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Human Virome

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The human virome is composed by the set of all viruses, eukaryotic and prokaryotic, present in the human body; as each body compartment constitutes a different microenvironment, the virome varies with the body part. Additionally, other factors influence the virome composition, such as age, diet, and the presence of other components of the microbiome. The study of the virome takes advantage of the development of next generation sequencing, and has allowed the discovery of novel viruses, and the characterization of the virome in healthy and diseased individuals, allowing the association of viruses with specific diseases. Perhaps the most interesting development of the study of the virome is the interplay that viruses can have with other components of the microbiome, specifically bacteria, that can either up- or down-regulate the antiviral immune response and can therefore modulate viral infectivity. This relationship is reciprocal since viruses can in turn modulate bacterial infections. The complex interactions of the virome with other members of the microbiome in the context of host genetics, and their influence in the health status of the patient have just begun to be investigated and are not completely understood, but the findings so far indicate that the regulation of the immune response by viruses and other members of the microbiome can affect the outcome of infections.

Key Words: Human virome, Phagome, Virome in health, Virome in disease, Virus-microbiome interaction, Clinical virome.

Definition of Virome and its Components

The virome can be described as the set of all the viruses that are found in a particular environment. In the context of the human body, several viromes have to be considered, as different compartments of the body harbor distinct viral communities. Also, the virome forms part of the microbiome, and when evaluating the effect of the microbiome on human health, all components need to be taken into consideration as a unity, as well as in the context of the relationships established with the host (1,2).

To better understand the virome researchers focus mainly on two groups of viruses, prokaryotic viruses or bacteriophages, and eukaryotic viruses. Bacteriophages have been studied for their role as modulators of the bacteriome (3), as well as for their direct impact on inflammation, as in the case of Crohn's disease (4). Bacteriophages can use two different cycles to infect and regulate bacterial communities, lytic and lysogenic, which can have different consequences in the host and microbiome composition. In the lytic cycle, the infection of bacteria results in the destruction of the infected cell and the release of viral progeny. Meanwhile, in the lysogenic cycle, the genome of the phage integrates into the host genome and stays there as a prophage replicating along with it. On the other hand, eukaryotic viruses are relevant as pathogens, either causing acute or persistent infections. Persistent viruses are of particular importance since they can establish a long-term relationship with their host and influence the inflammatory status of the body (5). Other eukaryotic viruses are found in healthy individuals without being associated with an apparent disease (6,7) but can play a role in the health and the physiology of the host (7–11).

Also, many viral sequences are integrated into the human genome, which in general are referred as endogenous

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viral elements (EVEs) (12), and include retroviral, which represent about 8% of the human genome, and nonretroviral families. In the latter case, only bornaviruses genes have been identified in the human genome, although viruses with different replication strategies have been found to be integrated into other animal genomes, including mammals. Interestingly some of these EVEs remain as complete ORFs and seem to be functional, possibly by providing exaptations to the genome into which they integrated. For instance, the inserted gammaretrovirus HERV-T envelope sequence apparently remained functional in primates for millions of years and could have been instrumental in driving this virus to extinction as the integrated sequence helped to block the interaction of the virus with its receptor (13).

Although less studied, the importance of other components of the virome to human health cannot be underestimated. These include viruses that infect protozoans, fungi or archaea. One recent study described a virus that infects *Trichomonas vaginalis* and modulates its pathogenicity (14), highlighting the relevance of these other components. Also, some viruses that infect fungi have been found in immunosuppressed patients (15). It remains to be determined the role of viruses in the control of these other microbes, and on the activation of the host immune response; so far this is a largely unexplored area of the human virome.

The development of next-generation sequencing (NGS) technologies has allowed the determination of all the viral sequences present in a given sample, in an expanding field called viral metagenomics. This area is quickly growing despite some technical difficulties that had been addressed with various degrees of success. For example, given the small amount of viral genetic material present clinical samples, it is often necessary to enrich the sample for viral particles to increase the number of viral reads obtained during sequencing. Also, given that virus genomes can be DNA or RNA, it is necessary to run both RNAseq and DNAseq protocols to get the full virome. Furthermore, identification and assembly of the viral sequences into the different viruses that conform the virome is not straightforward, largely because there is a bias in the sequence databases towards human pathogens. Therefore, many viruses remain unknown and show no sequence similarity with those reported in the databases. Despite these obstacles, viral metagenomics has become a useful tool to characterize the human virome, to investigate the presence of viruses in pathologies of unknown origin, to discover novel viruses, and to understand how as a whole the virome contributes to the state of health and disease of an individual.

Human Virome Diversity in Health and Disease

There are several aspects relevant to the diversity of the human virome. As mentioned, each body compartment harbors a distinct viral population, and therefore they have to be addressed separately. Furthermore, during an individual lifespan, the virome alters its composition and abundance in response to changes in the environment, such as exposure to infections or dietary modifications, and personal traits, such as age and immune status. The virome characterization from people around the world has served to highlight the importance of the environment in its composition, but also to establish that healthy people harbor different viruses without showing signs of disease. Moreover, individuals that recover from some acute infections can still carry the pathogenic viruses for varying periods of time (16,17).

In the human body, the presence of viral particles varies by anatomical site, from 10^9 particles per gram in the intestinal content, to 10^8 per milliliter of oral, nasal, pharynx, and saliva fluids, 10^7 in urine, and 10^5 in the blood, and 10^6 per cm^2 in the skin (18–20). A large part of these viruses are bacteriophages, whose distribution is somehow determined by the bacterial communities present in the host (19,21). These bacteriophages exhibit a more lysogenic lifestyle, in contrast to the active *kill-the-winner* predator-prey dynamic manifest in the marine environment (section 3-d-i for details).

To inspect inter-individual and intra-individual variation of phage communities, at the genus or species level the diversity is regularly measured using taxonomic matrices of the samples. This analysis is done at a single point or over time, or in different conditions. To this end, diversity indexes are used, such as dissimilarity distances (Bray-Curtis, Wiener), similarity distances (Euclidian, Pearson's, genusSorense, Hellinger), or phylogenetic distances (UniFrac). Also, other tools as agglomerative hierarchical clustering, principal component analysis or principal coordinate analysis are used to visualize and interpret the results.

On the other hand, the eukaryotic viruses' diversity is usually assessed by determining the viral families or species present in a sample, establishing the number of reads as a proxy of their relative abundance. Since usually, one or two viruses dominate the population, beta diversity is not usually determined.

