Metagenomics and the Human Virome in Asymptomatic Individuals

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Abstract

High-throughput sequencing technologies have revolutionized how we think about viruses. Investigators can now go beyond pathogenic viruses and have access to the thousands of viruses that inhabit our bodies without causing clinical symptoms. By studying their interactions with each other, with other microbes, and with host genetics and immune systems, we can learn how they affect health and disease. This article reviews current knowledge of the composition and diversity of the human virome in physiologically healthy individuals. It focuses on recent results from metagenomics studies and discusses the contribution of bacteriophages and eukaryotic viruses to human health.

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INTRODUCTION

Historically, viruses have been considered obligatory intracellular pathogens responsible for human, animal, and plant diseases. Viral diseases were reported long before viruses were discovered, but the history of virology is quite recent. It began in 1886, when Adolf Mayer (76) demonstrated that what he called the tobacco mosaic disease was transmissible to healthy plants by applying a paper-filtered liquid extract from crushed diseased plants. A few years later, in 1892, Dmitri Ivanovsky (56) used porcelain filters (20) to demonstrate that the causal agent of tobacco mosaic disease was ultrafilterable and speculated that this was due to spores or toxins. The concept of a virus ("poison" in Latin) was introduced in 1898 by Martinus Beijerinck (10), who definitively refuted bacteria and toxin theories when he discovered that the *contagium vivum fluidum* could not be replicated autonomously and needed a living host cell to multiply. The same year, Friedrich Loeffler and Paul Frosch were the first to discover an animal virus, the agent of cattle foot-and-mouth disease (71). In 1901, Walter Reed, James Carroll, and Aristides Agramonte (91) were first to discover a human virus, the agent of yellow fever. Finally, in 1915, Frederick Twort discovered viruses that infected bacteria (106). In 1917 Félix d'Hérelle (24) called them bacteriophages (phages). Thus, a century ago, the first reports of viruses that infected plants, animals, humans, and bacteria were published. Since then, major technological developments have considerably increased our knowledge of viruses and associated diseases.

Electron microscopy helped to clarify the nature of the tobacco mosaic virus in 1939 and continues to be a standard technique for visualizing viral particles and elucidating their ultrastructure and localization (44, 96). Clinical diagnosis of viral infections has also benefitted from progress in cellular and molecular biology (38). Polymerase chain reaction, a method to amplify nucleic acids, is now routine procedure—the gold standard in diagnostic virology. These techniques allowed for the discovery of several new viral pathogens in the past (97, 115) and will certainly continue to do so in the future. Another revolutionary development was next-generation sequencing techniques

