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Chapter 21

The Viruses of the Gut Microbiota

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The very rapid increase in untargeted Next Generation Sequencing usage in the field of microbiology has allowed to increase the knowledge of resident and pathogenic viruses, and characterize previously unknown or variant viruses. Technological pipelines uncover viruses referenced in databases with a threshold of sensitivity equivalent to that of quantitative PCRs, and are now becoming even more sensitive than PCR with the increasing depth of sequencing [1–4]. This type of pipeline also allows acquisition and assembly of de novo full-length genomes from biological samples, and thereby the discovery of new viruses, even when very distant from known viruses (for a recent review see Ref. [5]). These tools have begun to reveal the existence and composition of the human gut virome and unravel its intrinsic complexity, and interindividual variability in healthy individuals. Yet, the precise composition and potential impact on health of the human virome remains to be determined.

THE EUKARYOTIC VIROME, A COMPONENT OF THE GUT MICROBIOME

The gut virome is defined as the viral component of the gut microbiome, defined itself as the microbial communities of the gut. The gut virome is composed of eukaryotic viruses able to replicate in human cells, as well as bacteriophages that replicate in gut bacteria, which are the most abundant. Eukaryotic viruses also come from food intake, like plant [6] and animal [7] viruses. Systematic longitudinal studies are still lacking and it is therefore difficult to distinguish with certainty viruses that establish long-lasting infection and can be considered as members of a standard flora, from those responsible for acute infections, in particular for human eukaryotic viruses.

Regarding viruses of bacteria (bacteriophages), their presence is indeed modulated by the presence of their host bacteria and they also might regulate the bacterial contents. Recent reviews [8] have covered the human gut phage communities, showing that the population is highly individual and dominated by DNA phages exhibiting a temperate lifestyle. Nevertheless, phage can also lyze bacteria and impact relative bacterial counts [9,10]. The viral load is roughly similar to that of bacteria [11]. Phage may vector transduction (gene transfer) between strains and even bacterial species, and therefore deliver genes encoding toxins, virulence factors, or alternate metabolisms. We have focused this chapter on human eukaryotic viruses.

The history of human gut eukaryote viruses has until recently been dominated by the discovery of pathogenic viruses (among which *Enterovirus*, *Rotavirus*, *Norovirus* genus) that generally lead to transient and symptomatic infections, but some eukaryotic viruses seem resident of the human gut [12]. Most gut viruses are not cultivable and unbiased metagenomics studies have therefore contributed recently to their better characterization. The eukaryotic virome seems to be acquired progressively with age, in contrast to bacteriophage richness, which seems greatest early in life and then decreases [13]. Acquisition of these resident viruses is associated in healthy infants to no apparent underlying acute or chronic disorders [13]. Enteroviruses, parechoviruses, and sapoviruses were mostly detected. Comparison of the sequences of enterovirus and parechovirus strains from cotwins showed high identity, suggesting that twins harbor similar virome, in part linked to common exposure [13].

Later in age, persistent or intermittent shedding of resident enteric viruses from healthy people is well established. For example, human enterovirus (EV) [14] and parechovirus (HPeV) [15] are excreted by a large fraction of healthy children under the age of five. A 1-year NGS longitudinal study of the stool of two healthy infant siblings in samples taken at 1-week intervals demonstrated that viruses were continuously excreted [16]. The most frequently observed

viruses, in decreasing order, were anellovirus (*TTV* but also *TTMV*), picobirnavirus, and *HPeV types 1* and *6*. Bocavirus (*HBoV-1*), Adenovirus C and F, *Aichi virus*, astroviruses, and rotaviruses were less frequently detected. Surprisingly, other enteric viruses, such as noroviruses, coronaviruses, cardioviruses, cosaviruses, saliviruses, and sapoviruses, were not detected, although they are frequently detected in stool, indicating that the results of this survey only provide a first indication of the composition of the gut virome, which would benefit from the study of larger samples. Some viruses, including adenoviruses, anelloviruses, picobirnaviruses, parechoviruses, and *Human bocavirus*, were shed for months. These viruses are more likely to represent a significant portion of the normal human virome, owing to their ability to establish persistent infections. Among these viruses, another study showed that the presence of anelloviruses and circoviruses discriminate twin pairs that include one child with severe acute malnutrition from concordant healthy pairs, but not diseased versus healthy children within a given twin pair. So, it remains unclear if anelloviruses and circoviruses are markers of, or are responsible for, disease pathogenesis [17].

