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# INTRICATE INTERPLAY OF BACTERIA, VIRUS & OUR IMMUNE SYSTEM.

**Adhish Mishra[1], Adrian Shylla Handa[2], Dr. Anil Kumar Handa[3*]**

*[1](MSc Virology, Amity Institute of Virology and Immunology, Noida, Uttar Pradesh, India), Email id: adhishkisu.mishra@gmail.com, ORCID: 0009-0000-1648-2786*
*[2](B.Tech, Amity Institute of Virology and Immunology, Noida, Uttar Pradesh, India), Email id: andyshyllahanda@gmail.com*
*[3*](Professor, Department of Plant Pathology, Dr Y. S. Parmar University of Horticulture & Forestry, Nauni, Himachal Pradesh, India) Email id: anilhanda@msn.com*
*Address: 23, Serenity, Farm Scientist Colony, Khundidhar, P.O Shamti, Himachal Pradesh, India*

**Corresponding Author:*
*Email id: adhishkisu.mishra@gmail.com*

**ABSTRACT**

Over the past few years, the utilization of metagenomic sequencing techniques and analogous methodologies has significantly advanced our comprehension of the human virome's diversity and its implications for human health. The human organism is inhabited by a plethora of microbial communities, known collectively as the microbiome. Of comparable significance, yet seldom acknowledged, is the presence of a vast array of viruses, which are collectively termed the 'virome'. Viruses are widely regarded as the most prolific and diverse biological entities on Earth, with an estimation of 10^31 particles. The human virome harbours this vastness and intricacy, comprising approximately 10^13 particles per individual, exhibiting substantial variability among different body locations. This burgeoning field has elucidated the complex interplay between the virome and disease etiology, as well as the pivotal processes governing its establishment during early human life. It is now well established that the state of the viral community can correlate with deleterious consequences for the host, whereas certain states are indicative of a healthy individual. This Review aims to synthesize the current scientific discourse surrounding the human virome, focusing on the recent advancements in research that delve into the nuanced dynamics of virome assembly, its intricate composition, and the multifaceted interactions that unfold between the host and its viral counterpart.
These studies not only shed light on the mechanisms underlying the formation and maintenance of the human virome but also underscore the critical role that the virome may play in both the preservation and disruption of host homeostasis.

## 1. Introduction

### Overview of the Gut Microbiome and Virome

The human gut hosts a complex ecosystem of trillions of microorganisms, collectively called the gut microbiome, which includes bacteria, archaea, fungi, and protozoa (Backhed et al., 2005). Alongside the microbiome, the gut virome encompasses viruses, primarily bacteriophages that infect gut bacteria and eukaryotic viruses that interact with the host (Norman et al., 2015). The microbiome and virome form a dynamic community that plays a vital role in maintaining gut homeostasis. The gut microbiome is essential for various physiological processes, including digestion, nutrient metabolism, and synthesizing vitamins and short-chain fatty acids (SCFAs) (Rinninella et al., 2019). Similarly, the gut virome regulates bacterial populations, contributing to microbial diversity and stability (Manrique et al., 2016). Dysbiosis, or the imbalance of microbial and viral populations, is associated with numerous diseases, such as inflammatory bowel disease (IBD), diabetes, and cancer (Qin et al., 2010; Zuo & Ng, 2018).

The gut-associated lymphoid tissue (GALT), a key immune system component, interacts with gut microbes and viruses to regulate local and systemic immunity (Macpherson & Harris, 2004). Pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), recognize microbial-associated molecular patterns (MAMPs) and viral- associated molecular patterns (VAMPs) to mediate immune tolerance or initiate immune responses (Belkaid & Hand, 2014). These interactions are critical for maintaining a balance between inflammatory and anti-inflammatory signals in the gut. This review explores the intricate relationships between the gut microbiome, virome, and the immune system. Specifically, it will: a) examine the composition and roles of the gut virome, b) discuss how these ecosystems interact with the immune system to maintain health, c) highlight the implications of microbiome-virome-immune interactions in diseases, d) evaluate emerging therapeutic approaches targeting the gut microbiome and virome.