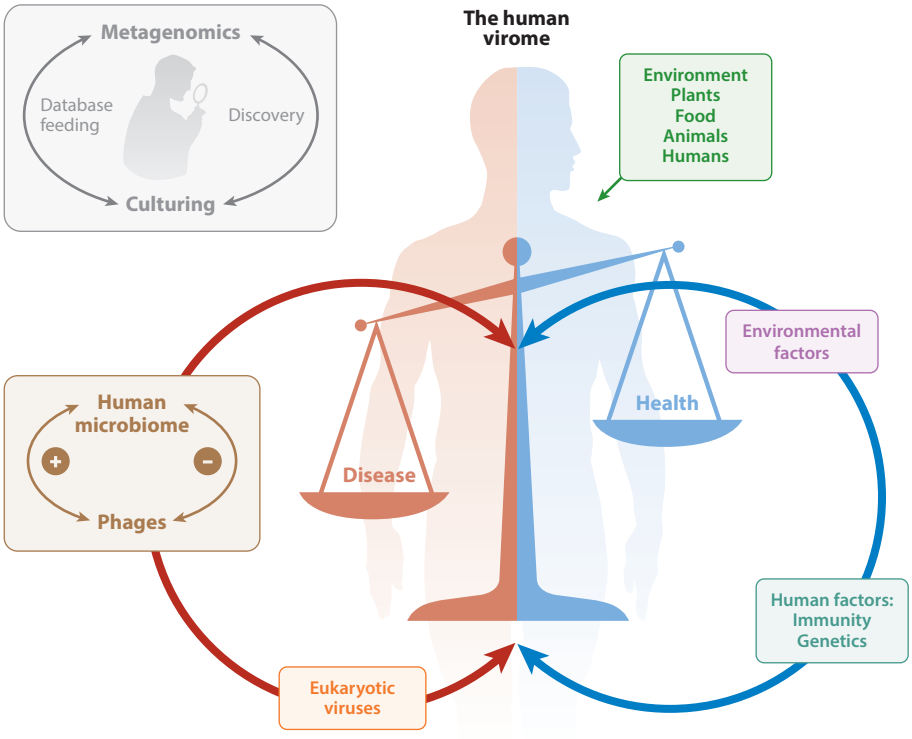

Figure 1

Highly diverse viral communities inhabit the human body, including eukaryotic viruses and a high abundance of bacteriophages. Both virus types are important to the balance between health and disease, not only because they infect human cells but also because they positively and negatively modulate the human microbiome. Viruses access the human body through food and through contact with plants, animals, and other humans. Environmental factors and internal factors (such as immunity and genetics) greatly influence the composition and dynamics of the human virome. Metagenomics is a powerful tool for analyzing the human virome and discovering novel viruses based on their similarities with known viruses. As information from cultured or characterized viral strains is added to sequence databases, the effectiveness of metagenomics increases.

for exploring the genomic composition of complex samples (metagenomes) by directly sequencing the nucleic acids they contain (50). Metagenomics overcome the main limitations of traditional virus-detection tools, which require precise genetic knowledge of previously isolated and/or characterized viruses. In 2001, the first viral metagenomics study on human stool revealed that the richness and diversity of the viruses associated with physiological conditions in humans were unexpectedly high and largely underestimated (16). We are now moving away from the traditional view of these as individual pathogens. Viruses are the most abundant biological entity on our planet. They are everywhere, colonizing all organic and inorganic, external and internal surfaces. We are constantly exposed to viruses through the air we breathe, our food, other humans, plants, and animals, and they can directly or indirectly (through other microbes) influence the equilibrium between health and disease (**Figure 1**).

In a previous work, we presented an exhaustive inventory of viruses detected by both molecular and metagenomic methods in several human body compartments under nonpathological conditions (88). Other recent works have also addressed different aspects of the human virome (18,

52, 65, 108, 117). Here we review current knowledge about the composition and diversity of the human virome in a physiological context, based on recent metagenomic studies. We emphasize bacteriophages not only because they are by far the most abundant viruses in the human body but also because they are the most common subject of metagenomic studies of healthy humans. We then summarize current knowledge and hypotheses on how bacteriophages and eukaryotic viruses can contribute to human health and disease.

THE HEALTHY HUMAN VIROME AND CURRENT LIMITATIONS

The human virome is defined as the repertoire of all viruses that are found on the surface of and inside our bodies in the absence of clinically significant symptoms of infection. This includes viruses that cause acute, latent, or persistent infections; viruses infecting eukaryotes, bacteria, and archaea; and endogenous viral elements integrated into host chromosomes.

More specifically, the human body is a composite of highly heterogeneous ecosystems with specific microbial communities at each body site (54, 122). As a consequence, viral communities differ in terms of abundance and composition within anatomical sites (3, 51, 93). The number of free virions varies from very high—10^9 particles per gram for body barrier sites (e.g., the gut, oropharynx, and skin)—to lower but still abundant—10^5 and 10^7 particles per milliliter of blood and urine, respectively (52, 98). The majority of these viral particles are from bacteriophages, and their distribution is to some extent determined by bacterial communities present in the host (89, 94). It has been estimated that the human body may contain up to 10^{15} bacteriophages (52) that regulate the structure and function of bacterial communities through their lytic and lysogenic cycles. Less often described in the literature, the virome of asymptomatic individuals also includes (*a*) circulating eukaryotic DNA and RNA viruses infecting the human host, its associated eukaryotic microbes, or other eukaryotes; and (*b*) viral elements that persist in cells: temperate bacteriophages; latent viruses in eukaryotic cells (109); and human endogenous retroviruses (HERVs), which are integrated into the human genome. It is estimated that HERVs constitute nearly 8% of our genome (13). Most are truncated and/or mutated and no longer encode functional genes, although some can still produce proteins (30, 103) or intact viral particles (14). Metagenomic studies describing HERVs in healthy humans are scarce and have been excluded from this review.

Having a complete and unbiased view of the human virome is challenging for many reasons. Results from shotgun sequencing on crude samples (without any viral purification and concentration) usually overwhelmingly comprise nucleic acids from cellular organisms (humans, bacteria, etc.). Thus, current protocols for generating a viral metagenome are based on enriching/purifying circulating viral particles by using filtration and centrifugation while depleting nonencapsidated contaminant nucleic acids through the use of nucleases (34, 104, 105), and these protocols are highly dependent on the nature of the sample. As a consequence, latent viruses (prophages and proviruses) that reside within cells long-term (in integrated or episomal forms) are largely missed, and their diversity/functionality is probably underestimated. Other missed constituents of the virome of humans in a physiological state are large double-stranded DNA (dsDNA) viruses (22, 88; reviewed in 49), which cannot be recovered after filtration, and RNA viruses—because most viral metagenomic studies conducted on RNA viruses targeted individuals with disease. Thus many eukaryotic viruses (both DNA and RNA viruses), including potential pathogens, have yet to be discovered.