It is interesting to note that a few families of DNA eukaryotic viruses have been found throughout the human body in conditions of health and disease (Table 1). Interestingly, many of these viruses can establish persistent infections as is the case of the *Herpesviridae* family, or have not been associated with a particular disease, as is the case of anelloviruses. This observation has not been made in RNA viruses, which tend to cause acute infections; however, many metagenomic studies have been focused solely on DNA viruses, and it is possible that as data accumulate, some RNA virus families could also be found widely distributed in the human body. For a summary of the

Table 1. Viruses widely distributed in the human body, described in asymptomatic and symptomatic individuals

Genus	Viral species	Body niche in asymptomatic subjects	Body niche in symptomatic subjects [disease]
Family: Herpesviridae			
Unspecified	HHV	Nasopharynx (22)	Blood [fever] (23) Nasopharynx [LRTI[a] and URTI[b]] (24)
Cytomegalovirus	Human cytomegalovirus	Nose, oral cavity, skin, vagina (25)	Nasopharynx [fever] (26) Oral cavity [periodontitis] (27)
Lymphocryptovirus	Epstein-Barr virus	Nose, oral cavity, vagina (25) Blood (28)	Oral cavity [periodontitis] (27) Respiratory tract [CF[c]] (29)
Rhadinovirus	HHV8		Blood [fever] (30) Respiratory tract [CF] (29)
Roseolavirus	HHV6	Blood (28,31) Nasopharynx (26)	Blood [fever] (26) Respiratory tract [CF] (29)
	HHV7	Blood (31) Nasopharynx (26) Oral cavity (32)	Blood [fever] (26)
Simplexvirus	HSV-1		Oral cavity [periodontitis](27)
	HSV-2		Oral cavity [periodontitis](27)
Family: Papillomaviridae			
Unspecified		Oral cavity (33) Skin (34,35)	Nasopharynx [LRTI and URTI] (24) Oral cavity [bone infection/brain abscess] (33)
Alphapapillomavirus		Gastrointestinal tract, nose, oral cavity, skin, vagina (25) Respiratory tract (29)	
Betapapillomavirus		Gastrointestinal tract, nose, oral cavity, vagina (25) Skin, (25,36,37)	
Gammapapillomavirus		Gastrointestinal tract, nose, oral cavity, vagina (25) Skin, (25,36,37)	
Family: Polyomaviridae			
Unspecified		Gastrointestinal tract, nose, oral cavity, skin, vagina (25)	Blood [fever] (26)
Alphapolyomavirus	Merkei cell polyomavirus	Skin (36)	Blood [fever] (30)
	HPyV9	Skin (36)	
Betapolyomavirus	KI polyomavirus	Upper respiratory tract (38)	
	WU polyomavirus	Upper respiratory tract (38)	
Deltapolyomavirus	HPyV6	Skin (36)	
	HPyV7	Skin (36)	
Family: Adenoviridae			
Mastoadenovirus		Gastrointestinal tract (39) Upper respiratory tract (38) Blood (28) Nasopharynx (22)	Gastrointestinal tract [AFP[d]] (40) Gastrointestinal tract [diarrhea] (41,42) Nasopharynx [fever] (43) Nasopharynx [RTI[e]] (22)
Family: Circoviridae			
Circovirus		Gastrointestinal tract (25,39) Oral cavity (25) Skin (36)	
Cyclovirus		Gastrointestinal tract (39)	
Family: Anelloviridae			
Unspecified		Blood, nasopharynx (26) Gastrointestinal tract (39)	Blood, nasopharynx [fever] (26) Gastrointestinal tract [diarrhea] (41,44)
Alphatorquevirus	Torque teno virus	Blood (28) Nasopharynx (22)	Blood [fever] (30) Gastrointestinal tract [AFP] (40) Nasopharynx [LRTI and URTI] (24) Nasopharynx [RTI] (22)
	SENV	Blood (28)	
Betatorquevirus	Torque teno mini virus	Blood (28) Nasopharynx (22)	Blood [fever] (30) Gastrointestinal [diarrhea] (42) Nasopharynx [LRTI and URTI] (24) Nasopharynx [RTI] (22)

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Table 1 (*continued*)

Genus	Viral species	Body niche in asymptomatic subjects	Body niche in symptomatic subjects [disease]
Gammatorquevirus	Torque teno midi virus	Blood (28) Nasopharynx (22)	Blood [fever] (30) Nasopharynx [LRTI and URTI] (24)
Gyrovirus	girovirus	Gastrointestinal tract (25,39)	

[a]Lower respiratory tract infection.
[b]Upper respiratory tract infection.
[c]Cystic fibrosis.
[d]Acute flaccid paralysis.
[e]Respiratory tract infection.

eukaryotic viruses that have been found, although without a widespread distribution in the human body Table 2.

Gastrointestinal Tract

The virome of the human gastrointestinal tract has been the most frequently studied and not surprisingly, phages have been found to be the most abundant viral entities. Gut phages are primarily classified as members of the double-stranded DNA virus families *Myoviridae, Podoviridae,* and *Siphoviridae* within the order *Caudovirales*, or to the single-stranded DNA *Microviridae* family (39,47–50). Members of the *Corticoviridae, Inoviridae, Leviviridae*, and *Tectiviridae* families have been less frequently identified; of interest, more than half of the phages detected remain unclassified at the family level (39,50–52). Most gut virome analyses have used fecal samples, where phage to bacteria ratios are low, between 1:10 and 1:1 (49), compared to intestinal mucosal surfaces that show ratios from 21:1–87:1 (53); this proportion is even higher than that found in aquatic environments, which ranges from 5:1–10:1 (54). It is noteworthy that most of the phages detected in the adult human gut showed evidence of having lysogenic lifestyles (section 3-d for details).

Opposed to bacterial communities, which are similar between housemates, gut viromes from healthy individuals have shown that bacteriophage populations are highly personalized (21,39,47–50,55,56). Also, they are preserved over time, with a 95 and 80% retention of the phage diversity over 1 and 2.5 year periods, respectively (21,55). Similar to the human gut bacteriome that is modified with changes in the diet routine, it was reported that the gut phage population of individuals following a particular diet could also experience significant alterations. Thus, the subjects on a similar eating regimen showed a more comparable, though not identical, virome composition at the end of the dietary intervention (55).

Ecological factors and even hereditary qualities also could play a role in phage populations' diversity (21,50,52,55). Interestingly, despite the personalization of phage communities and the high variability between individuals, 23 shared bacteriophages were found in more than half of 64 asymptomatic individuals from around the world (57). This study led to the proposal that there exist three classes of bacteriophages in the human population: a set shared among more than half of all people, a common set of 132 bacteriophages found in 20–50% of individuals, and a set that is either rarely shared or unique to a person. For example, the new crAssphage is highly abundant and ubiquitous in the human feces, across unrelated individuals (58).

Several studies have characterized the gut phage communities in newborn children, demonstrating a high richness and diversity and low stability during the first days of life. This diversity diminished over the first two years, in contrary to what has been observed in bacterial populations, which shift from low to high diversity (39,48,50,59). This phenomenon suggests negative predator-prey dynamics between phages and bacterial communities in infants, as opossed to what has been found in adults. This hypothesis is also supported by a shift in phage communities from *Shipoviridae* to *Microviridae* in the same period (section 3-d-i for details). Finally, during early childhood, the gut phages are more similar between infant twin pairs when contrasted to unrelated children (50).

