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Review

The virome in hematology—Stem cell transplantation and beyond

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ABSTRACT

The virome has been recently studied in hematology and mostly in the setting of allogeneic hematopoietic stem cell transplantation. However, in hematology (as in the setting of nonhematological disorders) the study of the microbiome (that indeed includes the virome) is a growing field. The overall field is moving beyond species catalogue to the understanding of the complex ecological relationship that microbes have with each other and with their host. Here we review the existing literature on the virome in transplant recipients and in other settings, and discuss potential applications of the virome study in hematology.

Introduction

Advances in molecular biology have provided an unprecedented opportunity to study the human virome. Transplant recipients and other immunocompromised hosts are at particular risk for viral reactivation, viral infection, and virus-related pathology (reviewed in [1,2]). After allogeneic transplantation, immunosuppression used to prevent and treat graft-versus-host disease (GvHD) results in a heightened and prolonged risk for opportunistic viral infections. Furthermore, in hematopoietic cell transplantation (HCT), conditioning regimens, delayed immune recovery, Human leukocyte antigen (HLA)-mismatch, among other factors contribute to susceptibility to virus-related disease. Pretransplant donor and recipient infection-related immune status, in the setting of prior immunosuppressive therapy for underlying diseases that prompt the need for transplantation, add to the risk in transplant recipients (reviewed in [3-5]). After a short summary of viruses in allogeneic HCT, we summarize herein advances in the study of the human virome, review the existing literature on the virome in transplant recipients, and discuss potential applications of the virome and next generation sequencing (NGS)-based findings in hematology.

Viruses and allogeneic stem cell transplantation

The recipient's susceptibility to viral infection is correlated directly with levels of immune deficiency (induced by GvHD and its treatment) and with immune reconstitution, and with previous immunization of the recipients and of the donor against viral agents. The immune deficiency after HCT is extremely complex and only partially decrypted in human; main cellular subsets involved in viral clearance include T, B, and NK cells (Fig. 1) (reviewed in [6,7]). The immune reconstitution against viruses is also influenced by the stem cell source (delayed reconstitution after cord-blood cell transplantation), T-cell depletion of the graft (and use of antithymocyte globulins or Campath) and might be influenced by the intensity of the conditioning regimen. Table 1 summarizes the main virus families and species associated with infection, or infectious disease post allogeneic HCT. However, the interaction of viruses with the transplanted host cannot be summarized only by infection/reactivation; there is compelling evidence that some virus's proteins may act through molecular mimicry or epitope spreading in the initiation or the amplification of the allogeneic reaction leading to GvHD.

Major advances have been made in the past 25 years in the diagnosis of viral infection. Initially based on culture, current methods for virus testing now rely on single target nucleic acid amplification tests by Polymerase chain reaction (PCR). PCR tools are rapid and more sensitive than viral culture and antigen tests and are the standard of care for screening, diagnosing, and monitoring viral infections in transplant recipients. While transplant recipients are at risk for multiple opportunistic viral infections, transplant-specific multiplexed syndromic panels are not currently available and a