Furthermore, a large proportion of the obtained sequences have no homologs in public databases (2, 69, 80, 81, 83, 92), which is a major obstacle for accurately describing a virome based on taxonomic composition. As more viruses are functionally characterized using traditional methods (cell culture, molecular biology, microscopy, etc.) and their genomes are added to

databases, a more accurate understanding of the human virome will emerge (**Figure 1**). Lastly, most metagenomic works have focused on the analysis of viruses at individual body sites (mainly the gastrointestinal tract, oral cavity, and skin), and very few have analyzed the differences in viral communities between body sites (body barriers or systemic compartments), geographic sites, disease states, or age groups, as has been done for microbial communities (e.g., the Human Microbiome Project, or HMP) (1, 84, 89). A recent analysis of HMP data from five body sites in 102 individuals revealed high interpersonal variability of the virome: An average of 5.5 eukaryotic virus genera were present in each person (116). Similar initiatives are warranted and will likely deepen our knowledge of factors influencing the composition, diversity, and dynamics of the human virome.

METAGENOMIC BIOGEOGRAPHY OF THE HUMAN VIROME

Gastrointestinal Tract

The gut is the most-studied site of the human microbiome in asymptomatic individuals. This is, to some degree, due to convenience sampling, but mostly it is because gut microbes play an essential role in human physiology and health. Viruses are highly abundant in the gut, and a growing body of evidence seems to indicate that the gut virome could also play significant physiological roles (21).

In most metagenomic studies, sequences classified as phages represent a much larger proportion than those classified as eukaryotic viruses. Phage communities are primarily composed of members of the *Myoviridae*, *Podoviridae*, *Siphoviridae* (order *Caudovirales*), and *Microviridae* families, with a very low representation of archaeal viruses (mainly from *Lipothrixviridae*) (69, 92). In fact, almost no studies have been able to detect archaeal viruses, probably because of their low abundance compared to bacterial phages and because less is known about this group of viruses. Members of the *Inoviridae*, *Corticoviridae*, and *Tectiviridae* families have also been identified, but a large proportion of phages (>50%) are still unclassified or unknown (15, 69, 80, 83, 92). The presence of these phage families varies between studies, probably owing to differences among biological samples and differences in sample preparation, sequencing technology and depth, and bioinformatics analyses. Despite these issues, several independent works have shown consistent common properties among gut phage communities.

It was shown that gut phages are maintained for long periods, and phage communities are very different between individuals (69, 81, 92, 93, 110). The same studies also found that environmental exposure, habits, and even genetics could play a role in shaping these communities. However, the mechanisms and processes contributing to interpersonal and intrapersonal phage dynamics are still not clear. Interestingly, despite the personalization of phage communities and the high variability and mutation rates of phages (80), it seems that some strains, such as the recently discovered crAssphage (cross-assembled phage), are widely distributed and conserved in the human population, across unrelated individuals (35).

The gastrointestinal tract is a highly heterogeneous environment that harbors different microbial (53, 66, 121) and viral communities both across its length and between the mucosa and lumen. Most analyses of gut viromes used samples from feces, where the reported ratio of bacteria to phages was between 10:1 and 1:1 (62, 81, 93). However, a much higher and more stable proportion was observed at the beginning of the gastrointestinal tract in humans and pigs, suggesting that the enrichment at this site could play a conserved role in human physiology (72, 123). Moreover, it has been shown that phages accumulate at the mucosa at a concentration 10 times higher than bacteria (7). The putative role of this accumulation is discussed below.

Several studies have characterized phage colonization and community development in the gut of infants (15, 69, 92, 100). One of these found that phage richness and diversity were greatest during the first days of life and decreased with age, accompanied by a shift from *Caudovirales* to *Microviridae* (69). This shift was confirmed by another independent work (92). Moreover, phage diversity was inversely correlated with bacterial diversity, suggesting predator-prey interactions (23) between phage and bacterial communities in early development of the infant gut, which could influence the development of the gut microbiome. In line with this hypothesis, it has been shown that phages might play a role during the microbiota succession necessary to recover from diarrhea by helping to stabilize the normal microbial flora (25).