Even though the presence of eukaryotic viruses in the intestinal tract is far less well characterized, some studies have shed light on the dynamic of these viruses, with at least 16 different DNA viral families and 10 RNA families having been detected in gut samples. A longitudinal study comparing the virome of healthy newborn twins showed that, in contrast to phages, the eukaryotic virus diversity has a tendency to increase over time, probably due to exposure to the environment, since the most common viruses found were enteroviruses and paraechoviruses. It is not surprising that anelloviruses, which are highly prevalent in the human population, were also found, with a maximum around 6–12 months of age and a progressive decreased presence around 18 months of age (39). Also, in twin infants, a variety of eukaryotic viruses were acquired gradually as maternal immunity waned (39), even though the specific strains showed more similarity between siblings, all children showed exposure to many of the same viruses over time. In contrast, viral diversity was one of the most important differences between children with acute diarrhea from two different geographical locations in Australia

Table 2. Viruses with a niche-specific distribution in the human body, described in asymptomatic and symptomatic individuals

Genus	Viral species	Body niche in asymptomatic subjects	Body niche in symptomatic subjects [disease]
Familiy: Coronaviridae			
Alphacoronavirurs	HCoV 229E		Nasopharynx [RTI[a]] (22)
	HCoV NL63		
Betacoronavirus	HCoV OC43	Nasopharynx (22)	Nasopharynx [LRTI[b] and URTI[c]] (24)
			Nasopharynx [RTI] (22)
	HCoV HKU1		Nasopharynx [RTI] (22)
Family: Paramyxoviridae			
Respirovirus	HPIV-3	Nasopharynx (22)	Nasopharynx [RTI] (22,45)
	HPIV-1	Nasopharynx (22)	Nasopharynx [RTI] (22,45)
Family: Pneumoviridae			
Metapneumovirus	hMPV		Nasopharynx [RTI] (22,45)
Orthopneumovirus	hRSV	Nasopharynx (22)	Nasopharynx [RTI] (22,45)
			Nasopharynx [LRTI] (24)
Family: Orthomyxoviridae			
Influenzavirus A	Influenza A	Nasopharynx (22)	Nasopharynx [RTI] (22,45)
Influenzavirus B	Influenza B		Nasopharynx [RTI] (22,45)
Influenzavirus C	Influenza C		Nasopharynx [RTI] (22,45)
Family: Parvoviridae			
Bocaparvovirus	Human bocavirus	Gastrointestinal tract (39)	Gastrointestinal tract [AFP[d]] (40)
		Nasopharynx (22)	Nasopharynx [LRTI and URTI] (24)
			Nasopharynx [RTI] (22)
Erythroparvovirus	Parvovirus B19		Blood [fever] (30)
Protoparvovirus	Bufavirus 1		Gastrointestinal tract [diarrhea] (44)
Family: Astroviridae			
Mamastrovirus	Human astrovirus		Gastrointestinal tract [diarrhea] (42)
			Nasopharynx [URTI] (24)
Family: Reoviridae			
Rotavirus	Rotavirus		Gastrointestinal tract [diarrhea] (41)
			Gastrointestinal tract [AFP] (40)
			Nasopharynx [LRTI and URTI] (24)
Family: Caliciviridae			
Norovirus	Norovirus C14		Gastrointestinal tract [diarrhea] (42)
	Snow Mountain virus		Gastrointestinal tract [diarrhea] (42)
	Unspecified		Gastrointestinal tract [diarrhea] (41)
Family: Picornaviridae			
Enterovirus	Human rhinovirus A, B and C	Blood (28)	Blood [fever] (30)
		Nasopharynx (22)	Nasopharynx [RTI] (22,45)
		Blood (28)	Nasopharynx [LRTI and URTI] (24)
	Human enterovirus A		Gastrointestinal tract [AFP] (40)
			Nasopharynx [URTI] (24)
	Human enterovirus B		Gastrointestinal tract [diarrhea] (41)
			Gastrointestinal tract [AFP] (40)
	Human enterovirus C	Gastrointestinal tract (39)	Gastrointestinal tract [AFP] (40)
Parechovirus	Human parechovirus type 3		Gastrointestinal tract [AFP] (40)
Cardiovirus	Saffold virus		Nasopharynx [URTI] (24)
Cosavirus	Cosavirus		Gastrointestinal tract [AFP] (40)
Kobuvirus	Aichivirus A		Gastrointestinal tract [AFP] (40)
			Gastrointestinal tract [diarrhea] (44)
Family: Picobirnaviridae			
Picobirnavirus	Picobirnavirus		Gastrointestinal tract [diarrhea] (42,44)
			Gastrointestinal tract [AFP] (40)
Family: Flaviviridae			
Flavivirus	Dengue virus		Blood [fever] (23,46)
Hepacivirus	Hepatitis C virus	Blood (23)	Blood [fever] (23)
Pegivirus	GBV-C	Blood (23,28)	Blood [fever] (23,46)
Family: Hepadnaviridae			
Orthohepadnaviridae	Hepatitis B virus	Blood (23)	Blood [fever] (23,46)
Family: Arenaviridae			
Arenavirus	Lassa virus		Blood [fever] (23)

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Table 2 (*continued*)

Genus	Viral species	Body niche in asymptomatic subjects	Body niche in symptomatic subjects [disease]
Family: Rhabdoviridae			
Tibrovirus	Ekpoma virus 1	Blood (23)	
	Ekpoma virus 2	Blood (23)	
Family: Marseilleviridae			
Marseillevirus	Marseillevirus	Blood (28)	
Unclassified	Smacovirus		Gastrointestinal tract [diarrhea] (41)

[a]Respiratory tract infection.
[b]Lower respiratory tract infection.
[c]Upper respiratory tract infection.
[d]Acute flaccid paralysis.

showing that samples from the more urbanized area showed a lower diversity (60). It is important to emphasize that in both reports the environment has a significant effect on the virome composition.

The remarkable advances in the characterization of the human gut microbiome and the possible implications of this knowledge in the clinical setting are still a subject of debate. Specifically, viral metagenomics offers the possibility of examining samples for which traditional diagnostics methods have not yield results. In this context, studies of viral communities of diarrheic specimens for unknown causes in individuals from different continents, such as America and Europe, coincide to find a common group of viruses. This group includes anelloviruses, picobirnaviruses, Aichi virus and a small circular DNA virus previously associated with fecal feces from asymptomatic animals (41,44). The role of these viruses in gut pathogenesis remains undetermined, as well as if their presence is the cause or a result of a diseased gastrointestinal tract. For example, the role of the immune status on the enteric virome has been investigated, by determining the virome of HIV-positive individuals that have developed AIDS. The results suggest that immunodeficiency correlates with the proliferation of the *Adenoviridae* and *Anelloviridae* families (61). This observation may indicate that some viruses can produce a more favorable environment for the establishment of other viral infections; additionally, the detection of these co-infecting viruses may have the potential use as a biomarker of poor immunological response.

On the other hand, the virome of diarrhea samples of pediatric patients allowed the detection of known human pathogens belonging to the families *Reoviridae, Caliciviridae, Picornaviridae, Picobirnaviridae, Astroviridae,* and *Parvoviridae*. Most of the patients showed a single predominant virus, in some cases, the virus identity could only be established at the family level, due to the lack of similarity to known viruses (42). In Peruvian children with diarrhea of unknown etiology novel small circular ssDNA viruses were detected in addition to other known pathogens (41), and in Asian children with acute flaccid paralysis, new viruses from the *Picornaviridae, Dicistroviridae, Nodaviridae,* and *Circoviridae* families were found (40).

