The role of virome in the gastrointestinal tract and beyond

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Abstract

The human gut virome is comprised of diverse commensal and pathogenic viruses. The colonization by these viruses begins right after birth through vaginal delivery, then continues through breastfeeding, and broader environmental exposure. Their constant interaction with their bacterial hosts in the body shapes not only our microbiomes but us. In addition, these viruses interact with the immune cells, trigger a broad range of immune responses, and influence different metabolic pathways. Besides its key role in regulating the human gut homeostasis, the intestinal virome contributes to disease development in distant organs, both directly and indirectly. In this review, we will describe the changes in the gut virome through life, health, and disease, followed by discussing the interactions between the virome, the microbiome, and the human host as well as providing an overview of their contribution to gut disease and disease of distant organs.

Introduction

The human virome is the assembly of viruses that can be found in the human body (Zarate *et al.* 2018). These viruses can be divided into eukaryotic viruses, which mostly infect human cells; prokaryotic viruses, otherwise known as bacteriophages or phages (Zarate *et al.* 2018); and archaeal viruses (Matijašić *et al.* 2020) (Table 1). Most archaeal viruses isolated so far have been double-stranded DNA (dsDNA) viruses (Prangishvili, Forterre and Garrett 2006). The interactions between archaeal viruses and their hosts as well as their impact on human health are still largely unexplored (Matijašić *et al.* 2020), and thus, will not be further discussed in this review. Eukaryotic viruses inhabiting the human body are the most studied compared to other members of the virome, as organisms that cause disease, and thus play a more obvious role in human health (Zarate *et al.* 2018). However, the majority of the viruses in the body are reported to be phages (Liang and Bushman 2021), which in the gut have an abundance of up to 10^8 virus-like-particles (VLPs) per millilitre of faecal matter (Dion, Oechslin and Moineau 2020). It has been suggested that phages play a significant role in maintaining gut homeostasis through regulating bacterial abundance, diversity, and metabolism (Mills *et al.* 2013).

Here, we will mainly describe the phages and eukaryotic viruses living in the human gut, changes in their community structure through life, the influencing factors that shape their composition, and their interactions with the human hosts, as well as their role in human health and disease. We believe that our understanding of the complex interactions between the gut virome and the human host remains insufficient, especially regarding the contribution of gut virome to pathogenesis and disease progression in distant organs.

Gut virome through life, health and disease

The gastrointestinal virome contains a higher abundance of *Caudovirales* or tailed phages compared to other virus families (Carding, Davis and Hoyles 2017). Amongst them, crAssphages, which infect *Bacteroides intestinalis*, are the most common phages in the human gut virome (Townsend *et al.* 2021; Beller *et al.* 2022), followed by their close relatives Gubaphages (Camarillo-Guerrero *et al.* 2021). Other members of the human virome include phages belonging to the families *Podoviridae* and *Siphoviridae*, all of which are dsDNA phages (Clooney *et al.* 2019), and are mostly temperate (Shkoporov *et al.* 2019), as well as the single-stranded DNA (ssDNA) *Microviridae,* which are mostly lytic (Minot *et al.* 2013). Our understanding of phage diversity is limited, as most phage genomes show no homology to existing viral databases (Aggarwala, Liang and Bushman 2017), and cannot be linked to a bacterial host (Fitzgerald *et al.* 2021). Therefore, composition estimates of the gut virome are difficult and are complicated further by bias introduced during sample processing (Kim and Bae 2011).

Gut virome composition and the role of its key regulators

Together with diet and health-status, age is one of the main factors influencing gut virome composition, starting from birth (Gregory *et al.* 2020; Liang and Bushman 2021) (Fig 1). The quantification of VLPs shows very few to no particles in the meconium, the first infant stool samples. However, the number of VLPs increases to 10^9 VLPs per gram of faecal matter after one month of life (Liang *et al.* 2020). Birth by vaginal or caesarean delivery has a significant impact on alpha and beta diversity of the infant virome at 12 months whilst no significant difference was observed in bacterial diversity (McCann *et al.* 2018). There is some evidence that the earliest phages in the infant gut are induced prophages originating from the first colonizing bacteria (Liang *et al.* 2020), such as *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Firmicutes* (Baumann-Dudenhoeffer *et al.* 2018), which themselves are acquired during vaginal birth (Shamash and Maurice 2022). For example, *Bifidobacteria* containing prophages have been proven to be transmitted from mother to child via breastfeeding (Duranti *et al.* 2017). The induced *bifidophages* were shown to belong to *Caudovirales* (Lugli *et al.* 2016) which dominate the early infant phageome (Sharon *et al.* 2013), which only contains a small percentage of *crAssphage* (Shamash and Maurice 2022). *CrAssphages* start to appear between 1 and 3 months after *Bacteroides* colonize the infant gut (Gregory *et al.* 2015). Similarly to the colonization by *Bifidobacteria* and their phages, *crAssphages* from the infant gut have been shown to have up to 99% genomic similarity to *crAssphages* from the maternal gut (Siranosian *et al.* 2020). With age, the eukaryotic viral richness increases whilst phage richness and diversity decrease in an inverse relationship to bacterial richness and

diversity (Lim *et al.* 2015). The *Caudovirales* dominated phageome shifts to *Microviridae* dominance in early childhood (Lim *et al.* 2015). In adulthood, *crAssphages* become the most abundant phages in the human gut virome (Guerin *et al.* 2018; Shkoporov *et al.* 2018; Beller *et al.* 2022)

Longitudinal studies (Reyes *et al.* 2010; Lim *et al.* 2015; Shkoporov *et al.* 2019) have shown that the individual virome is highly stable. Virome intrapersonal variation is less than 5% within a year in healthy adult individuals (Reyes *et al.* 2010), compared to the highly diverse and changeable infant gut (Garmaeva *et al.* 2019).