A very low abundance of eukaryotic viruses is typical of gut virome metagenomic samples from healthy individuals (15, 16, 80, 81, 93, 116). Anelloviruses are small single-stranded DNA (ssDNA) viruses from the *Anelloviridae* family and are the most common eukaryotic DNA viruses found in the gut, particularly in infant gut samples (58, 69, 92). There are three main viral groups among the *Anelloviridae* [Torque Teno Virus (TTV), Torque Teno Mini Virus, and Torque Teno Midi Virus] that are commonly found in the gut and several other compartments of the human body (29, 42, 48, 55). The prevalence of anelloviruses in humans is very high. Their role in human physiology and disease is still unclear, but anelloviruses are usually considered nonpathogenic commensals. In contrast to phages, eukaryotic viruses in infant twins showed low richness at birth and increased in richness thereafter, probably because of environmental exposure (69). Furthermore, two independent studies found that sequences related to anelloviruses peaked at 15–18 (92) or 6–12 (69) months of age, after which their abundance progressively decreased. It has been proposed that the peak correlated with the interval between maternal IgG protection and complete development of the infant's immune system (69). High persistence of anelloviruses during the first years of life has also been reported by molecular studies (58).

At least 15 other families of eukaryotic DNA viruses have been detected (58, 69, 80, 92, 93, 116). Of these, only 9 were identified by more than one metagenomic study: *Geminiviridae* (69, 92), *Herpesviridae* (92, 93), *Nanoviridae* (69, 92), *Papillomaviridae* (92, 116), *Poxviridae* (92, 93, 116), *Parvoviridae* (58, 69, 92), *Polyomaviridae* (69, 92, 116), *Adenoviridae* (58, 69, 92, 116), and *Circoviridae* (58, 69, 80, 92, 116). *Circoviridae*, in particular the *Circovirus* and *Gyrovirus* genera, seem to be ubiquitous and are consistently found in gut samples.

Metagenomic sequencing analyses have largely ignored RNA viruses. The RNA viral community is dominated by plant viruses that may originate in food (119). Various plant- and insect-related viral sequences were also detected in colon biopsy specimens from healthy subjects (112). Sequences from at least 10 families of eukaryotic RNA viruses have been found in gut samples (58, 69, 93, 119), although, surprisingly, some of these were from DNA metagenomes (93). Of these families, *Caliciviridae* (58, 69), *Picobirnaviridae* (69, 119), *Picornaviridae* [*Enterovirus* (58, 69)], and *Reoviridae* [*Rotavirus* (58, 93)] were detected by both metagenomics and molecular-based studies.

Finally, while some of the eukaryotic viruses (e.g., anelloviruses) seemed to be highly divergent, with high inter- and intraindividual variability (up to 47 anellovirus species), others (e.g., parechovirus and enteroviruses) were highly conserved among closely related individuals (69).

Oral Cavity and Respiratory Tract Viromes

Similar to the gut virome, the oral virome seems to be highly personalized and stable over time (1, 3, 74, 89). Metagenomic analyses have found a much higher proportion of phage sequences than eukaryotic virus sequences, and a high percentage of metagenomic sequences show no similarity to sequences in public databases. Interestingly, some studies have reported that, contrary to bacteria, phage communities are significantly more diverse and rich in the oral cavity than in the gut

(feces) (1, 26). Several studies have consistently classified phages in the *Siphoviridae*, *Myoviridae*, and *Podoviridae* families (1, 3, 74, 89). An interesting observation is that oral viromes were more similar between people with similar diet or oral bacterial communities and between people from the same household or family (95). Additionally, and similar to the gastrointestinal tract, the oral cavity was found to be a heterogeneous body compartment, with significantly different phage communities in the saliva, dental plaque, and subgingival and supragingival biofilms (74, 111). In fact, viral communities from the same oral site in different individuals seemed to be more similar than those from different oral sites in the same individual, suggesting that site-specific phage communities may modulate microbial dynamics in the oral cavity.

The first studies of the oral virome showed no direct relationship between the abundance of free viral phage particles and the abundance of their corresponding bacterial host (89). This was partially attributed to a large prevalence of lysogenic life cycles in most oral phages (89). Some studies were able, to some extent, to reconstruct bacteria-phage interactions and showed that phages probably have several hosts, forming interaction networks rather than predator-prey relationships (73, 111). Although some studies have observed shifts in oral viromes in individuals with oral diseases (74, 89), it is still unclear to what extent bacteriophage communities shape or modulate oral bacterial communities and whether this balance has a direct or indirect effect on human health.