INTERPLAY BETWEEN GUT VIROME AND IMMUNE SYSTEM

The interplay between the virome and the immune system is far from being fully understood. Phage and eukaryotic viral particles are translocated, and independently of replication can activate innate immunity. Physical abundance of phages over eukaryotic human viruses, nevertheless, suggests that such activation is mainly driven by phages. Detection of corresponding viral nucleic acids within cells through several pattern-recognition sensors for RNA (RIG-I and Toll-like receptors TLR7 and TLR8) and DNA (TLR9, cyclic-GMP-AMP) should lead to expression of type interferon (IFN)-α and -β with pleiotropic activities, and inflammatory cytokines, such as interleukin 1 and 6. It has been suggested that such activation of innate immunity could have a positive effect against pathogenic infections [18], as already demonstrated for systemic persistent cytomegalovirus infection [19].

On the other side, it has been shown recently that, quite surprisingly, viruses could also play a beneficial role in the control of gut inflammation. Resident viruses recognized by TLR3 and TLR7 favor intestinal homeostasis through antiinflammatory cytokines as IFN-β secreted mainly by plasmacytoid dendritic cells [20].

Immune impairment has been shown to modify the enteric virome [21,22]. The enteric eukaryotic virome expands during pathogenic SIV infection of rhesus macaques [21]. In HIV-infected patients, there is a relationship between low peripheral CD4 T cell counts and alteration of the virome [23]. Interestingly, an association was evidenced between enteric adenovirus and both advanced HIV/AIDS [23], and SIV-infected macaques [21,22]. This suggests a mechanism where adenovirus replication promotes mucosal lesions and enteropathy leading to bacterial and bacteriophage translocation that promotes chronic immune stimulation.

The extent by which immunosuppressed patients harbor pathogenic and opportunistic viruses in the gut is currently unknown. It is likely, however, that a large number of viral species coexist and persist, and may be transiently cleared to reappear later, in the absence of protective immunity. Prospective longitudinal studies aimed at characterizing at steady state in healthy and immunosuppressed individuals the dynamics of the gut virome are warranted, not only to better characterize the driving forces that shape the gut microbiome, but also to monitor and ideally predict pathogenesis associated with these viruses, and ideally prevent their transmission to other susceptible hosts.

PATHOGENIC VIRUSES OF THE GUT VIROME: ENTEROPATHOGENIC VIRUSES ASSOCIATED WITH SYSTEMIC INFECTIONS

Foodborne viral infections are associated with the presence of infectious viral particles in the gut, and enteropathogenic viruses are therefore either transiently, and in a prolonged manner, present in the gut and part of the gut virome. As for foodborne bacterial infections, which may lead to local intestinal infection, or systemic disease, viruses whose portal of entry is the gut may be associated with either local or systemic disease. Their transmissibility depends on the amount of infectious viral particles released in the feces, and the duration of the viral release. Rotavirus and noroviruses, which replicate in intestinal epithelial cells, lead to local replication in the digestive tract, and very high-level fecal shedding, which is favored by the associated diarrhea, which mechanism can result, as for bacteria, from the action of a toxin [24] or a direct enteropathogenicity, and accounts for their high transmissibility. In contrast, enteroviruses, the leading cause of meningitis, translocate at the gut level and disseminate systemically. While their cell tropism is broader, their replication in mucosal tissue and their release in the intestinal lumen lead to fecal shedding, release in the environment and transmission. The infective potential of enteric viruses depends on the species itself, microbiota, and many host factors, such as age, nutritional status, and immune functions, which are themselves dependent on the microbiota. This underlines the complex interplay between the host, and the bacterial and viral parts of the microbiome, which only begins to be deciphered.