Diet also influences the gut virome from birth. Infants fed with breast milk have a significantly different virome at 4 months when compared to infants fed with formula. Within the breast-milk-fed cohort, there was very little presence of eukaryotic viruses in the gut virome, and a high abundance in phages infecting *Bifidobacterium* and *Lactobacillus* (Liang *et al.* 2020). Later in life, diet still has an impact on gut virome. Dietary intervention studies (Minot *et al.* 2011; Garmaeva *et al.* 2021) showed that changes in diet, although moderate, had an effect on the gut virome, with the richness and diversity returning to pre-diet-intervention levels post-diet. However, recent studies have shown that common dietary ingredients such as coffee, cinnamon and oregano, amongst others (Boling *et al.* 2020), and orally taken medications, like NSAIDs (Sutcliffe *et al.* 2021), can induce prophages which could in turn alter the dynamics and composition of the gut microbiome.

Diet interconnects with ethnicity and geography as shown by a recent study of the gut virome in 6 different Chinese ethnic groups in both urban and rural environments. Ethnicity-specific diet had a significant effect on the gut virome composition between ethnic groups in the same environment. However, the most significant effect was between rural and urbanized environments, with higher urbanization being associated with a loss in virome diversity (Zuo *et al.* 2020). This has also been found in studies looking at the global abundance of *crAssphages*, finding that abundance was low in adults from traditional hunter-gatherer societies compared to modern industrialized populations (Honap *et al.* 2020). Similarly, in a study about the effect of breast milk and formula feeding on the infant gut, African-American cohorts were compared to a Botswanan cohort, which showed that in the Botswana cohort breast-milk-fed infants had a high number of *Enteroviruses* in their stool, which was not the case in the African-American cohorts (Liang *et al.* 2020). Other studies (Desai *et al.* 2020) confirmed that children in resource-poor regions have a higher diversity of enteric viral pathogens compared to those in resource-high regions.

Gut virome association with gut disease

Viral gastroenteritis is one of the most common illnesses globally as well as a leading cause of mortality, causing 2 to 3 million deaths annually (Oude Munnink and van der Hoek 2016). Interestingly, the main causative agent for viral gastroenteritis differs between adults and children. While children are primarily infected by *Rotavirus*, *Sapovirus* and *Adenovirus* (Eckardt and Baumgart 2011), adults are usually infected with *Norovirus* which accounts for 90% of viral gastroenteritis cases and roughly 50% of all viral gastroenteritis outbreaks

worldwide (Patel *et al.* 2009). However, in addition to causing acute infections, eukaryotic viruses are also estimated to cause approximately 8-12 chronic viral infections per person throughout their lifetime, most of which are asymptomatic (Virgin, Wherry and Ahmed 2009). One example is *Anellovirus*, which is abundant in infants but reduced in children older than 18 months (Carding, Davis and Hoyles 2017), and whilst it persists in many sites of the human body, its role in human physiology and disease is largely unknown (Virgin, Wherry and Ahmed 2009). Similarly, *Circovirus* is abundant in the human gut virome without being linked to any specific disease. (Rascovan, Duraisamy and Desnues 2016).

Increased viral diversity can also be found in colorectal cancer patients, mostly for eukaryotic viruses. Epstein-Barr virus (Coughlan *et al.* 2021) was reported to cause gastric cancer (Norman *et al.* 2015), while both Epstein-Barr and the human papilloma viruses have been linked to colorectal cancer (Prendergast *et al.* 2015; Aftab, Shah and Hashmi 2016; Prendergast and Kelly 2016; Ma *et al.* 2021). Gut phages are not considered direct causative agents of disease in humans, but are driving factors that modulate bacterial communities. Global shifts in the phageome are linked to the development of diseases including environmental enteric dysfunction (EED) (Reyes *et al.* 2015), inflammatory bowel disease (IBD) (Coughlan *et al.* 2021), which includes Crohn's disease (CD) (Norman *et al.* 2015) and ulcerative colitis (UC) (Clooney *et al.* 2019), irritable bowel syndrome (IBS), coeliac disease and oesophageal adenocarcinoma (Emlet, Ruffin and Lamendella 2020) (Table 2). These findings reflect the association of phages with the development of disease and imply that phages are actively involved in pathogenesis through their modulation of intestinal inflammation and bacterial communities (Norman *et al.* 2015; Nakatsu *et al.* 2018; Zuo *et al.* 2019). Therefore, they have the potential to act as biomarker for dysbiosis-associated diseases. In addition, phage genomes carry evidence of interactions between phages and their bacterial hosts, like a 'time capsule', which remains to be explored (Scanlan *et al.* 2011; Koskella and Brockhurst 2014). Many of these genes may play a role in bacterial metabolism and pathogenicity, thus contributing to different diseases (Scanlan *et al.* 2011; Koskella and Brockhurst 2014).

Mechanisms of Host-Virome interactions

Impact of gut phageome on host through gut microbiota modulation

Phage-mediated microbiota modulation in the gut. Phages are able to significantly impact and regulate the bacterial communities within the human body. They exert immense evolutionary pressure, which drives bacteria to evolve numerous resistance mechanisms (Barr, 2017).

Bacteriophages indirectly influence human health and disease through their contributions to the gut microbiome composition, structure, and function (Manrique, Dills and Young 2017; Tisza and Buck 2021). Shifts in the gut virome are linked to bacterial dysbiosis and intestinal inflammation in patients with IBD: either Crohn's disease or UC (Norman *et al.*, 2015). Observations of increased abundance of *Caudovirales* bacteriophages have also been reported in patients with IBD, although the viruses responsible for the microbiome shifts in each

disease were different, suggesting specific virome signatures for each disease (Norman *et al.*, 2015). Moreover, transplantation of phages from patients with UC alters the composition of the gut microbiome and increases colitis severity in human microbiota associated (HMA) mice (Levy *et al.*, 2017, Sinha *et al.*, 2021).

In addition to lysing their host bacteria, virulent phages can directly or indirectly shape the non-host bacterial community. For instance, lytic *Enterococcus faecalis* phages could inhibit non-host bacteria by inducing *E. faecalis* type VII secretion system upon their infection (Chatterjee *et al.* 2021). Moreover, virulent phages can cause shifts in gut bacteria through a cascading effect (Hsu *et al.* 2019), in which the phage-induced bacterial modulation impacts the gut metabolome, with reduced production of neurotransmitters and altered bile acid metabolism (Hsu *et al.* 2019), all of which are involved in interactions between the gut and distant organs (Sun and Chang 2014; Agus, Planchais and Sokol 2018). However, this study (Hsu *et al.* 2019) was performed *in vivo* with a consortium of 10 gut bacterial species, and hence further investigations are needed to determine the knock-on consequences of phage infection on the gut microbiome and human host.