Finally, the human lung and respiratory tract, which are now known to harbor a distinct microbiome (31), are populated by specific phage communities that can be altered by diseases such as cystic fibrosis (113, 118). Since the literature contains almost no studies to understand the distribution and putative effects of phages at these body sites, further research will be needed to determine if they are natural residents of these body sites and how they influence human health.

The relative abundances of eukaryotic viruses in the oral cavity and respiratory tract viromes were highly variable between studies. These results could reflect either natural variations or differences in sample preparation and bioinformatic analyses (74, 111). For instance, while one metagenomic analysis of oropharyngeal swabs could identify only Epstein-Barr virus (EBV) (114), another one on saliva was able to detect only TTV (89). A recent publication reported the presence of papillomaviruses in saliva and showed that their abundance increased after antibiotic treatment, suggesting an association between antibiotics and papillomavirus production (1). Another study reported that a gammapapillomavirus was steadily retrieved from nasal swabs during and after an acute episode of respiratory disease and is probably part of the commensal microflora in the nose (19). A virome analysis on the nasal aspirates of healthy individuals revealed the presence of several different viruses related to *Cytomegalovirus*; *Lymphocryptovirus*; *Roseolovirus*; herpes simplexvirus; alpha-, beta-, and gammapapillomaviruses and unclassified papillomaviruses; polyomavirus; *Mastadenovirus*; *Dependovirus*; *Alphatorquevirus*; and unclassified anelloviruses (116). Similarly, the respiratory tracts of healthy patients were heavily populated by anelloviruses and to a lesser extent by herpesviruses and papillomaviruses (118). The anellovirus load was higher in lung transplant recipients than in healthy controls and correlated with dysbiotic bacterial communities (118). Overall, these observations suggest that different eukaryotic viruses are found in different parts of the oral cavity and respiratory tract, with much higher richness and diversity in the nasal region. They also suggest that production of asymptomatic eukaryotic viruses is higher in the context of certain stresses.

Skin Virome

The skin, one of the main protections from external pathogens, is a significantly different environment compared to the body sites described above. Microbial diversity is much lower; the microbiota mostly comprise species and strains from two or three of the following bacterial

genera: *Staphylococcus*, *Propionibacterium*, and *Corynebacterium* (84). Accordingly, viral communities in the skin are significantly different compared to those at other body sites (51, 84). Contrasting results have been reported regarding the abundance of eukaryotic viruses and phages in the human skin (40, 51, 84). Similar to the gastrointestinal tract and oral cavity, the skin has very different viral communities at different sites.

In some studies phages were predominant in metagenomic viral sequences from the skin (51, 84). They also represented a significant proportion of the whole skin microbiome of the nose, nares, and adjacent alar creases (84). Phages at these sites showed similarities to *Staphylococcus* and *Propionibacterium* phages, which infect the two main bacteria genera present in human skin, suggesting that they could play a role in preventing skin bacteria from entering the respiratory tract. Another study performed on eight skin regions showed that the virome and bacterial metagenome of sebaceous sites were less diverse than those of moist and intermittently moist sites, respectively (51). It also suggested that the most significant source of skin virome variance is interpersonal variation, followed by body site variation with high variability over time at a given skin site, contrary to the temporal stability observed in the gastrointestinal tract and oral cavity.

Most of the sequences that could be classified as viruses corresponded to *Caudovirales*, a minor proportion to *Myoviridae* and *Siphoviridae*. The vast majority were from unknown families. Most of these phages were predicted to be lysogenic (51) with a capacity to infect a broad diversity of host strains and species (70). This would indicate that skin phages are not strain specific, as is normally assumed, and that they might be significant contributors to microbial gene exchange when inserted into bacterial genomes. However, up to 95% of the sequences obtained from virus-like particles did not show any similarity to known genomes, whereas 42% did not show similarity to any sequence in public databases. Therefore, although the bacterial fraction of the skin microbiome seems to be rather simple, further work is needed to determine the roles of the vast and diverse universe of unexplored viruses and viral genes (51).