These findings highlight the important role of metagenomic analyses in the discovery of novel viruses that can be related to the etiology of the disease; these new viruses can either belong to known families or genera or bear no resemblance to any known virus. In the latter case, it becomes necessary to carry out other tests that confirm the presence of these novel viruses and begin their characterization.

It is worth noting that plant pathogenic RNA viruses have also been found in human feces from healthy individuals, possibly ingested through the diet. On a study of the gut virome, most of the samples included pepper mild mottle virus and fluctuations in viral communities between individuals and over time were found, which might be due to differences in their diets (62). For instance, intra-individual differences observed in the genome of pepper mild mottle virus from different sampling times could be acquired by the ingestion of a variety of processed foods that will likely contain different strains of the virus, changing the detected virus throughout time. Also, a role of humans and other mammals in the spread of these plant pathogens was proposed, as the viruses remained infectious even after ingestion.

Oral Tract

The human oral virome is just beginning to be characterized and is mainly composed by bacteriophages classified mostly as members of the *Siphoviridae* family, and at a lower extent of the *Myoviridae* and *Podoviridae* families. Similar to the gastrointestinal tract, the oral virome seems to be also distinct for different individuals and stable over a long time without external disturbance (19,33,63,64). Different unique phage communities have been found in saliva, dental plaque and subgingival and supragingival plaques (64,65). In this regard, viral groups from a similar oral site in different individuals appeared to be more comparable than those from various oral destinations in the same person (19). Contrary to bacterial communities, phage groups are fundamentally more diverse and abundant in the oral virome than in the gut and have been related to gender (64). Additionally, it was demonstrated that

individuals living in the same house share a higher proportion of their oral viromes when contrasted to control subjects from different family units (66). Other factors could also have influenced this observation, such as eating regimen, bacterial diversity, and parentage of the individuals from the same households. Similar to gut virome, a significant proportion of oral phages are predicted to have lysogenic lifestyles in a dynamic harmony with their cellular hosts.

Most studies of the oral virome have not described the presence of eukaryotic viruses and those that have done it have focused solely on DNA viruses. An analysis of the salivary microbiome identified the presence of herpesviruses (32). Besides that, research on eukaryotic viruses in oral illnesses such as the periodontitis, a prevalent inflammatory disease, revealed an association between the presence of viruses and inflammation. The virome was constituted by human herpesviruses (HHV), herpes simplex viruses 1 and 2 (HSV-1, HSV-2), cytomegalovirus (CMV) and Epstein-Barr virus (EBV) (27). Furthermore, a pathological role for HSV-1 and EBV has been suggested (27,67). Therefore, this information must be taken into consideration when a treatment for this illness is prescribed since most of the times only bacterial etiological causes of the disease are considered. Finally, a recent study has identified herpesviruses, phycodnaviruses, poxviruses, mimiviruses, baculoviruses, and papillomaviruses in saliva from control individuals and with antibiotic therapy. Interestingly, in subjects on antibiotics papillomavirus was the most abundant viral population, even when considering phages (33).

Respiratory Tract

To date, few studies have focused on the virome of the lung and respiratory tract. It has been demonstrated that bacteriophages are abundant in the lung and that phage communities change together with the bacterial populations of the host (29). It has been suggested that there is a resident core set of 19 phages in the human respiratory tract and another random set that only was found in healthy people, possible coming from the local environment. Evidence for this variation originates from the similarities found in some phages, extra from the common core set, between a cystic fibrosis (CF) patient and her healthy partner, that was not observed among healthy individuals that did not live in the same house (29). Also, an additional group of 12 phages was identified only in individuals affected by CF corresponding to concurrent pathogenic bacterial species (29).

Regarding eukaryotic viruses, in patients with lower respiratory tract infections members of the *Paramyxoviridae, Orthomyxoviridae,* and *Picornaviridae* families have been commonly found, with some new viruses belonging to these and other families also been described (45). However, since this study was carried out only in symptomatic individuals, there is no baseline for comparison with a virome in individuals without respiratory disease. Additionally, in children presenting with acute respiratory infection, a metagenomic analysis showed the presence of viral families of known respiratory pathogens such as *Paramyxoviridae, Coronaviridae, Picornaviridae,* and *Parvoviridae*, despite previous negative tests to common respiratory pathogens (24). Moreover, viruses commonly found throughout the body such as *Herpesviridae, Papillomaviridae,* and *Anelloviridae* were also reported (24). Also, the same study found the presence of plant and nonhuman animal pathogens in the respiratory tract, probably acquired through the environment or food consumption. In another study of CF patients and controls, DNA viruses' diversity was assessed; it was found that non-CF individuals shared more similarity in their virome (sharing the presence of adenoviruses, herpesviruses, and poxviruses) pointing toward a possible core virome, although this has to be corroborated by further studies. On the other hand, CF patients showed some shared viruses, including HHVs, such as EBV, HHV-6B, and HHV-8P, but with differences in their relative abundance (29). It must be noticed that in genetic and chronic diseases such as CF where the respiratory tract is commonly affected by viral infections, the complexity of the virome is higher, as expected (68).

The respiratory virome of healthy individuals has also been characterized; interestingly in nasal, and nasopharyngeal samples, several pathogenic viruses have been found from the families *Adenoviridae, Parvoviridae, Paramyxoviridae* (26), *Picornaviridae* (43), and *Orthomyxoviridae* (38). It is not clear how long these viruses remain in the respiratory tract in healthy individuals, or if they can establish persistent asymptomatic infections (69).

The study of the respiratory virome has shown that the complexity of the viral community is lower in healthy individuals. For instance, children and adults suffering from different respiratory conditions harbored a variety of eukaryotic viruses (22,45,70). In contrast, in healthy children, viral populations were mainly composed of members of *Anelloviridae* family and in minor proportion HHV. It is worth to notice that viral communities in this kind of respiratory affections are quite similar between children and adults.

Skin

It has been harder to study viral groups of the skin due to the low biomass obtained from skin samples. While bacterial communities have been found to inhabit the skin (71), there is limited information on phage populations. In a recent study (34), up to 94% of the viral sequences did not match a known viral genome in reference databases. Most of the remaining viral sequences could not be identified at the family level, just to the *Caudovirales* order, the few phages that were characterized at the family level

included *Myoviridae* and *Siphoviridae*. The vast majority of these phages were predicted to be lysogenic. It was also suggested that intra-personal and inter-personal variations assume a more prominent role in cutaneous viral composition, followed by body site and a high temporal variability, contrary to the temporal stability observed in the gastrointestinal tract and the oral cavity.