Commensal bacteria such as *Faecalibacterium prausnitzii* are generally depleted in IBD patients (Cornuault *et al.* 2018). Previous studies reported an increase in the abundance or prevalence of *F. prausnitzii* phages in the faecal samples of IBD patients compared to healthy controls, suggesting a temperate-phage mediated killing of the bacteria that aggravates intestinal inflammation and promotes dysbiosis (Cornuault *et al.*, 2018). Comparably to virome shifts in IBD patients, IBS patients have been shown to have significantly lower alpha diversity of phages compared to healthy controls (Coughlan *et al.* 2021). However this occurs without the switch in core phage lifecycles from lysogenic to lytic as has been described in IBD, where an individual-specific shift towards induced temperate phages replaced the healthy core virome, and their induction was presumably due to environmental stress factors associated with the inflamed gut (Clooney *et al.* 2019). Furthermore, gut inflammation and enteric disease were shown to trigger induction of the *Salmonella Typhimurium* (S.Tm) prophage, SopEΦ (Diard *et al.* 2017). This is a consequence of the bacterial SOS response being triggered by inflammation, which promotes prophage induction, leading to bacterial lysis and increased dysbiosis (Diard *et al.* 2017).

Translocation of gut viruses to distant organs. Some gut viruses can reach distant organs through multiple pathways. Specifically, gut phages are capable of adhering to the mucosal surfaces and crossing the epithelial cells by specific uptake, in which viruses are recognized by the epithelial cell receptors, which allows their crossing and migration into the body (Bomsel and Alfsen 2003; Cavarelli and Scarlatti 2014; Fu *et al.* 2017; Gilsdorf 2019; Good, Wells and Coyne 2019; Oechslin, Moradpour and Gouttenoire 2020), or nonspecific transcytosis (Nguyen *et al.* 2017), including macropinocytosis (Miernikiewicz and Dabrowska 2022) as shown in Fig. 2. They can spread throughout the human body to reach blood and lymph, as well as major organs such as the liver, kidneys, lungs, urinary tract, and even the brain (Keller and Engley 1958; Weber-Dabrowska, Dabrowski and Slopek 1987; Barr 2017; Nguyen *et al.* 2017). In the lymphatic system, phages interact with macrophages

and circulating dendritic cells (DCs), which triggers the humoral immune responses and stimulates cytokine production (Miernikiewicz *et al.* 2013; Van Belleghem *et al.* 2017) (Fig. 2). The presence of phage DNA was found inside extracellular bacterial membrane vesicles (MV) in circulation, suggesting another route for phage transport and dissemination outside of the gut (Champagne-Jorgensen *et al.* 2021: 1).

Additionally, viruses can pass through the epithelium at sites of cellular damage and punctured vasculature into the interstitial matrix via a phenomenon characterized by increased intestinal permeability called 'Leaky Gut' (Handley *et al.* 2012; Karimi *et al.* 2016) (Fig. 2). Evidently, this phenomenon complicates underlying diseases; for example, gut dysbiosis-associated inflammation could allow small molecules to bypass the epithelium and enter the body where they can affect various organs (Hartmann, Chen and Schnabl 2012). It is still unclear whether gut leakage is a causative factor of gut diseases or simply occurs in association, however more studies support the latter hypothesis (Camilleri 2019).

Once these viruses enter the interstitial matrix and drain into the lymphatic system, they become circulating viruses, and can then access the regional lymph nodes and disseminate to organs throughout the body (Wiig, Keskin and Kalluri 2010; Choi, Lee and Hong 2012) according to their host tropism and receptor distribution (Jang *et al.* 2009; Jiao *et al.* 2014; Op de Beeck and Eizirik 2016; Majer, McGreevy and Booth 2020; Oechslin, Moradpour and Gouttenoire 2020).

Impact of gut phages on distant organs. Changes in gut phage composition and increased abundance of intestinal pathobionts and their infecting phages influence the development of diseases in distant organs, including pulmonary arterial hypertension (Kim *et al.* 2020), Parkinson's disease (Baizabal-Carvallo and Alonso-Juarez 2020), non-alcoholic fatty liver disease (Lang *et al.* 2020), type 1 diabetes (T1D) (Cinek *et al.* 2017; Zhao *et al.* 2017) and type 2 diabetes (T2D) (Chen *et al.* 2021) (Table 2). Increased gut permeability can result from phage-induced microbiota alterations (Tetz and Tetz 2016) and triggers or intensify diseases in distant organs (Bosi *et al.* 2006; Baizabal-Carvallo and Alonso-Juarez 2020). Increased intestinal permeability has been linked to leakage of substances produced by gut microbiota into the central nervous system, which accelerates CNS inflammation and degeneration in neurodegenerative diseases (*e.g.* Parkinson's disease) (Baizabal-Carvallo and Alonso-Juarez 2020).

Gut phages can also positively impact distant organs. Increased levels of *Caudovirales* and specifically the *Siphoviridae* virus family in humans were linked to improved functioning in verbal memory and executive processes, while subjects with increased *Microviridae* levels exhibited impaired executive abilities (Mayneris-Perxachs *et al.* 2022). Comparably, *Siphoviridae*-rich microbiota transplantations from human donors improved memory function in both mice and *Drosophila* (Mayneris-Perxachs *et al.* 2022). Adding to the positive effect of gut phages on distant organs; applying lytic phages from *Picovirinae* subfamily to target cytolytic *E. faecalis* in the mammalian gut decreased cytolysin expression and attenuated alcoholic liver disease (Duan *et al.* 2019).

Temperate *E. coli* phages have a protective effect from autoimmune reactions like T1D, in which they reduce *E. coli* abundance and induce bacterial amyloids, which could function as antigens for plasmacytoid dendritic cells (pDCs) and trigger disease progression (Tetz *et al.* 2019). These examples validate the important role that gut phages play in modulating disease progression in distant organs. Further research is required in order to assess the exact role phages play in distant disease pathogenesis.

Auxiliary metabolic genes. Phages regulate bacterial community structures in most ecosystems both by predation and horizontal gene transfer. They contribute to host virulence, colonization, replication, and transmission (Hampton, Watson and Fineran 2020). Through phage-mediated horizontal gene transfer (HGT), phages supply their hosts with functional genes that were acquired during ancestral infections and allow fitness advantages for their host (Mara *et al.* 2020).