Different studies have shown that eukaryotic viruses in the skin belong to several families (*Papillomaviridae*, *Polyomaviridae*, *Circoviridae*, *Adenoviridae*, *Anelloviridae*, *Herpesviridae*, and *Poxviridae*); of these, *Papillomaviridae* were predominantly detected (40, 51, 75, 84, 116). *Papillomaviridae* are small, nonenveloped dsDNA viruses infecting a wide range of hosts. To date, approximately 200 types of human papillomavirus (HPV) have been described (27). Based on sequencing data, HPVs were predominate in HMP skin samples, with at least 12 known species and many unclassified HPVs (75). Virome analysis of cutaneous swabs collected from different anatomical locations in healthy individuals revealed that the composition and diversity of skin viromes were not equal at all sites and in all individuals (51). Indeed, HPV sequences were more abundant at the palm, forehead, retroauricular crease, and occiput sites than at other skin sites, suggesting that they might be differentially distributed on the human skin (51). Ultimately, these studies showed that HPVs are common inhabitants of the human skin and that they have a worldwide distribution, thus confirming previous molecular biology–based work (4).

Polyomaviruses and circoviruses have also been frequently found in skin samples (40, 51, 84, 99). Polyomaviruses belong to *Polyomaviridae*, a family of ubiquitous, small dsDNA viruses found in a wide variety of hosts, including birds and mammals. The group of hosts where they have been detected dramatically expanded in recent years owing to the development of high-throughput sequencing technologies. This family includes 13 human polyomaviruses (HPyVs), only 2 of which were known before 2007. Four are recognized human pathogens: Merkel cell carcinoma polyomavirus, human JC polyomavirus (causing progressive multifocal leukoencephalopathy), trichodysplasia spinulosa–associated polyomavirus, and polyomavirus BK (causing nephropathy) (37, 43, 85, 107). We also recently observed human polyomavirus 6 (HPyV6) infecting lymph nodes of a patient presenting a chronic inflammatory disorder known as Kimura disease (90). The

results suggested that HPyV6 could either be playing a role in the development of the disease or be an opportunistic virus that replicates when the immune system is depressed. Polyomavirus species are highly conserved among individuals and showed very low genetic diversity among viruses collected from individuals of different geographic regions (40, 90, 99). Similar to HPVs, these viruses were not evenly distributed across skin regions and were not detected in all individuals (51, 84). Sequence hits for other viruses belonging to the *Poxviridae*, *Mimiviridae*, and *Phycodnaviridae* families were also detected in all skin regions (51). However, in all cases the relative abundances were highly variable between locations and individuals (51).

Blood Virome

Healthy human blood has often been considered sterile, but metagenomic studies have demonstrated that it harbors substantial viral communities. A pioneering viral metagenomic study on blood found sequences related to many eukaryotic viruses, including new TTVs, with only weak similarities to sequences in the databases (17). The great genetic diversity of anelloviruses in the blood was confirmed by several studies on blood DNA viromes (41). Anelloviruses can be transmitted through transfusions (11) and have been proposed as biomarkers of immunocompetence because increases in the anellovirus load in the blood have been associated with immunosuppression levels in transplant recipients and patients with HIV (28, 67, 118). Another recent metagenomics study on RNA viruses in the blood of 328 healthy individuals led to the discovery of two novel rhabdoviruses (102). Serological surveys showed very high exposure rates to these viruses (10–50%), and the authors suggested that human infection with rhabdoviruses might be common (102). Other viral sequences found in the blood of asymptomatic individuals are related to members of the *Herpesviridae*, *Picornaviridae*, *Poxviridae*, *Flaviviridae*, *Marseilleviridae*, *Mimiviridae*, and *Phycodnaviridae* families (41, 64, 87, 102).

Phage DNA sequences were found in blood in studies analyzing virus-like particles and circulating DNA: *Myoviridae*, *Siphoviridae*, and *Microviridae* in the former (17, 68) and *Myoviridae*, *Siphoviridae*, *Podoviridae*, and *Inoviridae* in high abundance in the latter (32). Nevertheless, the corresponding bacterial host DNA was also found in the circulating blood, which could indicate that only prophages were detected. Dinakaran et al. (32) and Li et al. (68) reported a higher abundance of phage DNA in blood of patients with cardiovascular disease and HIV, respectively, compared to healthy individuals. Since the type of phages found in blood corresponded to bacterial hosts that are normally present in the gut and skin, the possibility of free DNA spreading from these body sites cannot be ruled out. Although no direct evidence of phage particles in blood has been reported, some authors have suggested that phage translocation from the gut to the bloodstream could be frequent and have a certain degree of immunomodulatory effects (47).

Genitourinary Tract Virome

Metagenomics studies of the genitourinary tract virome are scarce, but all have found that HPVs were much more prevalent in urine and vaginal swabs than any other eukaryotic virus (75, 98, 116), again indicating that these are probably part of the commensal microflora. Half of the HPVs detected in the vagina were organ specific, whereas the other types were also present in the skin, oral cavity, and gastrointestinal tract (75). Herpesviruses and polyomaviruses were detected in the urinary tracts of healthy males and females, without sex-specific differences in sequence abundances (98).