GUT AS A MAJOR SOURCE OF NEUROTROPIC VIRUSES, WHEN PATHOGENESIS AND SHEDDING IS FAVORED BY HUMORAL IMMUNE DEFICIENCY

The composition of the gut virome may vary according to multiple factors, such as exposure to viral species, the composition of the microbiota, and host factors, such as immune functions. It is well known by clinicians in charge of immunodeficient patients that diarrhea is a frequent symptom associated with opportunistic viral infections. Patients under immunosuppressive therapy for organ transplantation may present with chronic diarrhea associated with noroviruses and sapoviruses, leading to chronic fecal shedding, and nosocomial transmission [25]. Patients with congenital agammaglobulinema or profound hypogammaglobulinemia are prone to chronic and recurrent severe enterovirus infection, associated with encephalitis. These patients are frequently persistently colonized with enteroviruses, which can persist for years in the gut, and be associated with multiple episodes of CNS disease. In these patients, the occurrence of encephalitis in the absence of detectable enterovirus by RT-PCR methods in the CSF does not rule out their presence. NGS is indeed able to detect enterovirus genome when RT-PCR is negative, because of its intrinsic sensitivity, and its ability to detect variants that may not be amplifiable by PCR (our unpublished results). Interestingly, astroviruses, which are known to induce enteritis in human have also recently been associated with encephalitis in the context of profound humoral deficiency, underlining the intestine as a reservoir for neurotropic viruses [25a,25b,25c]. Another example of virus associated with gastrointestinal infections leading to meningitis and encephalitis are parechoviruses, and in particular human *Parechovirus 3* [26].

Yet the large number of enteric viruses species, their broad host tropism, the quasispecies nature of *Picornaviridae* within a given host, their capacity to recombine, their high transmissibility, and their interactions with the host microbiota and immune functions illustrate not only the fascinating evolutionary success of enteroviruses, but also the complexity of understanding all the factors to take into account to make sense of the contribution of a single viral species to the gut virome.

AN UNCERTAIN STATUS FOR DIET-DERIVED ANIMAL VIRUSES

The human gut virome is also composed of animal viruses transmitted by the oral route by consumption of contaminated food. *Hepatitis E virus* (HEV) is typically responsible for acute hepatitis in humans. Genotypes 1 and 2 are human specific but type 3 and type 4 have a reservoir in pigs [27]. The virus can be seen as a typical zoonotic virus in the context of acute infections. Nevertheless, long-term chronic infections have been described in immunocompromised patients, highlighting the importance of the host-immune status on virome composition and its potentially pathogenic properties [28,29]. Animal genotypes 3 and 4 frequently infect humans from the animal reservoir (up to 50% of the population is antibody positive in some areas [30]), and might be adapting to humans. The situation is puzzling for gyroviruses. The first gyrovirus in humans (HGyV), which is part of the *Circoviridae* family, was initially found by NGS at the surface of the skin of healthy people [31]. It was later shown that different gyroviruses, including a very similar virus, AGV2, are very prevalent in chickens and in human stools [32,33]. This owns to cross-species transmission of gyroviruses and their replication in humans, or alternatively to passive transit of animal viruses via food intake, as has been observed for plant viruses [6]. Within the *Circoviridae* family, the cycloviruses constitute a new genus and are found in the feces of humans and other animal species, pose similar questions as gyroviruses. [34]. It is noteworthy that gyroviruses harbor an apoptin gene, which encodes a protein that has the rare property of being specifically cytotoxic for cancer cells. Indeed, natural infection could be of benefit in controlling the development of tumor cells [35], in particular colon cancer (Table 21.1).

TABLE 21.1 Eukaryotic Viruses of the Human Gut

Family	Genus	References
Picornaviridae	*Enterovirus*	[36]
	Parechovirus	[36]
	Cardiovirus	[37]
	Salivirus	[38]
Picobirnaviridae	*Picobirnavirus*	[16]
Astroviridae	*Astrovirus*	[39]
Reoviridae	*Rotavirus*	
Caliciviridae	*Norovirus*	[40]
	Sapovirus	[41]
Adenoviridae	*Mastadenovirus* C and F, and others	[42]
Anelloviridae	*Anellovirus*	[16]
Cycloviridae	*Circovirus*	[43]
	Cyclovirus	[43]
Parvoviridae	*Bocavirus*	[44,45]

Recent reviews or informative papers are cited when available.

REFERENCES

[1] Mee ET, Preston MD, Minor PD, Schepelmann S. CS533 Study Participants. Development of a candidate reference material for adventitious virus detection in vaccine and biologicals manufacturing by deep sequencing. Vaccine 2016;34:2035–43.

[2] Cheval J, Sauvage V, Frangeul L, et al. Evaluation of high-throughput sequencing for identifying known and unknown viruses in biological samples. J Clin Microbiol 2011;49:3268–75.

[3] Wylie KM, Mihindukulasuriya KA, Sodergren E, Weinstock GM, Storch GA. Sequence analysis of the human virome in febrile and afebrile children. PLoS ONE 2012;7:e27735.