One example of this is auxiliary metabolic genes (AMGs). These are genes that have originated in bacteria and are mainly described for marine phages (Chevallereau *et al.* 2022). AMGs are involved in reprogramming the host metabolism, which indirectly influences several biogeochemical cycles important to living organisms, including carbon, nitrogen and sulphur) (Hurwitz, Hallam and Sullivan 2013; Anantharaman *et al.* 2014; Kieft *et al.* 2021a).

Dissimilatory Sulphur Metabolism (DSM) genes are encoded by 'sulphur phages' within the order *Caudovirales* and more specifically, the *Myoviridae, Siphoviridae* and *Podoviridae* families in marine environments (Kieft *et al.* 2021b). However, these AMGs are involved in the production of hydrogen sulphide (H_2S), a gas that mediates various biological processes, such as metabolism, neurodegeneration, regulation of inflammation, and blood pressure (Fike, Bradley and Rose 2015; Hellmich and Szabo 2015; Kieft *et al.* 2021a). The main sources for H_2S production in the human gut are 1) cysteine catabolism by colonocytes and 2) sulphate reduction by sulphate-reducing bacteria (Guo *et al.* 2016; Blachier *et al.* 2021). Increased levels of H_2S were found in cancer cells of various tissue types including colorectal cancer (CRC) (Shackelford *et al.* 2021). Additionally, the resistance of colon cancer cells to chemotherapy is linked to an increase in H_2S synthesis, while inhibition of H_2S synthesis increases the sensitivity of the cancer cells to chemotherapeutic agents (Shackelford *et al.* 2021) (Fig. 3). Although to date, DSM genes have only been described in marine phages, based on their considerable contribution to sulphur metabolism (Kieft *et al.* 2021b) and the important role that sulphate-reducing bacteria play in H_2S synthesis in the human gut (Guo *et al.* 2016), it is highly likely that these genes are also carried by gut phages, yet this remains to be explored.

However, AMGs are found to be prevalent in the genomes of many gut phages identified by metagenomic analyses (Mathieu *et al.* 2020; de Jonge *et al.* 2021; Ma *et al.* 2021; Nayfach *et al.* 2021; Pratama *et al.* 2021). For example, individuals at risk of developing rheumatoid arthritis (RA) have been shown to harbour distinct gut phages compared to their healthy counterparts. In addition, these phages encode AMGs associated with immunomodulation and disease progression, including genes involved in outer membrane glycan metabolism (e.g. LPS) (Mangalea *et al.* 2021). Glycoside hydrolase family 32 (GH32), an AMG found in

Bacillus subtilis phages, is involved in the metabolism of fructo-oligosaccharides (FOS) (Maaroufi and Levesque 2015; Ozaki *et al.* 2017), which retain intestinal permeability and tight junctions (Carvalho *et al.* 2021; Tanno *et al.* 2021).

AMGs not only assist in microbial cellular processes, but also in extracellular virulence by encoding bacterial exotoxins. These exotoxins are one of the most described virulence properties encoded by phages (Wagner and Waldor 2002). For example, bacterial exotoxins such as spyA, tccC, entB and entD encoded by phages are found to be associated with the development of oesophageal diseases (Ma *et al.* 2021). In addition, CTXφ-encoded zonula occludens toxin (Zot) in *Vibrio cholerae*, that resembles the activity of zonulin structurally and functionally, can regulate the intestinal permeability and cause multiple diseases in distant organs (Fasano 2011; Pérez-Reytor *et al.* 2018).

The effects of AMGs can be significantly expanded through phage-mediated horizontal gene transfer (Tyler *et al.* 2013; Fasano 2002; Muniesa and Schmidt 2014; Mai-Prochnow *et al.* 2015). Considering the high abundance of prophages in the gut, we expect AMGs to play a significant role in human health and disease. Therefore, more studies are warranted to explore the function of phage-encoded genes in gut bacteria, especially those that are unknown.

Interactions between the gut virome and the mammalian immune system

Interactions between bacteriophages and the immune system. Bacteriophages are important components of the virome and have a great potential to shape and regulate mammalian immunity. *In vitro* incubation of purified *Staphylococcus aureus* and *Pseudomonas aeruginosa* phages with peripheral blood monocytes could induce immune responses, such as increasing the transcription of IL-1, IL-6, and tumour necrosis factor (TNF) (Van Belleghem *et al.* 2017). The oral treatment of germ-free mice with purified and lipopolysaccharide(LPS)-free *E. coli* phages resulted in the expansion of IFN-γ-producing CD4+ T cells and of CD8+ T cells in the Peyer's patches of the treated mice (Gogokhia *et al.* 2019) (Fig. 2). M13 phages could trigger interferon production and protect mice from tail lesions caused by vaccinia virus (Mori *et al.* 1996), and the *Staphylococcus aureus* phage A20/R induced the production of pro-inflammatory cytokine IL-6 (Zimecki *et al.* 2003). Lactobacillus, Escherichia, and Bacteroides phages were capable of exacerbating colitis via their activation of IFN-γ through a toll-like receptor 9 (TLR9)-dependent pathway (Gogokhia *et al.*, 2019). Moreover, altered viral signatures in mice were correlated with the release of pro-inflammatory cytokines as well as decreased production of neurogenesis markers (Seth *et al.* 2019).

Nevertheless, phages are weak immunomodulatory agents, and they generally have poor pro-inflammatory effects. Research on the effects of T4 phages on the immune system showed that LPS-induced reactive oxygen species (ROS) production by peripheral blood polymorphonuclear leukocytes (PMNs) in response to bacterial infections was reduced when the cells were treated with purified phages with low endotoxin levels (Miedzybrodzki *et al.* 2008). Additionally, phages are capable of inhibiting human T-cell activation and proliferation as well as obstructing other pathogenic viral infections when present in the medium by inhibiting nuclear transcription factor NF-κB activation in response to the viral

attack (Górski *et al.* 2006). However, it is important not to oversimplify the nature of interactions between phages and the immune system, because their effect on the immune system is not exclusively suppressive, as mentioned above.