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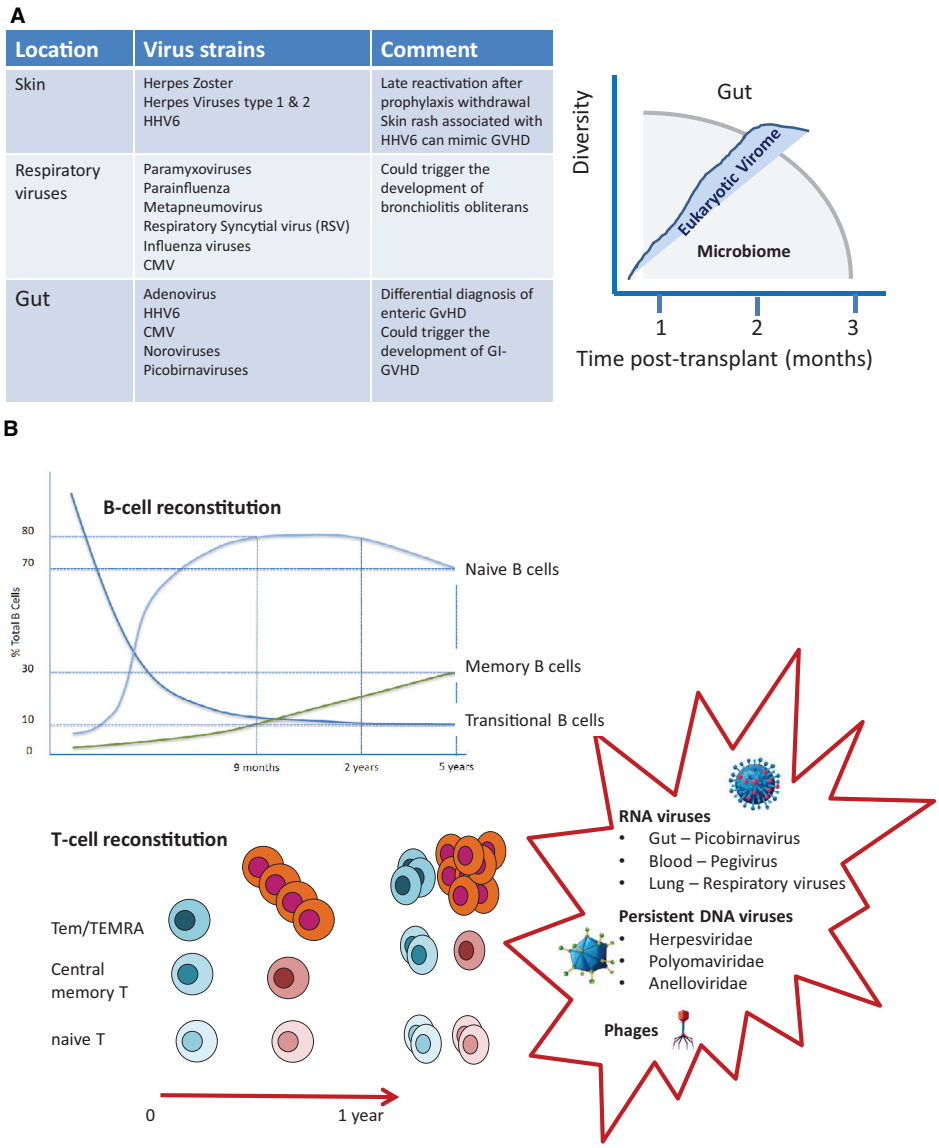

Fig. 1. (A) schematic representation of the microbiome and virome diversity over time post-transplantation The gut microbiota diversity decrease post-transplant while there is a bloom of eukaryotic virus reactivation /infection early post-transplant. (B) The bloom of viruses must be put within the context of the immune reconstitution; T-cell reconstitution is characterized by early TEMRA expansion with latter reconstitution of the naïve and memory cell subsets. The B-cell reconstitution is even slower with naïve and memory cell subsets reconstitution taking years.

Table 1
Main virus strains associated with infection and diseases post-transplantation according to main clinical sites.

Location	Virus strains	Comment
Skin	Herpes Zoster	Late reactivation after prophylaxis withdrawal
	Herpes Viruses type 1 & 2	Skin rash associated with HHV6 can mimic GVHD
	HHV6	
Respiratory viruses	Paramyxoviruses	Could trigger the development of bronchiolitis obliterans
	Parainfluenza	
	Metapneumovirus	
	Respiratory Syncytial virus (RSV)	
	Influenza viruses	
	CMV	
Gut	Adenovirus	Differential diagnosis of enteric GvHD
	HHV6	Could trigger the development of GI-GVHD
	CMV	
	Noroviruses	
	Picobirnaviruses	

large number of potentially disease-causing viruses remain uncharacterized [8]. Multiplexed real-time PCR assays are restricted to a limited number of viruses per assay, due to fluorescent reporter molecule availability, the complexity involved with optimizing primer and probe concentrations, and the difficulty with quantitating multiple targets in a single reaction [8].

Viruses lack a common gene that can be easily targeted for sequencing and differentiation among virus species. To overcome this, metagenomic approaches are employed to sequence the breadth of nucleic acid content in a sample. However, clinical samples are heavily dominated by nucleic acids of human origin and viral genomes represent a minute proportion of the total nucleic acids (see infra). Sequencing at higher depth can overcome some of these challenges, but at higher costs. Despite this, metagenomic NGS approaches can still fail to detect viral nucleic acids that have been demonstrated to be present by a targeted molecular assay [8].