[4] Greninger AL, Chen EC, Sittler T, et al. A metagenomic analysis of pandemic influenza A (2009 H1N1) infection in patients from North America. PLoS ONE 2010;5:e13381.

[5] Chiu CY. Viral pathogen discovery. Curr Opin Microbiol 2013;16(4):468–78.

[6] Zhang T, Breitbart M, Lee WH, et al. RNA viral community in human feces: prevalence of plant pathogenic viruses. PLoS Biol 2006;4:e3.

[7] Gia Phan T, Phung Vo N, Sdiri-Loulizi K, et al. Divergent gyroviruses in the feces of Tunisian children. Virology 2013;446:346–8.

[8] Ogilvie LA, Jones BV. The human gut virome: a multifaceted majority. Front Microbiol 2015;6:918.

[9] Abeles SR, Robles-Sikisaka R, Ly M, et al. Human oral viruses are personal, persistent, gender-consistent. ISMEJ 2014;8:1753–67.

[10] Duerkop BA, Clements CV, Rollins D, Rodrigues JLM, Hooper LV. A composite bacteriophage alters colonization by an intestinal commensal bacterium. Proc Natl Acad Sci USA 2012;109:17621–6.

[11] Minot S, Sinha R, Chen J, et al. The human gut virome: inter-individual variation and dynamic response to diet. Genome Res 2011;21:1616–25.

[12] Reyes A, Haynes M, Hanson N, et al. Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature 2010;466:334–8.

[13] Lim ES, Zhou Y, Zhao G, et al. Early life dynamics of the human gut virome and bacterial microbiome in infants. Nat Med 2015;21:1228–34.

[14] Witsø E, Palacios G, Cinek O, et al. High prevalence of human enterovirus a infections in natural circulation of human enteroviruses. J Clin Microbiol 2006;44:4095–100.

[15] Kolehmainen P, Oikarinen S, Koskiniemi M, et al. Human parechoviruses are frequently detected in stool of healthy Finnish children. J Clin Virol 2012;54:156–61.

[16] Kapusinszky B, Minor P, Delwart E. Nearly constant shedding of diverse enteric viruses by two healthy infants. J Clin Microbiol 2012;50:3427–34.

[17] Reyes A, Blanton LV, Cao S, et al. Gut DNA viromes of Malawian twins discordant for severe acute malnutrition. Proc Natl Acad Sci USA 2015;112:11941–6.

[18] Duerkop BA, Hooper LV. Resident viruses and their interactions with the immune system. Nat Immunol 2013;14:654–9.

[19] Barton ES, White DW, Cathelyn JS, et al. Herpesvirus latency confers symbiotic protection from bacterial infection. Nature 2007;447:326–9.

[20] Yang JY, Kim MS, Kim E, et al. Enteric viruses ameliorate gut inflammation via Toll-like receptor 3 and Toll-like receptor 7-mediated interferon-β production. Immunity 2016;44:889–900.

[21] Handley SA, Thackray LB, Zhao G, et al. Pathogenic simian immunodeficiency virus infection is associated with expansion of the enteric virome. Cell 2012;151:253–66.

[22] Handley SA, Desai C, Zhao G, et al. SIV infection-mediated changes in gastrointestinal bacterial microbiome and virome are associated with immunodeficiency and prevented by vaccination. Cell Host Microbe 2016;19:323–35.

[23] Monaco CL, Gootenberg DV, Zhao G, et al. Altered virome and bacterial microbiome in human immunodeficiency virus-associated acquired immunodeficiency syndrome. Cell Host Microbe 2016;19:311–22.

[24] Lorrot M, Vasseur M. How do the rotavirus NSP4 and bacterial enterotoxins lead differently to diarrhea? Virol J 2007;4:31.

[25] Roos-Weil D, Ambert-Balay K, Lantemier F, et al. Impact of *Norovirus/Sapovirus*-related diarrhea in renal transplant recipients hospitalized for diarrhea. Transplantation 2011;92:61–9.

[25a] Frémond ML, Pérot P, Muth E, Cros G, Dumarest M, Mahlaoui N, Seilhean D, Desguerre I, Hébert C, Corre-Catelin N, Neven B, Lecuit M, Blanche S, Picard C, Eloit M. Next-generation sequencing for diagnosis and tailored therapy: a case report of astrovirus-associated progressive encephalitis. J Pediatric Infect Dis Soc 2015;4(3):12.