On the other hand, the skin of healthy individuals can harbor several eukaryotic viruses for varied periods of time. These viruses belong to the *Adenoviridae, Anelloviridae, Circoviridae, Herpesviridae, Papillomaviridae, Phycodnaviridae, Poxviridae, and Polyomaviridae* families (34−37,72). The most common resident viruses found in the skin are alfa, beta and gamma human papillomaviruses (25,36,37), human polyomavirus 6 and 7, and also Merkel cell polyomavirus. This polyomavirus had only been isolated from Merkel cell carcinoma and is regarded as the etiological agent of this type of skin cancer (73); it is interesting that it can be found in healthy cells. Other viruses, with not know pathologic association, have been found, such as circoviruses (36,74). It is worth noting that as these studies have focused solely in the DNA virome; the RNA virome remains uncharacterized.

Blood

The human blood was thought to be a sterile compartment, but metagenomic studies have demonstrated that it can contain large viral communities. Phages classified in the *Myoviridae, Siphoviridae, Podoviridae, Microviridae,* and *Inoviridae* families have been found (75−77). Interestingly, in healthy individuals, lower proportions were reported as compared to people with cardiovascular disease (76) or immune suppression (77), which suggests the existence of immune components that could restrict the access of phage to the bloodstream.

Many eukaryotic viruses were identified in the blood of 8,000 individuals, commonly including HHVs, anelloviruses, polyomaviruses, papillomaviruses, parvovirus B19, and human T-cell leukemia virus. Interestingly viral sequences of Mollivirus were also detected (31). Moreover, in asymptomatic individuals, members of the *Herpesviridae, Flaviviridae, Marseilleviridae, Mimiviridae, Phycodnaviridae, Picornaviridae* and *Poxviridae* families, have been found (23,28,78). The presence of viruses in the blood of the majority of the individuals sampled raises concerns regarding blood safety.

Additionally, the analysis of blood of individuals from different geographic regions that had the same illness showed a set of viruses that apparently differs significantly among localities. For example, a blood virome analysis of American febrile children identified viruses from the *Anelloviridae* family, specifically TTV and TTMDV that were absent in healthy controls (79). On the other hand, blood samples from Kenya from adults with unexplained fever were mainly positive for parvovirus B19, anellovirus, pegivirus C, dengue virus, HIV-1, and herpesvirus (30). This kind of studies could outline viral communities in the blood of febrile patients that could be taken into account in early diagnostic tests and treatment design depending on the patient's origin.

Genitourinary Tract

Phage populations in genitourinary tract remain largely unexplored since there are just a few reports of this compartment. The viruses described in the urinary tract have been mainly phages (>99%) with no significant differences regarding health status (20). The high proportion of integrase genes identified in phages suggests lysogenic lifestyles in this tract (Section 3-d-i).

Regarding eukaryotic viruses, only DNA viruses have been characterized. It is not surprising that most studies found the presence of many members of the *Papillomaviridae* family, followed by members of *Herpesviridae, Anelloviridae* and *Poliomaviridae,* in urine and vaginal samples (20,25).

The study of viral communities and their influence on human health has led to determine that the balance in diversity and abundance of specific viruses strongly depends on environmental factors such as diet, living conditions, access to potable water and sewage network, pollution, among other factors. However, many studies need to be replicated in various countries to determine the relative contribution of environmental and genetic differences that can restrict the generalizations of the conclusions reached so far.

Interactions of the Virome with other Members of the Microbiota

As mentioned earlier, symptomatic and asymptomatic individuals can harbor diverse eukaryotic viruses that can play a role in health and disease and influence the physiology of the host (7−11). Some of these effects can be induced by a direct interaction of viruses with the host, while others may require the interplay of the virome with other components of the microbiome.

Most of the interactions between the virome and the bacteriome have been characterized in the gut, which is not surprising, since, from the microbiological point of view, the intestine is a complex system densely populated by different components of the microbiome and often by parasites. In this body compartment, there are around 10^{14} bacteria that represent between 500 and 1,000 different species (46,80). Thus, when entering the body, enteric viruses find a large population of resident microorganisms in the intestinal lumen, mainly commensal bacteria, but also other viruses, archaea, fungi, and protozoans, among other organisms, with which they can potentially interact. These interactions may occur, regardless of whether the

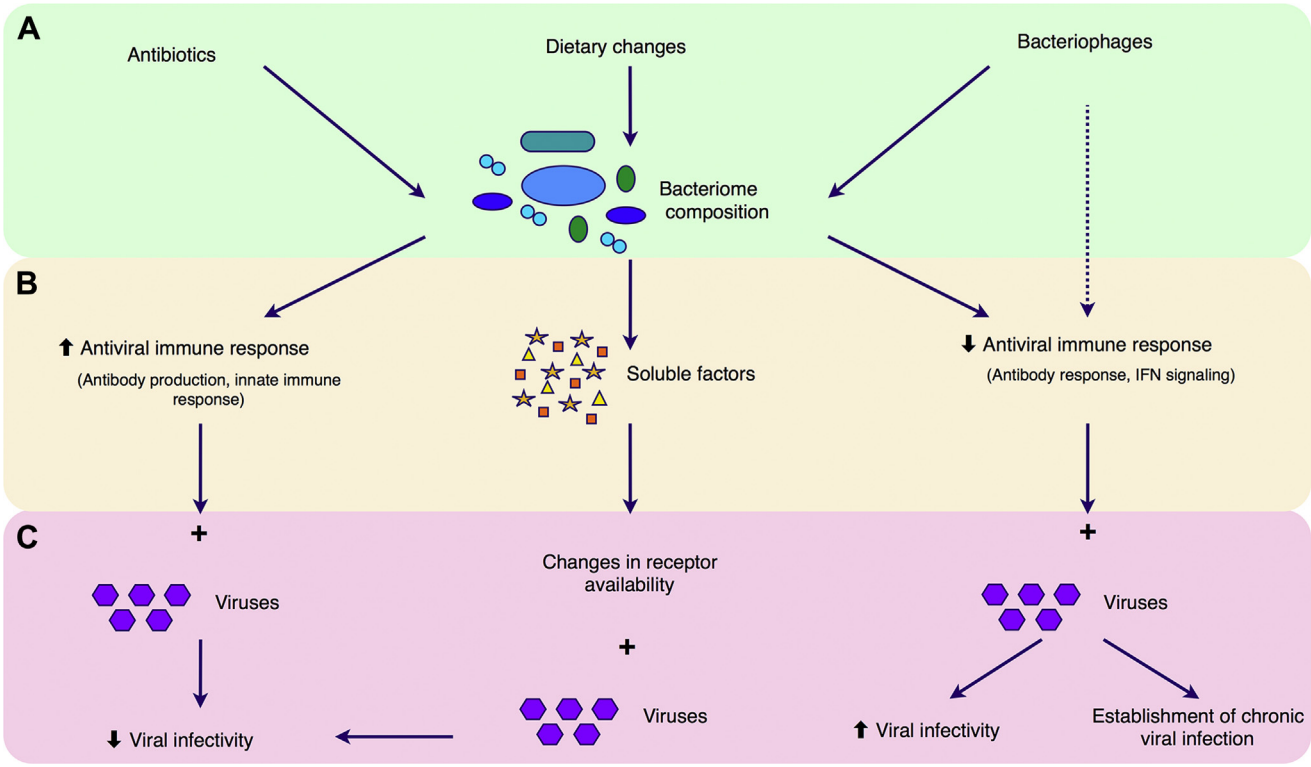

Figure 1. The bacteriome composition regulates viral infectivity. (A) Several external variables can alter bacteriome composition, including the presence of bacteriophages (81), changes in the diet, and the use of antibiotics (82), among others. (B) The composition of the bacteriome may influence the immune status of the host either by decreasing the antiviral antibody response (83,84) and the virus-induced IFN signaling (83,85), or by increasing the antiviral antibody production (86) and activating the innate immune response (87). In addition, there is evidence that indicate that bacteriophages could inhibit activation and proliferation of human T cells, although this has to be further investigated (88). (C) The resulting microenvironment can affect viral infectivity; for instance, an impaired immune response can increase viral infectivity and/or facilitate the establishment of chronic infections (83–85), whereas an activated immune system can block viral infections (86,87). Also, bacteria can produce soluble factors, such as glycans, which can compete with receptor binding and decrease viral infectivity (89). (A color figure can be found in the online version of this article).