The advent of the virome study

In his seminal review in the Journal Cell, Virgin stated that; "*The virome contains the most abundant and fastest mutating genetic elements on Earth. The mammalian virome is constituted of viruses that infect host cells, virus-derived elements in our chromosomes, and viruses that infect the broad array of other types of organisms that inhabit us. Virome interactions with the host cannot be encompassed by a monotheistic view of viruses as pathogens.*"

The mammalian virome [9] includes viruses that infect eukaryotic cells; bacteriophages that infect bacteria; and virus-derived genomic segments integrated into the human genome (endogenous retroviruses, endogenous viral elements) [9,10]. Eukaryotic viruses and bacterial viruses both have lytic life cycles in which the host cell is destroyed during viral replication and latent life cycles in which the virus stably resides within a living cell. During latency the viral genome is either integrated into the host chromosome (eg, prophages) or as an episome (eg, herpesviruses) until the need arises to reactivate and become infectious.

In mammals, the virome inhabits all mucosal surfaces. After clearance of acute viral infection, viruses can also persist in systemic niches especially in neurons, hematopoietic cells, stem cells, vascular endothelial cells. This is a significant difference between the virome and the bacterial microbiome, members of which are not known to persist systemically. The size of the mammalian virome is not known. Although our own cells are outnumbered about 10-fold by our bacterial microbiome, the number of viruses may be still 10-fold higher [11]. Human feces alone contain ca. 108 to 109 viruses per gram [11,12]. Additionally, the mammalian virome is continuously "updated" through rapid evolution of viruses [13] and through exposure to viruses [9,10]. The gastrointestinal tract is characterized by the apposition of the mucosal immune system with myriad of viruses and other microorganisms. Most of these viral particles correspond to phages with extreme inter-individual variation [14,15]. Humans and other mammals harbor animal viruses in the intestine that remain to be characterized [1,2,16]. Metagenomics studies of the intestine and other anatomical sites thus represent attractive tools to detect new viruses.

The virome is far more than a source of disease. Asymptomatic hosts carry multiple viruses, and interactions between the virome and the host do not always involve the death of virus-infected cells. This concept has led to the emerging paradigm that the virome can influence the host in profound ways independent of classical viral disease [10]. Furthermore, viruses are frequently inherited from parents very early in life by transmission soon after birth, [9]. The immune system is in dynamic equilibrium with all components of the microbiome, including the virome, in the asymptomatic state. Many components of the mucosal microbiome stimulate low-level immune responses in asymptomatic individuals. Including the virome that continuously stimulates the immune system. We are constantly re-infected by viruses that repeatedly stimulate mucosal host responses, with potential consequences for host resistance to other infections. Increasingly, these viruses are recognized to persistently infect a proportion of our population. This chronic carrier state for viruses has likely been underestimated due to the lack of tools to detect and quantify extremely diverse members of the virome [10]. In addition to these mucosal components of the virome, the immune system is continuously stimulated by chronic systemic viruses. This aspect of host-microbiome interactions appears specific to the virome. It is estimated that healthy humans harbors >10 permanent chronic systemic viral infections [9]. Viruses that fall into the categories of commensal and opportunistic pathogen include many that are detected in the majority of the adult human population, such as members of the herpesvirus, polyomavirus, adenovirus, circovirus, and anellovirus families [9,17-19]. These infections drive continuous activation of the immune system as they reactivate from latency or continuously replicate. The role of such active immune surveillance in control of these systemic additions to our individual genetic identities is clear from the fact that these viruses often cause disease when immuno-surveillance fails as, in the setting of transplant-associated immunodeficiency [10].

Methods for deciphering virome

As there is no common gene across all the different virus families, unbiased strategy with random amplification is mandatory to detect any kind of virus in different compartments and body fluids. As virus genome size is hundred to ten thousand times smaller than bacteria or eukaryote genomes, sensitivity to detect viruses in poly-microbial human samples is much lower than that for bacteria. Therefore, particular attention in sample preparation, next generation sequencing library generation and sequencing depth has to be given to get a representative picture of inhabiting viruses.

To enhance sensitivity several approaches may be considered. One strategy is to increase the proportion of viral particles, either with density gradient ultra-centrifugation or by using filtration to remove bacteria and human cells. Another, and complementary, approach is to degrade free contaminating bacterial and human DNA with nuclease digestion that leaves viral genomes protected by the viral capsids. The stability of capsid was also used to specifically degrade phospholipid membranes of bacteria and eukaryotic cells by cycles of freezing and thawing before nuclease digestion. Those strategies may be combined to increase rates of both viral like particles and viral nucleic acids.