Phages are therefore double-edged immunomodulators; they can trigger both pro- and anti-inflammatory immunological pathways, which further solidifies their role as modulators of disease development, either through their regulation of the microbiome or through stimulation of immune responses, the extent to which is still obscure and requires further research.

Interactions between eukaryotic viruses and the immune system. The immune system plays an essential role in regulating the intestinal microbiota via its control of the density and composition of the resident microbial and viral communities (Salzman *et al.* 2010; Duerkop and Hooper 2013). Host cells can recognize the invasion of viruses through the detection of viral components such as genomic material or viral proteins. The innate immune system first detects pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs) (Akira, Uematsu and Takeuchi 2006).

Viruses can be beneficial to human health and can act as mutualistic symbionts, conferring advantages to their host instead of being strictly harmful parasites. For example, latent infection with *herpesvirus* increases resistance to *Listeria monocytogenes* and *Yersinia pestis* through the production of IFN-γ. Systemic activation of macrophages (Barton *et al.* 2007) and natural killer (NK) cells in mice results in higher protection against tumour grafts (White *et al.* 2010).

Consistent with the positive effects of the virome on host health, commensal viruses in healthy mice were shown to contribute to the development of intraepithelial lymphocytes (IELs) which are a key component of the defence system of the mammalian gut (Lee and Baldridge 2019). Conversely, an altered gut virome leads to increased intestinal inflammation and permeability as well as interleukin 6 (IL-6), IL-1β and interferon gamma (IFN-γ) production (Seth *et al.* 2019).

Gut eukaryotic viruses contribute to the recruitment of immunosuppressive regulator T cells (Treg) and the decrease of T cell activation (Pearson *et al.* 2019), which means that the immunomodulatory role of viruses is likely heavily influenced by the complex equilibrium between Tregs and affected cell populations in order to maintain immune tolerance to commensal viruses and prevent inflammation (Li, Handley and Baldridge 2021).

Viral infectivity of *poliovirus* and *reovirus* was reduced in antibiotic treated mice, suggesting a potential role that bacteria play in increasing viral pathogenicity (Kuss *et al.* 2011). These findings suggest that enteric viruses exploit intestinal microbes for increased pathogenicity and infectivity (Robinson and Pfeiffer 2014).

Viruses can encode host antigen-like proteins to elicit autoimmune responses, such as viral tyrosine phosphatase (IA-2) encoded by *enterovirus* and *rotaviruses* causing cross-reactive

immune responses against β-cells (Härkönen *et al.* 2002; Honkanen *et al.* 2017). Additionally, host-like hormones and enzymes encoded by viruses can function similarly to those produced by the host. For example, *Iridoviridae* viruses produce insulin/insulin growth factor (IGF)1-like peptides in the gut, which can interact with human and murine insulin and IGF1 receptors, activate cell proliferation and increase glucose uptake (Altindis *et al.* 2018; Huang, Kahn and Altindis 2019).

Influenza-induced type-I IFN production in the lungs promotes the reduction of obligate anaerobic bacteria and the increase of Proteobacteria in the gut, leading to a dysbiotic intestinal microenvironment (Deriu *et al.* 2016). A recent study found that viral dsRNA induces NLRP6 inflammasome activation, which is involved in anti-microbial defence in the intestine and liver (Shen *et al.* 2021). Influenza infection also limits antimicrobial and inflammatory responses to *Salmonella*-induced colitis in the gut, thus escalating *Salmonella* intestinal colonization and systemic dissemination (Deriu *et al.* 2016). Conversely, the commensal microbiota in the intestine can regulate the production of CD4+ and CD8+ T cells and antibody responses following influenza virus infection and this can lead to a higher viral replication in the lung (Ichinohe *et al.* 2011).

These findings confirm the complexity of interactions between viruses and the human host, both locally and in distant organs.

Concluding remarks

Given the complexity of virome studies, from the known unknowns to the unknown unknowns, many challenges need to be overcome in order to reach a comprehensive understanding of the direct and indirect interactions between the virome and its hosts.

It seems promising to focus more efforts into looking at the associations between the gut and the rest of the human body, because, as the Greek physician Hippocrates said in the 5th century BCE: “All diseases begin in the gut.”

'Omics' approaches, including metagenomics, meta-transcriptomics and metabolomics, are specifically helpful for understanding the mechanisms by which the gut virome affects distant organs by providing unprecedented resolution of the interactions between viruses and their hosts. Therefore, we expect an inevitable transition from mono-omics to multi-omics in virome research in the near future to address the current shortcomings with virome analyses in revealing the mechanistic link between the virome and human health.

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Author Contributions

All authors listed have contributed significantly to this work and approved it for publication.

Declaration of interests

There are no interests to declare.

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Glossary

Alpha diversity: The measure of microbiome variation within a single community (sample).

Auxiliary metabolic genes (AMGs): Genes expressed during phage infections to regulate metabolic processes and modulate virion production by directing intracellular nutrients towards viral replication.

Beta diversity: The measure of microbiome similarities or differences between two communities (samples).

Homeostasis: A self-regulating process in which biological organisms maintain stable internal physical and chemical conditions while adjusting to unstable external conditions.

Leaky Gut syndrome: A condition characterized by increased intestinal permeability and loosening of intestinal wall tight junctions. It is a digestive condition in which viruses, bacteria, toxins and cytokines are able to "leak" through the intestinal wall.

Lysogenic cycle: A state in which bacteriophages integrate their nucleic acids into the host bacterium's genome or form a circular replicon in the bacterial cytoplasm. In this cycle, phage DNA is termed prophage.

Lytic cycle: A state in which bacteriophages use bacterial hosts upon infection to manufacture more viruses, with the viruses then bursting out of the cell.

Pathobionts: Organisms, which, under homeostatic conditions, are non-harmful symbionts but can potentially become pathological when specific environmental conditions are altered.

Peyer's patches: Groupings of organized lymphoid follicles in the mucus membrane that lines the small intestine.

Sulphate reduction: A process in which sulphate-reducing bacteria reduce sulphate into hydrogen sulphide (H_2S) that is a toxic gas synthesized by both sulphate-reducing bacteria as well as endogenous enzymes in the human body.