enteric viruses replicate locally in the intestine or whether they use the gut as a portal of entry to the organism and show their pathology systemically or in organs different from the gastrointestinal tract (Figure 1).

The Microbiota Modulates Viral Infection

The interactions that occur among the components of the microbiome and between them and the host have been mainly characterized for bacteria. However, the relationship between commensal bacteria and viruses, and in turn with the host has received little attention, until recently. These first reports have shown that these interactions can modulate the infectivity of viruses (83,90,91).

Interaction with the microbiota can enhance virus infectivity. The microorganisms that compose the microbiome, or parts of them, can affect enteric viruses through a direct, physical interaction. In this regard, it has been reported that the interaction of commensal bacteria with poliovirus and reovirus increases viral infectivity (92). In the case of poliovirus, it was further shown that it binds the bacterial surface polysaccharides, what enhances its stability and its attachment to cells through increasing its binding to the cellular receptor (91). Also, it was more recently shown that bacteria facilitate poliovirus co-infection of mammalian cells and promote virus genetic recombination (93).

In another example, it was shown that infection of B cells *in vitro* by human norovirus requires histo-blood group antigen-expressing enteric bacteria, while the replication of murine norovirus in mice is reduced by depleting the gut microbiota by antibiotic administration (82). Rotavirus replication and diarrhea in a mouse model were also shown to be stimulated by enteric bacteria (84).

Also, the successful transmission of the murine mammary tumor virus from an infected mother to her pups was prevented by antibiotic treatment of the mother and, similarly, the transmission did not occur in germ-free mothers, and it was restored by reconstitution of the gut microbiota (94). These findings show the relevance of commensal microbiota for the efficient infection of several enteric viruses in mice. In addition to the direct mechanisms through which enteric bacteria stimulate virus infection, the microbiota can also enhance virus replication or

facilitate the establishment of a chronic infection by attenuating the antiviral host immune response to induce a tolerogenic environment (83,94). This attenuation can be due to the suppression of the antiviral antibody response (83,84), or by modifying the virus-induced interferon (IFN) signaling (83,85).

The microbiota can negatively affect virus infection. In addition to the positive effect that the microbiota can have on virus infection, components of the enteric microbiome can also reduce viral infectivity. Rotavirus attachment to cells requires the interaction with glycans on the cell surface, and it was found that the addition to human intestinal cells in culture of bacterial soluble factors that increase cell-surface galactose, like those produced by *B. thetaiotaomicron* and *L. casei*, efficiently block rotavirus infection (89). In a different study, the probiotic bacteria *L. reuteri* shortened the duration of rotavirus diarrhea in a neonatal mouse model, what correlated with an augmented enterocyte proliferation and villus repopulation by enhanced migration, and with an increase of rotavirus-specific antibodies (86).

In another example, flagellin, the principal component of bacterial flagella and a potent immune activator in the gut, prevented rotavirus infection and resolved chronic rotavirus infection in mice, independent of adaptive immunity and IFN. It was found that flagellin protected by activation of two distinct innate immune signaling pathways that elicited the production of cytokines IL-22 and IL-18. Of interest, administration of these cytokines to mice recapitulated the effect of flagellin on the replication and clearance of the virus (87).

The microbiota can modulate virus infection at Distal sites. The intestinal microbiota can also negatively affect virus infection at a different mucosal surface, and even systemically. Thus, the intestinal commensal microbiota was shown to regulate the establishment of CD4 and CD8 T cells and the IgA antibody response to influenza virus infection, predisposing the mice to high viral replication in the lung (69). These results show the relevance of commensal microbiota in the regulation of the respiratory tract immune response. Likewise, manipulation of intestinal commensal bacteria of mice by antibiotic administration dysregulated the adaptive immune response and the macrophages' reaction to type I and II IFNs. This dysregulation resulted in a degraded protective immunity after infection with either lymphocytic choriomeningitis (LCMV) or influenza, responsible, respectively, for systemic and mucosal infections.

Helminths have also been shown to modulate the immune response of the host in a way that influences the control of pathogens. Intestinal helminths are known to induce in the host a strong Th2 response that reduces the activity of the antiviral cytokine IFN-gamma and activates macrophages with an immunoregulatory rather than a pro-inflammatory phenotype (95). In this regard, it was recently found that acute infection with the helminth *H. polygyrus* or administration of *S. mansoni* eggs to mice latently infected with gammaherpesvirus, induced reactivation of the virus. Investigation of the immune mechanism of reactivation showed that the helminth infection resulted in the production of cytokine IL-4, which promoted viral replication and, at the same time, blocked the antiviral effects of IFN-gamma. Of interest, administration to mice of exogenous IL-4, plus blocking the activity of IFN-gamma, induced the replication of the latent gammaherpesvirus infection (96).

Influence of the Virome on Host Immunity

Infection with multiple herpesviruses is common during childhood. After acute infection, the virus remains in the organism in a state of latency which persists for life, and this dormant virus can be reactivated under different conditions, resulting in disease. However, it was shown that virus latency could also be beneficial for the host by establishing an upregulated basal immune state that controls subsequent bacterial infections. Thus, mice latently infected with murine herpesviruses were shown to be resistant to infection with *L. monocytogenes* and *Y. pestis* through a long-term production of IFN-gamma and systemic activation of macrophages (97). The interaction of latent herpesvirus infections with the host immune system was also evident when it was shown that a chronic herpesvirus infection complements a genetic immunodeficiency in mice having a mutant HOIL-1 gene. These immunodeficient mice die when infected with certain bacteria and parasites, but they can tolerate infections if chronically infected with herpesvirus, apparently by restoring the capacity of the mice to produce protective cytokines that were decreased in the non-infected animals (98). These findings suggest that a latent virus infection could be responsible for some of the variability observed in the symptoms of different individuals having the same hereditary immunodeficiency when they are infected with pathogenic microbes (98).