As virome includes both phages and eukaryotic viruses, amplification methods have to target different nucleic acids types such as single strand or double strand, linear or circular, RNA or DNA genomes. Some studies claiming to analyze virome used methods that preferentially amplified circular DNAs. Especially, as most phages are DNA viruses, some studies did not attempt to amplify RNA genomes [14,20]. To overcome cellular DNA and ribosomal RNA contamination and to amplify either DNA or RNA viral genome, specific RNA and DNA preparation may be considered: (i) removal of cellular DNA by specific binding of methylated DNA followed by DNA library amplification for DNA viruses, (ii) DNAse treatment and ribosomal RNA depletion followed by random cDNA synthesis and DNA library amplification for RNA viruses. Some studies have compared different methods of virus purification to assess efficacy to remove cells and reproducibility for qualitative and quantitative detection of the intestinal virome. The results suggest that density gradient centrifugation with cesium chloride while efficient for cell depletion was not the most reliable technique to provide representative virome description and reproducible quantitative results [21].

Despite dramatic increase in the rate of viruses detected with these strategies, human and bacteria DNA remain the most representative reads sequenced in samples such as feces. Although the yield of viruses exceeds 10^9 particles per gram of stools, viral sequences may remain as low as one percent of total reads sequenced. In stool samples eukaryotic viruses may represent less than 1% of viral sequences. Therefore, the depth of sequencing is a critical issue to be able to detect eukaryotic viruses. With the current methods, a minimum depth of ten millions of reads should be considered to analyze the gut virome.

To overcome the poor sensitivity to detect viruses by metagenomics, a technique using ~2 million of specific probes targeting viral nucleic amplicons has shown a 100- to 10,000-fold increase in eukaryotic viral reads from blood and tissue homogenates compared to previously mentioned virus enrichment strategies [22]. If such an approach is powerful to decipher eukaryotic viruses, this misses the main part of the virome and likely the understanding of interactions with bacterial microbiota. In addition, the probe set is designed with available sequences of known viruses. Even though, it was reported to detect divergent viruses, it seems unlikely to capture and thus to report unknown families or species.

Last but not the least critical point is the bio-informatics pipelines used to decipher viral taxa. Virome characterization and dynamics require algorithms that exclude misidentified reads [23]. Several pipelines for virome analysis or viral discovery have been published. They differ from one to each other according to their speed, their ability to identify divergent strains and integrate updated databases. The choice of a pipeline has a clear impact on the final data output [24-26].

Even with the use of tools enabling the identification of divergent viruses, a significant proportion of sequences remain unclassified, especially in stool samples [27]. Depending on the sample, viral dark matter can make up from anywhere between 40 and 90% of sequences [16]. Thus according to the sample tested and the question raised, a special attention has to be drawn to reduce the extent of viral dark matter.

The virome after allogeneic HCT

Gut virome

Very few studies have reported results of virome analysis with a metagenomic approach after Hematopoietic stem Cell Transplantation (HSCT). We previously reported a longitudinal analysis of the gut virome after HSCT in 44 patients [23]. The results showed significant differences in the gut virome between patients with or without graft versus host disease. In this study a median of 29 million reads were sequenced per stool sample. Despite several steps to remove cell and bacteria DNA, the median percentage of viral reads overall was 0.22%. Eukaryotic viruses accounted for 1.76 % of total viral reads with a wide range between 0 and 99.9%. Previous reports in population of patients with specific disease, including diarrhea and flaccid paralysis already showed that eukaryotic viruses could dominate the gut virome [28,29]. In our series, eukaryotic viruses exceeded phages in 15% of samples. Phage analysis showed that individuals with enteric GvHD had both decreased richness and a higher abundance of *Microviridae*, a single strand DNA family, prior to and during the first few weeks following transplantation. Conversely, the abundance of Microviridae decreased significantly over time in patients with enteric GvHD.

Among vertebrate viruses, a total of 20 families, 52 genera, and 347 species were identified. The most frequent viral families observed in stool samples were Anelloviridae (37.8% of samples), Polyomaviridae (14.9%), Picobirnaviridae (13.9%), and Herpes viridae (10.9%). Persistent DNA viruses (Anelloviridae, Herpes viridae, Papilloma viridae, and Polyomaviridae) were detected in half of samples (49.8%). Exogenous viruses were less frequently detected (35.8% of samples). Overall, persistent viruses yielded higher read numbers than exogenous viruses. The proportion of vertebrate viruses in stool samples increased progressively during the weeks following transplantation in patients with or without enteric GVHD.