[25b] Brown JR, Morfopoulou S, Hubb J, Emmett WA, Ip W, Shah D, Brooks T, Paine SM, Anderson G, Virasami A, Tong CY, Clark DA, Plagnol V, Jacques TS, Qasim W, Hubank M, Breuer J. Astrovirus VA1/HMO-C: an increasingly recognized neurotropic pathogen in immunocompromised patients. Clin Infect Dis 2015;60(6):881–8.

[25c] Naccache SN, Peggs KS, Mattes FM, Phadke R, Garson JA, Grant P, Samayoa E, Federman S, Miller S, Lunn MP, Gant V, Chiu CY. Diagnosis of neuroinvasive astrovirus infection in an immunocompromised adult with encephalitis by unbiased next-generation sequencing. Clin Infect Dis 2015;60(6):919–23.

[26] Renaud C, Harrison CJ. Human *Parechovirus* 3: the most common viral cause of meningoencephalitis in young infants. Infect Dis Clin North Am 2015;29:415–28.

[27] Smith DB, Purdy MA, Simmonds P. Genetic variability and the classification of hepatitis E virus. J Virol 2013;87(8):4161–9.

[28] Lhomme S, Abravanel F, Dubois M, et al. Hepatitis E virus quasispecies and the outcome of acute hepatitis E in solid-organ transplant patients. J Virol 2012;86:10006–14.

[29] Koning L, Pas SD, de Man RA, et al. Clinical implications of chronic hepatitis E virus infection in heart transplant recipients. J. Heart Lung Transplant 2013;32:78–85.

[30] Kamar N, Bendall R, Legrand-Abravanel F, et al. Hepatitis E. Lancet 2012;379:2477–88.

[31] Sauvage V, Cheval J, Foulongne V, et al. Identification of the first human gyrovirus, a virus related to chicken anemia virus. J Virol 2011;85:7948–50.

[32] Phan TG, Li L, O'Ryan MG, et al. A third gyrovirus species in human faeces. J Gen Virol 2012;93:1356–61.

[33] Chu DK, Poon LL, Chiu SS, et al. Characterization of a novel gyrovirus in human stool and chicken meat. J Clin Virol 2012;55:209–13.

[34] Delwart E, Li L. Rapidly expanding genetic diversity and host range of the Circoviridae viral family and other Rep encoding small circular ssDNA genomes. Virus Res 2012;164:114–21.

[35] Los M, Panigrahi, Rashedi I, et al. Apoptin, a tumor-selective killer. Biochim Biophys Acta 2009;1793:1335–42.

[36] de Crom SCM, Rossen JWA, van Furth AM, Obihara CC. Enterovirus and parechovirus infection in children: a brief overview. Eur J Pediatr 2016;175(8):1023–9.

[37] Himeda T, Ohara Y. Saffold virus, a novel human Cardiovirus with unknown pathogenicity. J Virol 2012;86:1292–6.

[38] Yip CC, Lo KL, Que TL, et al. Epidemiology of human parechovirus, Aichi virus and salivirus in fecal samples from hospitalized children with gastroenteritis in Hong Kong. Virol J 2014;11:182.

[39] Kapoor A, Li L, Victoria J, et al. Multiple novel astrovirus species in human stool. J Gen Virol 2009;90:2965–72.

[40] Ahmed SM, Hall AJ, Robinson AE, et al. Global prevalence of *Norovirus* in cases of gastroenteritis: a systematic review and meta-analysis. Lancet Infect Dis 2014;14:725–30.

[41] Koopmans M, Vinj J, Duizer E, de Wit M, van Duijnhoven Y. Molecular epidemiology of human enteric caliciviruses in The Netherlands. Novartis Found Symp 2001;238:197–214.

[42] Kosulin K, Geiger E, Vécsei A, et al. Persistence and reactivation of human adenoviruses in the gastrointestinal tract. Clin Microbiol Infect 2016;22. 381.e1-388.e1.

[43] Li L, Kapoor A, Slikas B, et al. Multiple diverse circoviruses infect farm animals and are commonly found in human and chimpanzee feces. J Virol 2012;84:1674–82.

[44] Ong DSY, Schuurman R, Heikens E. Human bocavirus in stool: a true pathogen or an innocent bystander? J Clin Virol 2016;74:45–9.

[45] Paloniemi M, Lappalainen S, Salminen M, et al. Human bocaviruses are commonly found in stools of hospitalized children without causal association to acute gastroenteritis. Eur J Pediatr 2014;173:1051–7.