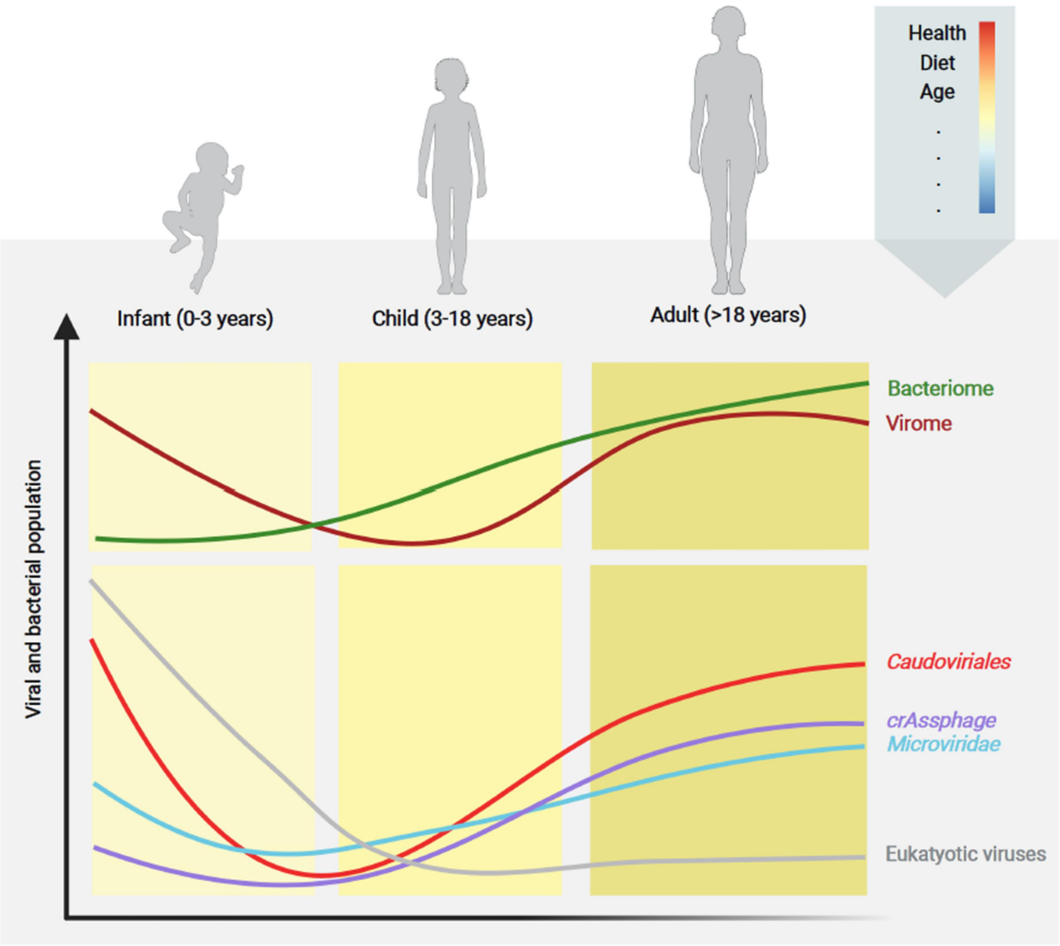

Figure 1.

Changes in bacterial and viral populations inside the human gut across the lifespan of healthy, individuals. Impact of different factors is illustrated: Health status, diet and age are the most influential factors. Figure is adapted from (Gregory *et al.* 2020).

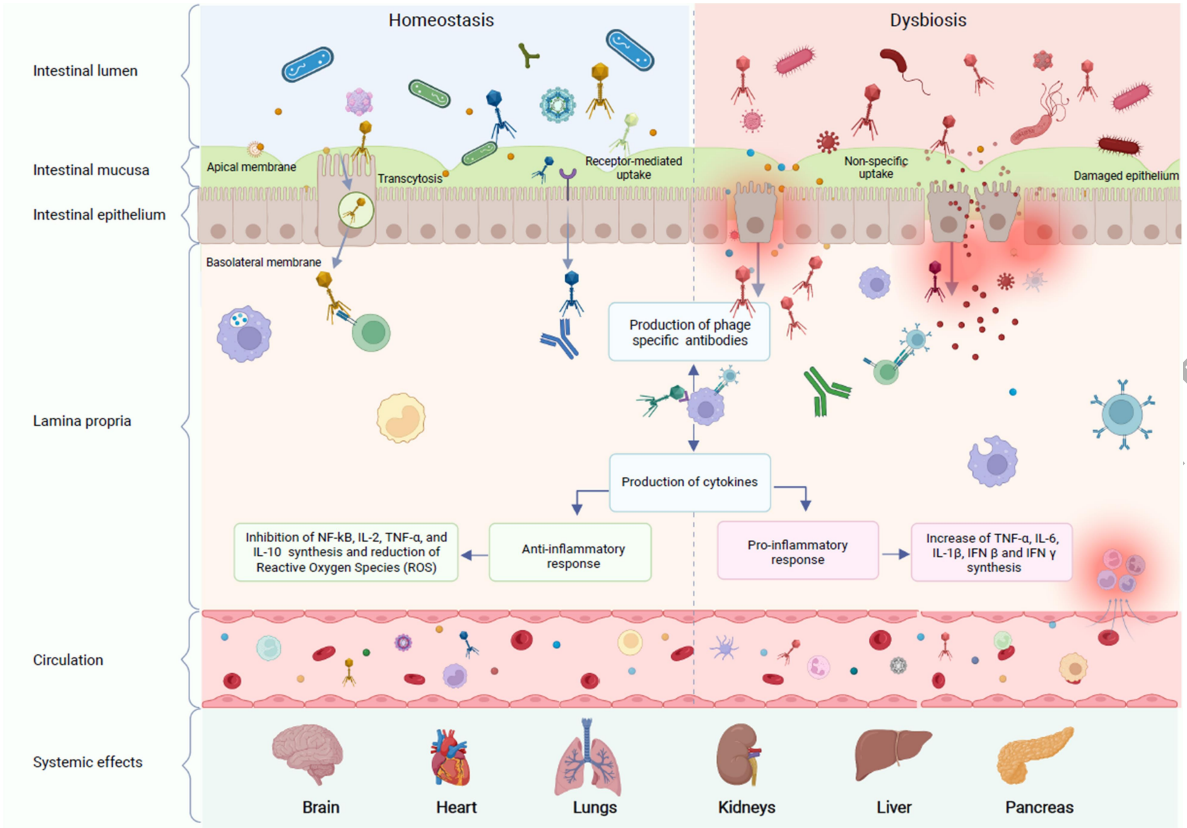

Figure 2.