Interestingly, the rates of detection and the number of reads of persistent DNA viruses in stool samples, especially those of Herpes viridae family, were increased after week 3 following HSCT in patients with enteric GVHD. In addition, more frequent detection of cytomegalovirus (viral load > 3.5 log10 IU/ml) and human herpesvirus 6 (HHV-6) was seen in blood from individuals with enteric GVHD. However these observations were not predictive of subsequent development of enteric GvHD.

Persistent viruses might have been reactivated by GVHD-associated inflammation and/or immunosuppression. In contrast, the reactivation of persistent DNA viruses in the gut during weeks 1 to 3 after transplantation was seen mainly in individuals without enteric GVHD. This could suggest a potential protective role for persistent DNA viral replication in the regulation of gut inflammation predisposing to enteric GVHD, as previously postulated in a mouse model of IBD [30]. Further investigation is needed to understand the role of persistent virus in immune gut homeostasis.

The most surprising finding of this work was the association of picobirnavirus infection with subsequent development of GVHD. The detection of PBV was predictive of the occurrence of both enteric and of any type of GvHD. In all patients who eventually developed enteric GVHD, the detection of PBV occurred before disease onset. Higher levels of fecal calprotectin and α1-antitrypsin, 2 biomarkers associated with severity and response to corticosteroids in individuals with GvHD [31-33], were observed in stool samples positive for PBV suggesting an association between PBV and gut inflammation. Repeated detection of PBV in successive samples was observed in 12 patients.

Data on the pathogenicity of Picobirnaviridae remain limited and inconclusive. PBV have been poorly reported in the literature as their detection has been hampered by the high genetic variability of the virus. Previous reports relied mainly on the visualization on polyacrylamide gel of the 2 double strand RNA segments. Attempts of generic PCRs failed to detect a broad range of diverse PBV. The advent of NGS has recently unveiled their frequent detection in feces of various vertebrate animals [22,34,35]. These results confirm some previous reports showing that PBV may persist in the gut over time, as in HIV patients experiencing diarrhea [36]. Whether persistent replication of PBV may promote gut inflammation or trigger dysregulated immune response from the mucosa or donor T cells needs further work.

Blood virome

Vu et al [37] analyzed the plasma virome of 40 allogeneic HSCT patients 1 month after transplantation. In plasma specimens collected at a median of 33 days after transplantation, three samples were negative for any virus sequences. Thirty-2 (80%) and 19 (47.5%) had at least 1 detectable DNA and RNA virus sequence, respectively. Among the 37 positive patients, the number of detected viruses per sample ranged from 1 to 5 viruses. The most frequent DNA viruses were polyomaviruses (50% of samples positive), anelloviruses (40%), herpesviruses (35%), human papillomaviruses (27.5%), and adenoviruses (5%). The authors do not report (whether or not) differences of DNA virus detection was detected according to GvHD status or other clinical outcomes. The DNA viruses found in blood are similar to those reported in stool and confirmed the high rate of reactivation of persistent DNA viruses after HSCT in different compartments.

The authors also report the detection of RNA viruses. The most frequent was pegivirus (HPgV; 35%). Sequences of hepatitis C virus (5%) and Rubella virus (2.5%) were also reported. Because HPgV was highly prevalent, the authors completed their study by a 1 year screening of 122 patients by a specific real-time PCR assay. Fifty-one patients had plasma positive for HPgV with persistent detection up to 1 year after transplantation. Most of the patients (47) were positive before transplant. HPgV was detected only after HCST in 7 patients. Median viral loads ranged between 1.7 10^4 and 5.2 10^5 copies/mL of plasma. The comparison between patients with or without HPgV infection at time of transplantation did not show any significant difference regarding infections, engraftment, survival, GvHD, relapse, or immune reconstitution. HPgV detection was associated 12 months after transplantation, with lower numbers of CD4 and CD8 and higher NK cell counts and with lower proportions of CD8 TEMRA T cells. However, these differences did not persist after multivariable analysis.