Phages were also found in the urinary tract, representing 27% of the metagenomic contigs obtained from urine, although at an abundance one order of magnitude lower than in saliva (98).

Detection of phages in human urine and urinary tracts has largely been ignored in the literature (47), although some authors have proposed that their presence in the urine could defend against invading bacteria (77). Phages were found in the vagina (59, 86), including several strains of *Lactobacillus* that exhibited lysogenic and lytic phenotypes, depending on the infected *Lactobacillus* strain (59). Nevertheless, the resident phage communities on human genitals remain largely unexplored.

THE ROLE OF PHAGES IN THE EQUILIBRIUM OF THE HUMAN MICROBIOME AND HUMAN HEALTH

Genomic Reservoirs of Bacterial Metabolic Functions

Phage communities found in the human body are likely dominated by lysogenic phages (16, 51, 74, 81, 89, 93, 98, 101). Prophages can activate under certain types of stress in vitro and inside the human body (79, 86, 120). Perhaps the best-studied example of this phenomenon occurs during response to antibiotics. Phages are important reservoirs for antibiotic resistance genes and facilitate their exchange between bacterial species (1, 36, 51, 81, 82, 93). After antibiotic treatment, the abundance and diversity of antibiotic resistance genes increase in the gut phage communities of mice (82). The expansion of the phage resistome after long-term antibiotic treatment was also observed in the human gut virome, but not in the oral cavity (1).

Interestingly, after antibiotic treatment, there was an increase in diverse genes that could indirectly increase bacterial fitness (82). However, since most phage genes lack a known function (2, 3, 51, 69, 80, 81, 83, 92, 93), it is often hard to interpret what fitness or physiological roles are associated with shifts in the vast majority of phage genes. Phage genomes are full of genes of bacterial origin, which are also much more divergent than the bacterial encoded counterparts and normally have a very high mutation rate (80). Phages can acquire enough mutations to generate new species after short periods (80). Since phages seem to be a major source of horizontal gene transfer and gene exchange among bacteria and, simultaneously, represent a vast universe of genomic information, we might speculate that they also confer metabolic plasticity to bacterial communities. There is also evidence that new links are established between bacteria and phages after antibiotic treatment (and other stresses), promoting a wider connectivity network, which is also accompanied by greater phage integration (82). In fact, multiple studies have shown that bacteria and phages form wide and promiscuous networks rather than highly specific strain-strain interactions, as is generally believed (70, 74, 82, 89, 111). All together, these findings suggest that the genomic and metabolic functions of bacterial communities in the human microbiome could be modulated and readapted in response to stresses by obtaining genes from the extensive reservoirs contained in lysogenic phages.

The Revised BAM Model and Nonhost Innate Immunity

Phages accumulate at high densities at the mucosae of different tissues and even in extremely different animal species, from corals to fish to humans (7). Inspired by this finding, a new model of the nonhost innate immune system was proposed, called bacteriophage adherence to mucus (BAM) (7). According to this model, lytic phages accumulating at the mucosae provide a defense against bacteria approaching the cell walls. Although it was initially proposed that phages would accumulate by binding immunoglobulin-like proteins (IGLP) to the multilayered mucus, which is primarily composed of gel-forming mucin glycoproteins (7), it now seems that this interaction does not entirely explain the higher phage concentration at the mucosae sites (8). A recent revised version of the BAM model proposes that the IGLP-mucins interaction does not primarily

determine the accumulation of phages at the mucosae but rather increases the efficiency of phage infectivity. Phage-bacteria encounters would be favored by subdiffusion dynamics of phages at the mucosae, produced by transient interactions between glycoproteins binding proteins and the mucins (8).

Under this model, lytic phages might prevent entry of bacteria into the organism by killing those approaching human cells at mucosal sites, whereas lysogenic phages might contribute to shaping and controlling microbiome functioning. Phages are extensive reservoirs for genomic information (80, 82) that could be used to increase bacterial fitness when conditions change (82, 86). They are also stable over time within individuals (1, 3, 69, 74, 81, 89, 92, 93); they form infectivity networks, which are expanded under adverse conditions; and, since they are not living entities, they can probably resist wipeout events when microbiome diversity is severely affected by resting trapped at the mucosae. Taking all these observations into account, it is certainly possible that phage communities serve as microbiome buffers as well as genomic storage for human microbiome metabolic capacities, which could be restored after major diversity-loss events.