Direct interactions between phages and the immune system in homeostasis compared to dysbiosis. Phages cross the intestinal epithelium via specific transcytosis and receptor-mediated uptake in homeostatic conditions; or non-specific and non-controlled uptake in the case of a damaged epithelium. Once they have crossed the intestinal epithelium, phages interact with circulating immune cells and can elicit different responses and modulate inflammation. Phages can then drain into lymph nodes and disseminate to different organs throughout the body.

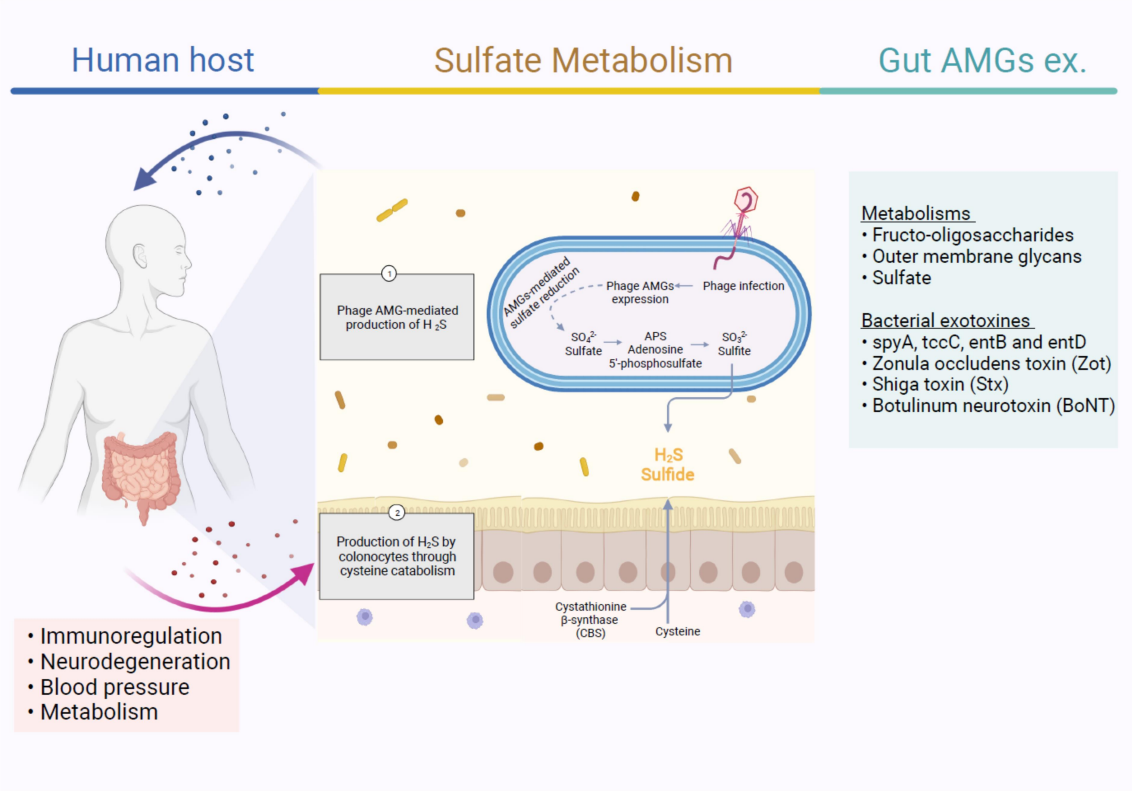

Figure 3.

Overview of Hydrogen Sulfate (H_2S) production pathways: Cysteine catabolism inside human colonocytes and potential phage AMG-mediated sulfate reduction within sulfate-reducing bacteria (SRB) and its effect on human health. H_2S regulates DNA replication, metabolism, oxidative stress and inflammation in various organs of the body (e.g. brain, liver, heart, kidneys…). Other examples of AMGs in humans include genes that are involved in metabolisms of Fructo-oligosaccharides, Outer membrane glycans, etc., and bacterial exotoxins such as Zonula occludens toxin (Zot), Shiga toxin (Stx), and Botulinum neurotoxin (BoNT).

Table 1. Virus communities in the human gut

Virus type	Genome	Infant	Adult
Bacteriophages	Double-stranded DNA	*Myoviridae, Podoviridae, Siphoviridae, Corticoviridae, Tectiviridae, Amandaviridae, Sisseviridae, Picoviridae, Skunaviridae, β-crassviridae, Jeppeviridae, Alberteviridae, Hannahviridae, Flandersviridae, Evaviridae*	*Myoviridae, Podoviridae, Siphoviridae, crAssphages*
	Single-stranded DNA	*Inoviridae, Microviridae, Gokushoviridae, Alpaviridae, Inesviridae, Almaviridae, Noraviridae, Circoviridae*	*Inoviridae, Microviridae*
Eukaryotic viruses	Double-stranded DNA	*Adenoviridae**, *Polyomaviridae**	*Adenoviridae*, Herpesviridae*, Iridoviridae*, Marseilleviridae, Mimiviridae*, Papillomaviridae*, Polyomaviridae*, Poxviridae**
	Single-stranded DNA	*Anelloviridae*, Circoviridae, Geminiviridae, Nanoviridae, Parvoviridae*, Genomoviridae**	*Anelloviridae*, Circoviridae, Parvoviridae**
	Double-stranded RNA	*Picobirnaviridae *, Chrysoviridae, Reoviridae**	*Picobirnaviridae*, Reoviridae**
	Single-stranded RNA	*Caliciviridae*, Astroviridae*, Virgaviridae, Picornaviridae*, Alphaflexiviridae, Tombusviridae*	*Caliciviridae*, Astroviridae*, Virgaviridae, Picornaviridae*, Retroviridae*, Togaviridae*, Alphaflexiviridae, Bromoviridae, Luteoviridae*
Archaeal viruses		*Lipothrixviridae*	