A physiological benefit to the host by chronic viral infections was also observed in mice infected with murine cytomegalovirus since it was found that the infection increased the epithelial turnover in multiple tissues, mediated by the production of type I antiviral IFN cytokines (99). In this regard, it is of particular interest the recent report describing a common enteric virus (murine norovirus), non-pathogenic for mice, that can replace the beneficial effects of commensal bacteria in the animal gut. It was shown that this virus could promote the proper development of intestinal morphology and the immune function of the host in the absence of bacteria, and also prevent the intestinal damage caused by antibiotics. This evidence suggests that eukaryotic viruses have the potential to contribute

to the intestinal homeostasis and the maturation of the host immune response, similar to what has been previously observed for commensal bacteria (100).

The effect of viruses on the immune status of the host can also be appreciated distantly from the infected organ. Thus, influenza virus infection in the respiratory tract can generate an intestinal dysbiosis through a systemic mechanism dependent on type 1 IFNs. Also, the IFNs produced in the respiratory tract can lead to the inhibition of the antimicrobial and inflammatory response in the gut favoring the intestinal colonization and systemic dissemination of Salmonella in a colitis mouse model (101). This report, together with the negative effect the microbiome has on influenza virus infection (69), exemplify the reciprocal influence of the intestinal and respiratory tract microbiota. This interaction occurs through a constant stimulation of the immune system (91,102,103).

Interactions with Host Genetics

The interactions of the virome with other components of the microbiome, in concert with the genetic background of the host, can also play a role in the outcome of infection. Thus, it has been shown that, in distinct genetic backgrounds, a virus infection can trigger or modify diseases, other than those directly related to the infection and sometimes even destruct susceptible cells (102). For instance, it has been reported that a persistent infection with murine norovirus of mice that carry a mutant version of the autophagy *Atg16L1* gene, which is associated with susceptibility to Crohn's disease in humans, causes a manifestation of intestinal pathologies, while the disease does not develop when wild-type mice are infected. Of interest, the intestinal anomalies were prevented by treatment with broad spectrum antibiotics, indicating that the virus acts in concert with commensal bacteria to cause the inflammatory disease (102). In an additional example, murine norovirus infection in an IL10-deficient mouse model of inflammatory bowel disease resulted in an increased paracellular permeability and inflammation of the mucosa, while norovirus-infected wild-type mice remained asymptomatic. The presentation of the disease in these mice was largely dependent on the microbiota since the inflammatory lesions were not observed in germfree IL10-deficient mice (104). These examples provide evidence of the manifestation of a disease determined by the combination of a specific virus infection with a particular host gene allele and the enteric microbiota.

In an additional observation, it was found that infection with a lymphotropic strain of LCMV prevents the development of type 1 diabetes in the non-obese diabetic mouse model, through abrogation of the lymphocyte-caused autoimmune response that leads to severe disease and death (105). However, the potential involvement of the microbiota and the genetic basis responsible for this protective phenotype are not known.

Bacteriophages Regulate the Structure of Bacterial Populations

Phages are important bacteria limiting elements; for instance, they account for up to 80% of bacterial death in the oceans (3). Each phage can usually interact with a small variety of bacterial strains of the same species, and replicate by either lysogenic (temperate) or lytic life cycles. Therefore, bacteriophages can have a significant and direct impact on the structure of bacterial populations. Also, bacteria can use their prophages to kill related bacteria or prevent overgrowth of certain bacterial species or strains that use the same ecological niche, thus assisting the bacterial survival and propagation (106).

Phage-bacteria predation dynamics. Phage-bacteria interactions generally exhibit a predator-prey interaction called *kill-the-winner* where, in a particular ecosystem, phages kill only the dominant bacterial strains to reestablish a microbiological equilibrium. This phenomenon is characterized by episodes of rapidly growing bacteria species followed by blooms of their phages, generating periodic changes in phage and bacterial abundances. These interactions between phages and bacteria are known to be predominant in aquatic environments, such as oceans (107). In contrast, different human niches seem to contain stable communities of phage and bacteria, what appears to be the consequence of a predominant phage lysogenic cycle (3,21,48,55). This hypothesis is supported by: a) the high frequency of integrase genes identified in bacteria-phage genomes, which are used as markers for temperate phages, since they allow the integration of the phage genomes into the bacterial DNA; b) the number of phage sequences identified by comparison to known prophages; and c) the small number of sequences that show similarity to bacterial genomes sequences bounded by CRISPRs (short repeated sequence that flanks the viral insertion site) that are considered genomic records of phage predator interactions (21,34,50,55).

Moreover, when mice containing an artificial community of bacteria derived from the human gut were inoculated with pooled virus-like particles enriched from human feces, the predominance of only two phages, with the concomitant decrease of their bacteria host density was observed. Afterwards, bacteria levels recovered to a stable state when phage abundance decreased (81). Interestingly, at latter times, the bacterial genomes showed no immunity sites to the infecting phages (CRISPR elements). This observation might be due to a phenomenon where bacteria refuge from phages (81,108), according to the Red Queen hypothesis of co-evolution, where escape strategies in the prey population are countered by adaptations of the predator (109).

Additionally, it was shown that phages belonging to *Myoviridae* family, which are typically considered lytic phages and with relative broad bacteria host ranges, were

more abundant in subgingival plaque of individuals with periodontal disease. In contrast, in healthy subjects, phages from the *Siphoviridae* family, generally recognized as lysogenic phages, were predominant (110). This evidence suggests a more active role of lytic phages into the development of the disease and modulation of the microbial community.

In this regard, other studies have shown non-stable phage-bacteria relationships. For example, the oral phages in periodontitis patients tend to have a homogeneous community structure compared to healthy individuals; however, this phenomenon was not observed in the bacterial population (110). Also, a clear difference was found in the bacterial diversity between Crohn's disease and ulcerative colitis versus control groups, but not in the phage population (111). Moreover, phage communities were found to be obviously gender-consistent in the oral cavity, whereas similar patterns were not detected for the bacterial communities (64). These examples of phage and bacteria interactions show that more studies are needed to understand their dynamics in the human virome.

Phage-induced bacterial genome modification. Phages offer evolutionary advantages to bacteria by stimulating mutations, adaptations, and the introduction of antibiotic resistance genes and virulence factors that can enhance their survival. For instance, there is a direct correlation between prophage presence and *Enterococcus feacalis* adhesion to human platelets, what is the first step to infective endocarditis (112). In another example, *E. faecalis* strain V583, a commensal bacteria of the human intestine, has been shown to harbor a prophage whose production is controlled by nutrient availability, allowing these bacteria to be more competitive and dominant over other *E. faecalis* strains *in vitro* and *in vivo*. Also, phage SopEΦ increases the virulence of *Salmonella typhimurium* through the horizontal gene transfer of the membrane-associated effector SopE (113), an inducer of intestinal inflammation and activator of several innate immune signaling pathways. In this context, a moron gene of prophage HK97 is expressed in *Escherichia coli* excluding it from superinfection with similar phages that do not contain this particular insertion (114). In fact, about 70% of sequenced bacterial genomes harbor prophages and could contain multiple prophages in their chromosomes, as in the case of *E. coli* O157:H7 that has 18 prophage genome elements, representing 16% of its total genome content (115).

