages and viruses during FMT may contribute to treatment efficacy in inflammatory disorders. This concept was underlined by a recent study demonstrating that transfer and effective colonisation of donor Caudovirales bacteriophages correlates with treatment efficacy of FMT in *Clostridioides difficile* infection (CDI).[62] Others demonstrated that sterile faecal filtrates from donor faeces were effective in treating recurrent CDI in five patients and the gut virome at several months post-FMT related to the donor virome.[63 64] These findings indicate that apart from live bacteria, other components of the microbiota such as bacteriophages, antimicrobial compounds or metabolites contribute to re-establishment of the intestinal microbiota in FMT. These findings

have important implications for future design of FMT trials in patients with inflammatory disorders and suggest that careful characterisation of the gut virome should be considered for FMT donors. In addition, the potential of selective transfer of viral communities should be explored. For instance, a recent study[65] described that selective transfer of caecal viral communities from mice with a lean phenotype into mice with an obese phenotype leads to reduced weight gain and normalised blood glucose parameters, suggesting that faecal virome transfer might be used in the future for treatment of diseases.

Another important concept arising from studies in patients with viral infections relates to the gut as potential reservoir for recurrent disease or continuous immune cell stimulation (figure 1). For instance, the intestine has been identified as a crucial reservoir for HIV-infected immune cells that may reach the bloodstream via immune cell trafficking, thereby potentially impairing the efficacy of ART in affected patients. Furthermore, more recent studies of patients with COVID-19 on SARS-CoV-2 infection uncovered a potentially important role of persistent infection of intestinal epithelial cells for prolonged memory immune responses and prevention of reinfection. Collectively, these data shed new light on the role of the intestine as reservoir for viral infections and provide fascinating insights into the function of intestinal epithelial and immune cells in controlling host responses during infections.

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