Protection from Incoming Bacteria

Phages accumulate at significantly higher concentrations at all routes of bacterial entry. This has mostly been observed at the oral and nasopharynx cavities (with surprising preponderance at the nose) and the ileum. The presence of phages in the vagina and urinary system may be evidence of a similar role at these body sites (59, 77, 86, 98). Together, these studies suggest that phages might act in symbiotic association with humans as protective barriers against incoming bacteria.

Human Immune System and Phages

Phages interact with human immune system cells both directly, by inducing a humoral response, and indirectly, through nonspecific immunomodulatory effects on innate and adaptive immune responses (33, 45, 46). They can interact directly with human antibodies (which may inhibit phage activity) (33, 39, 46), be engulfed by dendritic cells in vitro (6), and inhibit activation and proliferation of human T cells in vitro (45), and they may promote tonic stimulation of the antiviral immune response (39). Phage enzymes may change bacterial recognition by the immune system by modifying the outer membrane lipopolysaccharide (26). However, despite these diverse phenomena, the role of phages as modulators of the human immune system remains largely unexplored.

HOST–EUKARYOTIC VIRUS INTERACTIONS: FROM COMMENSALISM TO MUTUALISM

Metagenomics has detected viral sequences from small circular DNA viruses belonging to four families (*Papillomaviridae*, *Polyomaviridae*, *Anelloviridae*, and *Circoviridae*) in the gastrointestinal tract, oral cavity/respiratory tract, skin, and blood of asymptomatic individuals; however, their real abundance cannot be determined because most viral metagenomics relies on multiple-displacement amplification, which preferentially amplifies circular DNA (60, 61).

The significance of the large prevalence of these viruses in humans is unknown, but their temporal persistence among individuals is more consistent with (subclinical) persistent infection than with transitory exposure. These viruses have either dsDNA (*Papillomaviridae*, *Polyomaviridae*) or ssDNA (*Anelloviridae*, *Circoviridae*) genomes ranging from 2 kb to 8 kb (63). Within each viral family, multiple types or strains have been genetically characterized, and some are recognized

human pathogens. Another frequently detected and large family is *Herpesviridae*. These viruses have linear dsDNA (125 to 240 kb) and infect a wide range of hosts. Eight of them are well-characterized human pathogens (5): herpes simplexvirus types one and two (HSV1 and HSV2), varicella-zoster virus, EBV, cytomegalovirus (CMV), and human herpesvirus types six to eight (HHV-1 to HHV-8). All humans are infected with multiple herpesviruses during childhood. Severe infections are observed mainly in very young infants, fetuses, and immunocompromised individuals. After clearance of the acute infection, herpesviruses can establish latent infection within tissues specific to each virus (5).

The physiological role of most of these viruses in humans has yet to be defined. One hypothesis, for instance, might propose that some of them promote human health indirectly, by eliminating viral pathogens through cross-immunity, and/or directly, by viral interference. Indeed, it has been shown that latent infection with murine gammaherpesvirus 68 (a model for EBV) or murine CMV (a model for human CMV) confers protection against *Listeria monocytogenes* and *Yersinia pestis* (9) in mice. It has also been shown that in individuals with HIV, coinfection with the hepatitis G virus (GB virus type C) delays progression to AIDS (12). In contrast, by maintaining chronic inflammation, some viruses may increase the risk of secondary infection with a similar but more virulent genotype or other microbial/viral agents and/or promote viral carcinogenesis in individuals with a specific immunologic disorder (78). Indeed, several members of the *Papillomaviridae* (e.g., HPV5, HPV8, HPV16, HPV18), *Herpesviridae* (e.g., EBV, HHV8), and *Polyomaviridae* (e.g., MCPyV) families have been linked to human cancers (78).

It is thus clear that viral interactions with humans go beyond simple parasitism and that several eukaryotic viruses have established persistent interactions with their host. The adverse or beneficial effects of these interactions probably depend on the anatomical site of infection, the host genotype/immune status, and the presence of other viruses and microbes.

CONCLUSION

Although next-generation sequencing and metagenomics have drastically accelerated the discovery and characterization of human-associated viruses, the diversity and physiological roles of eukaryotic viruses and phages remain largely unexplored. Nevertheless, a fast-growing body of evidence indicates that these viruses constitute persistent communities in the human body and establish complex symbiotic relationships with their hosts. Some of these interactions likely play site-specific or general physiological roles directly affecting human health. The great advances we have made in understanding human-associated viruses in the last few years will undoubtedly inspire new techniques and approaches to access the human virome and understand its contribution to health and disease.

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