In humans, phages are suppliers of virulence and antibiotic resistance genes and by horizontal gene transfer can exchange them between different bacterial species, contributing to the survival and pathogenesis of their hosts (33,34,116,117). Interestingly, after antibiotic medication, new interactions are established between phages and many other bacteria, rather than a specific phage-bacteria interaction. This interaction network is promoted by the increase in the frequency of phage integration and the stimulation of broad host range (19,65,110,117). Beside prophages with antibiotic resistant genes, other genes involved in complement and immunoglobulin degradation have been found (19,64). In addition, many diseases are caused by toxins encoded by phages, which contribute to bacterial pathogenesis.

Phages provide evolutionary pressure on bacteria. An *in vitro* study demonstrated that after less than 200 generations, a 10–100-fold increase in mutation rates of bacterial populations that coevolved with their phages occurred, with no significant change in bacterial populations grown in the absence of phages (118). These mutations can confer metabolic benefits to bacterial communities. For example, the main differences between two strains of *Streptococcus pyogenes*, which are associated with different types of disease, seem to be mainly in the region of their prophages (119). Nucleotide substitution rates are different in each phage family; *Microviridae* has been associated with the highest rate of mutation ($>10^{-5}$ per nucleotide per day), which can even generate new phage species over a short 2.5 year period (55).

Phage-Human Immune System Dynamics

Phages and microbiota colonize mucosal surfaces and are considered as part of the innate immune system as they provide physical and biochemical antimicrobial defenses (53). It was shown that a high presence of phage adhered to the mucous layer results in a dramatic decrease in bacterial colonization with a significantly reduced pathology (53). Nonetheless, as previously discussed, phages can also contribute to the development of some bacterial diseases. Free bacteriophages can access the host immune system through bacterial translocation across mucosal surfaces such as those in the mouth and gut (53,110). They can interact with the human immune system directly by inducing a humoral antibody response (which may obstruct phage action) or indirectly, through nonspecific immunomodulatory effects on the immune response (88,120). For instance, it has been reported that phages inhibit activation and proliferation of human T cells *in vitro* (88), or change bacterial recognition by modifying the outer membrane lipopolysaccharide (121). Also, T-even phages were found to facilitate transplant and exert antitumor activity by reducing oxygen species production, suppressing T-cell and activating NF-κB (122,123). In the same way, phage T4 is believed to be responsible for immunosuppressive and antitumor activities (88). Despite these studies, little is known of phage dynamics with the human immune system.

Applications of the Virome in the Clinical Practice

Viral metagenomic analyses can provide much information regarding the communities present in a particular sample. It

is clear that this information can have clinical relevance and it has been proposed to include viral metagenomics and NGS technologies for different purposes in the clinical practice. The most straightforward application will be the diagnosis of diseases of unknown origin, where the survey of the viral and bacterial populations will give clues about the etiological agents of the disease. In this case, several practical and ethical considerations need to be made. In the first place, metagenomics studies can reveal the presence of viruses that are not related to the illness for which the patient is seeking care; for instance, they can unveil sensitive information, such as HIV status. Therefore, guidelines and protocols must be put in place to address these concerns (124). In the second place, assumptions about etiology are easily made; however, it is important to emphasize that the presence of a virus in a sample does not imply its contribution to the disease. For instance, a metagenomic analysis was carried out in a patient with a respiratory tract infection that identified a gamma papillomavirus; however, it was demonstrated to be unrelated to the disease (125). In the third place, there is a bias in the sequences represented in the reference databases that can lead to uncertainty about the identity of the sequences found in a sample. Also, there is a lack of virome baselines to use as a reference to interpret the results. Finally, it is necessary to benchmark these methodologies against the current gold standards and estimate false positive rates, sensitivity, and other statistics before metagenomics can be used as a diagnostic tool.

Besides the characterization of the virus community present in a clinical sample, the use of metagenomics allows the discovery of novel viruses, sometimes even permitting the determination of the full genome sequence of viruses that are new or poorly characterized (126). This technology has two advantages over more classical virus discovery methods. First, it does not require viral culture and, second, it does not use sequence-specific probes, allowing for the detection of divergent viruses or viruses unrelated to previously described agents. However, given that usually no cell culture or animal models for these newly discovered viruses exist, their clinical relevance cannot be rapidly established.

A related application of NGS in the clinical setting is the use of ultra-deep sequencing in the characterization of viral populations, with the aim of looking for antiviral-resistant variants, co-infection, and viral diversity and evolution driven either by therapy or by the host immune response, among other factors. The rapid accumulation of mutations in the genome of RNA viruses allows the formation of a diverse viral population over short periods of time. Many characteristics of the viral populations cannot be explained by the consensus sequence, and depend upon the presence of low-frequency variants, which presence can be only detected by ultra-deep sequencing. These low-frequency variants can have clinical significance because they may lead to drug resistance, and the knowledge of their presence in a patient can help decide the best course of treatment. Moreover, in the case of HIV, ultra-deep sequencing can aid in the detection of CXCR4-tropic viruses that are considered a marker of disease progression (127). Ultra-deep sequencing has also been applied in the molecular epidemiology of HCV to identify transmission clusters, detect recombinant viruses, and subtyping virus strains (128).

On the other hand, the information resulting from viral metagenomic analysis can have practical applications, for instance, understanding the interplay between the bacteriome and the virome in the gut can help to design phage therapies aimed to control bacterial infections through the use of specific lytic phages in combination with traditional antibiotics. Phages can also be used to modulate resident bacteria, and the use of genetically modified phages has been proposed as a way to regulate nutrient metabolism in obese and dysmetabolic patients (129). Also, the use of a personalized mixture of bacteriophages as an alternative in treating bacterial infections has been proposed (130). Additionally, phages can be used as biocontrol to increase food safety by treating food-processing facilities and by direct application of phages in harvested food (131).

Conclusions

The human virome remains largely uncharacterized despite the fact that it has become a hot topic of research. The limited number of patients used in the different studies and the fact that they are limited to certain human populations difficult drawing general conclusions. Nevertheless, some interesting findings are worth taking into consideration, from the discovery of how viral diversity changes throughout the lifetime of an individual, to how geographical differences result in distinct virome compositions. One thing to bear in mind is that, so far, in most studies, the search has been directed to DNA viruses, and among these, the bacteriophage diversity has been better characterized. Thus, the RNA virome has remained unexplored in many studies. Additionally, the sequence data is biased in current databases, since they contain sequence information of known viruses, mainly those of clinical or economic importance. This bias results in the fact that around 50% of the sequences obtained in viral metagenomics analyses cannot be assigned to any virus family. However, and noteworthy, the NGS approach for studying viral diversity has led to the discovery of new viruses, although until now, in just a few cases the relationship with a disease has been established.

One of the most exciting areas of research goes beyond the characterization of viral diversity and focus on the interaction of viruses with other organisms in the microbiome. Thus, the interaction of viruses with bacteria can modulate viral infectivity and pathogenesis. Also, the modulation of the immune system carried out by some chronic viral infections can affect pathogenicity of other microorganisms, either increasing it or decreasing it. These studies have

highlighted the importance of understanding these complex interactions that can impact the human health.

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