* Eukaryotic viruses reported to be associated with human diseases

Table 2. Gut viral and bacterial population alterations in human diseases

Disease	Major gut virome alteration	Major gut bacterial microbiome alteration	Ref.
Diseases in the gut			
IBD (Crohn's disease and ulcerative colitis)	Increased *Caudovirales* abundance; Increased *Caudovirales* richness; Increased ratio of *Caudovirales* to *Microviridae;* Increased *Hepadnaviridae* and *Hepeviridae*, reduced *Polydnaviridae*, *Tymoviridae* and *Virgaviridae*	Reduced bacterial diversity and richness; Reduced *Bacteroides, Ruminococcus* and *Blautia*, increased *Haemophilus* and *Streptococcus*	(Ungaro *et al.* 2019) (Norman *et al.* 2015) (Liang *et al.* 2020) (Fernandes *et al.* 2019)
Ulcerative colitis	Increased *Caudovirales*, phage and bacteria virulence functions, and loss of viral–bacterial correlations		(Zuo *et al.* 2019)
Crohn's disease	Moderate alterations; The virulent phages are replaced with temperate phages	Reduced bacterial alpha diversity	(Clooney *et al.* 2019) (Pérez-Brocal *et al.* 2015)
Colorectal cancer	Increased viral diversity	Reduced bacterial diversity	(Nakatsu *et al.* 2018)
Coeliac disease autoimmunity	Increased *enteroviruses* Viral dysbiosis: increased *Human polyomavirus 2*, *Enterobacteria* phage mEpX1, and *Enterobacteria* phage mEpX2, reduced *Lactococcus* phages ul36 and *Streptococcus* phage Abc2		(Lindfors *et al.* 2020) (Mouzan *et al.* 2022)
Cystic fibrosis	Different beta-diversity，reduced *Myoviridae*, *Faecalibacterium* phage FP Taranis and unclassified *Gokushovirinae*	Bacterial dysbiosis	(Coffey *et al.* 2020)
Enteric graft-versus-host disease	Increased *picobirnaviruses*	Increased *Lactobacillales* spp,decreased *Clostridiales* spp.	(Legoff *et al.* 2017)
Oesophageal adenocarcinoma	Abundant and rare phage communities in the gut may contribute to the progress of oesophageal carcinogesis		(Ma *et al.* 2021)
Disease in distant organs			
Type 1 diabetes	Reduced viral diversity； Increased *E. coli* phage/*E. coli* ratio	Bacterial dysbiosis； Depletion of amyloid-producing *E. coli*	(Zhao *et al.* 2017) (Tetz *et al.* 2019)
Type 1 diabetes during pregnancy	Increased *picobirnaviruses* and *tobamoviruses*		(Seth *et al.* 2019) (Wook Kim *et al.* 2019)
Type 2 diabetes	Increased putative phage scaffolds, complex core interaction between bacteria and phages		(Ma *et al.* 2018)
Obesity and type 2 diabetes	Reduced viral richness and diversity, weakened viral-bacterial correlations	Significant change in bacterial communities	(Tetz *et al.* 2018) (Yang *et al.* 2021)
AIDS	Increased enteric *adenoviruses*	Shift from *Bacteroides* to *Prevotella* dominance, increased *Enterobacteriaceae* spp	(Monaco *et al.* 2016)

Gulf War Illness	Increased viral richness and alpha diversity; DNA bacteriophage dysbiosis	Bacterial dysbiosis	(Seth *et al.* 2019) (Seth *et al.* 2020)
Parkinson's disease	Reduced virus abundance; Increased *Lactococcus* phage, (increased phage/bacteria ratio in lactic acid bacteria)	Increased *Verrucomicrobiaceae* (*Akkermansia muciniphila*) and unclassified *Firmicutes*, reduced *Prevotellaceae* (*Prevotella copri*) and *Erysipelotrichaceae* (*Eubacterium biforme*); Bacterial dysbiosis, depletion of *Lactococcus* spp.	(Bedarf *et al.* 2017) (Tetz *et al.* 2018)
Coronary heart disease	*Virgaviridae* and *Microviridae* dominate in the enteric virome		(Guo *et al.* 2017)
Hypertension	*Erwinia* phage ΦEaH2 and *Lactococcus* phage 1706 may be associated with hypertension, increased pervasive virus-bacteria linkages		(Han *et al.* 2018)
Atherosclerotic cardiovascular disease	Increased *Enterobacteriaceae*- and *Streptococcus* phages	Increased *Enterobacteriaceae* and *Streptococcus* spp.	(Jie *et al.* 2017)
Pulmonary Arterial Hypertension	Increased *Enterococcal* phage, reduced *Lactococcal* phage	Alteration of *Enterococcus* associacted with *Enterococcal* phage, increased *Lactococcus*	(Kim *et al.* 2020)
Pneumonia	Reduced *Lactococcus phage*, increased *Staphylococcus*- and *Enterococcus* phages	Loss of obligately anaerobic bacteria, increased *Staphylococcus* and *Enterococcus* spp	(Kullberg *et al.* 2021)
Pancreatic cancer	Cancer stage was positively correlated with relative abundances of *Cyprinivirus*	Bacterial dysbiosis, increased *Klebsiella pneumoniae* and *Alistipes purtredinis*	(Matsukawa *et al.* 2021)
Alcohol-associated liver disease	Increased viral diversity, increased *Escherichia-, Enterobacteria-,* and *Enterococcus* phages and *Parvoviridae* and *Herpesviridae*	Reduced bacteria diversity	(Jiang *et al.* 2020)
Non-alcoholic fatty liver disease	Reduced viral diversity, proportionately fewer phages, decreased *Lactococcus*- and *Leuconostoc* phages, increased *Lactobacillus* phage phiAT3	Reduced bacterial diversity, low *Lactococcus* abundance	(Lang *et al.* 2020)
Cirrhosis	Different beta-diversity, altered phage and bacterial linkages centered on *Streptococcus* spp	Worsen bacterial alpha/beta diversity	(Bajaj *et al.* 2021)
Rheumatoid arthritis	*Streptococcaceae, Bacteroidaceae*, and *Lachnospiraceae* phages	Increased *Lachnospiraceae*	(Mangalea *et al.* 2021)