These two studies highlight frequent reactivation of persistent DNA viruses after HSCT. Although well designed, the limited number of patients tested warrant independent validation before drawing any definitive conclusion. The 2 studies also raise unexpected high frequency of RNA viruses. Human pegivirus and picobirnavirus are not associated with organ disease. PBV have been reported in patients with diarrhea but they are also detected in stool of asymptomatic patients. Detection of HPgV in blood was found also in healthy blood donors (1%-5%) in developed countries and transmission through blood transfusion is known [38,39]. Even though HPgV primarily infects hematopoietic stem cells and is found in T, B, and NK cells and in monocytes and that an immunomodulatory impact is suspected, HPgV has not been associated with any defined hematological or immunological disorder. In the 2 aforementioned studies persistence was reported over weeks in stool for PBV and months in blood for HPgV.

These observations underline the need to consider both DNA and RNA viruses and the use of unbiased approaches to unravel the complex interplay between viruses, microbiota, and the host and to understand their potent impact on clinical outcomes after HSCT.

Virome in CSF

Pou et al explored [40] the virome in cerebrospinal fluid (CSF) of 35 HSCT patients with neurological complications by using also unbiased NGS. HSCT patients had mainly headache or seizures and nine of them presented with a raised white cell count in the CSF at time of symptoms. Thirty controls subjects were also tested. Controls were nontransplanted children, aged 0 to 17 years, who had a lumbar puncture because of suspected CNS infection but without any etiological agents found among enterovirus, HSV, VZV, TBE, and Borrelia Burgdorferi. Approximately, 23 and 40 million paired-end reads were generated from the control and patient pools respectively. This resulted in a set of 3 million nonhuman reads from the control subjects and 10 million reads from NC patients. Patients with NC harbored higher number of viral reads matching anelloviruses. No link between viral detection and symptoms could be determined. These results are in agreement with the blood and gut virome studies after HSCT where frequent detection of anelloviruses has been reported. The CSF study thus confirms that replication of anelloviruses in a context of deep immunosuppression occurs in every body compartment.

The virome beyond allogeneic HCT

Beyond HSCT, the virome has been investigated in solid organ transplant recipients. De Vlaminck analyzed the virome in blood of Heart and Lung transplant patients. In this study, 656 samples from 96 patients (41 adult heart transplants, 24 pediatric heart transplants, 31 adult lung transplants) were tested up to 14 months after transplantation [41]. Overall nonhuman sequences were mainly viruses (73%) followed by bacteria (25%) and fungi (2%). Among viruses, the predominant family was Anelloviridae, accounting for 68% of the total population. As observed in HSCT other blood viruses were mainly persistent DNA viruses, Herpes viridae (13%), and Polyomaviridae (5%). Phages represented a minor population, with the clade Caudovirales being the most prevalent (5%). The virome composition and dynamics was closely related to immunosuppressive and antiviral treatments. The total viral load increases with immunosuppression, whereas the bacterial composition and load remained relatively stable over time. Herpesviruses and Caudovirales dominated the virome when patients received a low dose of valganciclovir and tacrolimus. In contrast, Anelloviridae became the main component of the plasma virome when patients receive high dose of immunosuppressants and antivirals. Finally the anellovirus loads were inversely correlated with the risk of acute organ rejection. The authors suggest that total burden of anelloviruses could be used as a surrogate marker of the overall state of immunosuppression and to predict the risk of graft rejection in solid organ transplant recipients. This study did not suggest that the virome *per se* could modulate pathogen specific or alloreactive immune responses.

Another study conducted in lung transplant patients showed a bloom of anelloviruses in upper and lower respiratory secretions compared to healthy controls and HIV infected individuals without lung disease or respiratory symptoms [42]. The raise of anelloviruses in lung transplant patients was associated with bacterial dysbiosis. Of sequences assigned to viruses, a large majority (>68%) belonged to the Anelloviridae family. Other eukaryotic viruses included Epstein-Barr virus, Human Herpesvirus 7 and Human Papillomavirus, all with low viral loads. No correlation was found between respiratory virome, anellovirus increase and any clinical outcome or graft rejection.

Similar results were reported in the urine of kidney transplant patients [43]. The virome in the urine of 7 patients with positive (BK+) and 15 with negative BK virus PCR (BK-) were analyzed by metagenomic shotgun sequencing. Sequences-matching with the BK virus was found in the 2 groups. BK virus was the predominant virus in the BKV+ group. Other viruses were detected, including the human polyomavirus JC (JCV) and anelloviruses. A higher genetic diversity among BK virus strains was seen in the BK+ group. Other virus families detected were Herpes viridae, Adenoviridae, Papilloma viridae.

Altogether after transplant of either hematopoietic stem cell or solid organ, there is a dramatic expansion of persistent DNA viruses, dominated by anelloviruses. If high levels of replications of herpesviruses and polyomaviruses have been associated with specific end-organ disease, the true impact of changes in virome composition and dynamics remains elusive. The number of studies remains limited and, in most of reports, population sizes were quite small. Further investigations need to confirm these first observations and attempt to go beyond just temporal associations between viruses and clinical or biological changes.

The virome in immune disorders and inflammatory diseases

The analysis of the gut virome in HSCT patients revealed links between the virome composition and the occurrence of GvHD. Previous virome analyses in inflammatory bowel diseases showed also significant alteration in the gut virome. In three independent cohorts of patients with ulcerative colitis (UC) or Crohn's disease (CD) and household controls without inflammatory bowel disease in UK and USA, Norman et al showed an inverse correlation

between the Caudovirales and Microviridae [20]. A significant expansion of Caudovirales was observed in patients with CD and UC compared to household controls. The virome of CD and UC patients were disease-, and cohort-specific. Virome alterations did not seem to be secondary to changes in bacterial populations. Of note, methods for sample preparation and sequencing depth did not enable to investigate eukaryotic viruses.

In a mouse model of colitis induced by dextran sulfate sodium Yang et al [30] found that a cocktail of antiviral drugs (ribavirine, lamvidune, acyclovir) worsened colitis. Antivirals altered the abundance and composition of gut viral and bacterial communities. The control of viruses by antivirals actually disrupted TLR3 and TLR7 activation induced by viruses. The use of Toll Like Receptors (TLR) 3 (TLR3)(Poly I:C) and TLR7 (Imiquimod) agonists ameliorated gut inflammation. In a cohort of patients CD or UC a combination of genetic variants of TLR3 and TLR7 had a higher cumulative rate of hospitalization [30]. Overall, the results suggested that gut resident viruses induce TLR3 and TLR7 signaling pathways which played a significant role in the regulation of intestinal inflammation. The viral families mainly impacted by antiviral drugs were not detailed and most of viral sequences remained unclassified. Thus differences in virome communities before and after antiviral treatments remain to be elucidated.

Experimental design and methods significantly differ between the 2 studies and results seem opposite. Actually, both depict complex interactions between gut viruses and host immunity and suggest that enteric viruses are, as bacteria, a key component of immune homoeostasis. Recent studies on asymptomatic reoviruses indicate that gut infection in predisposed genetic settings could trigger gluten intolerance, by upregulating Th1 inflammatory response and inhibiting proliferation of Treg cells [44]. Therefore, both resident viruses and exogenous viruses need to be considered.

The field of virome is in fast and wide expansion. Many unclassified sequences, especially in the intestine environment, required to be characterized and assigned. Recently, a new family named Redondoviridae was discovered [45]. Those viruses were mainly identified in upper respiratory tract samples and less frequently in feces. Redondoviridae detection was associated with periodontal disease, and patients admitted in a medical intensive care had high in respiratory samples. This new virus family need to be investigated in transplant patients and virome analyses in such settings should be run again with databases integrating Redondoviridae.

Viruses may present direct toxicity into tissues and their detection may be contemporary to tissue damages. However they may also trigger delayed impacts and may have disappeared at the time of disease occurrence. In the field of HSCT, lung dysfunctions, including bronchiolitis obliterans syndrome, may be induced by viral infections [46-48]. Respiratory function declines may occur a long time after infection and one may hypothesize that viruses triggered a dysregulated immune response. To circumvent such issues, longitudinal studies of virome would be needed along with the investigation of host immune response profiles by the use of transcriptomics or metabolomics [49].

Conclusion & perspectives

Alterations of the human microbiome, the virome and probably of the fungome have generated huge interest in the scientific community in the past 10 years [50]. In the setting of Hematology, studies have mainly focused on the role of the microbiota after hematopoietic HCT [51,52] and few attention have been paid to the role of the virome. Host-microbiome/virome have both localized and systemic effect. The interplay between viruses/bacteria/host tissues/acquired and innate immunity is highly complex (Fig. 1B) and only very recently integrative studies aiming integrating different “omics” tools have been published [53]. Such integrative approaches and search for new pathogenic agents will most probably be developed in Hematology, not only in the field of HCT, but far beyond in non-malignant and malignant hematopoietic disorders.

Declaration of Competing Interest

None relevant to this manuscript.

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