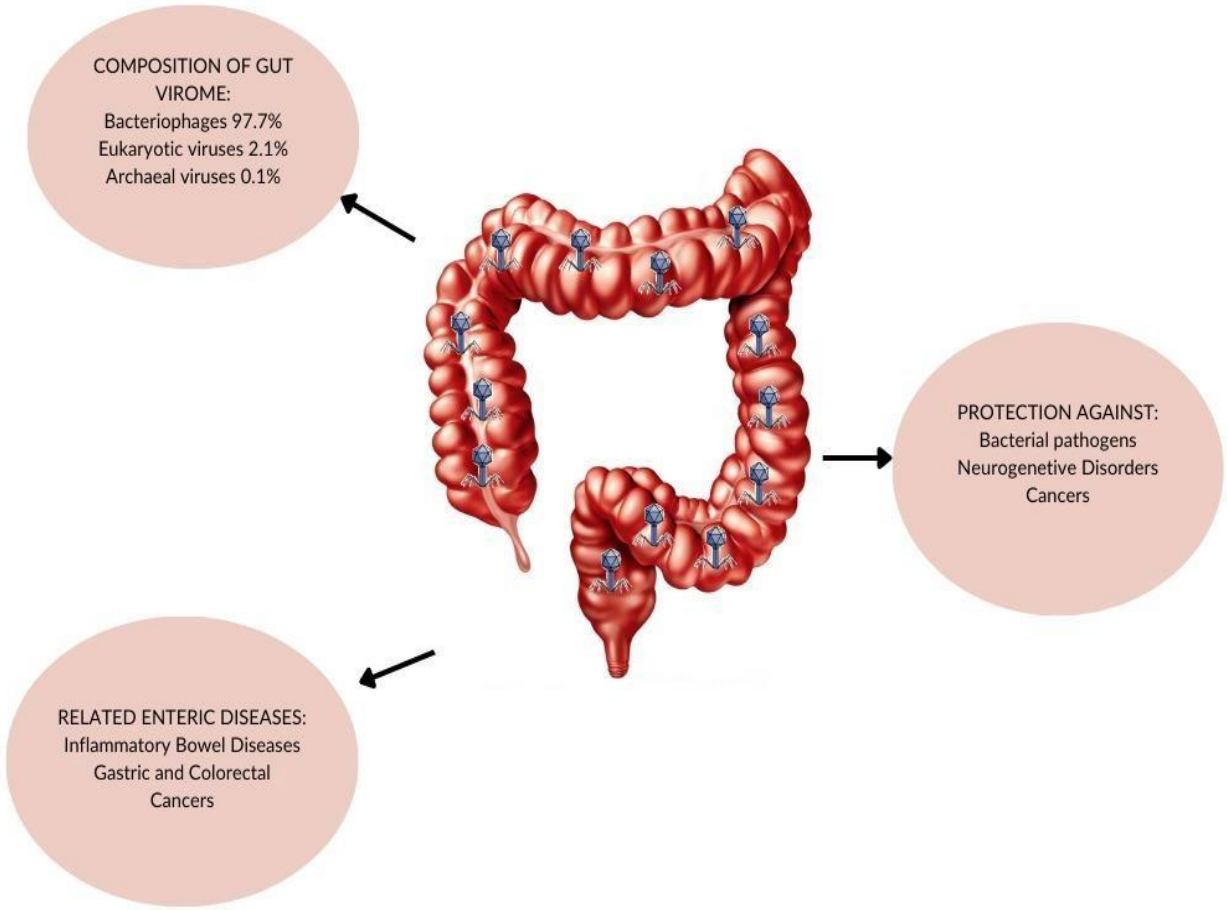


**Fig 1:** Human gut virome (Spencer L. et al)

## 2. Human Gut Virome

### 2.1. Composition and Diversity

The human gut virome consists of trillions of microorganisms, predominantly bacteria, distributed among several major phyla, including *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* (Arumugam et al., 2011). According to the Global Virome Database, human gut virome consists of 97.7% Phages (out of which 88% are unclassified), 2.1% are Eukaryotic Viruses, and 0.1% are Archaeal Viruses. The diversity of the virome varies and is affected by the individual's age group throughout life. The gut virome is an integral component of the human gut ecosystem, consisting predominantly of bacteriophages (viruses that infect bacteria) and eukaryotic viruses that can infect host cells (Norman et al., 2015). Bacteriophages, the most abundant members of the virome, play a crucial role in shaping the gut microbiota by lysing specific bacterial populations and facilitating horizontal gene transfer among microbes. Families *of Podoviridae, Myoviridae, Microviridae, and Siphoviridae* were analyzed in the adult human gut. (Manrique et al., 2016). The eukaryotic viruses in the gut, such as adenoviruses and enteroviruses, typically coexist with the host in a symbiotic or commensal relationship. However, they may occasionally contribute to disease under certain conditions (Wylie et al., 2014). The virome is highly dynamic, and its composition varies across individuals, influenced by factors such as age, diet, and geographic location (Reyes et al., 2010). Interactions between the virome and the gut microbiota are intricate, with bacteriophages modulating bacterial diversity and metabolic function, impacting host health (Lepage et al., 2008).

A study showed infants (0-3 years) and adults (18-65 years) showed higher phage diversity than children (3-18 years) and elderly (65 years and above). However, this trend is not observed in the case of eukaryotic viruses (Gregory et al., 2020). Phages discovered in the gut are characterized by three major groups based on their most prominent phenotype, i.e. their tail structure: a) *Myoviridae* with an extensive and complex tail, b) *Siphoviridae* with a non-extensive and long tail and c) *Podoviridae* with a short and non-extensive tail (Veesler & Cambillau, 2011). Single-stranded DNA bacteriophages, especially *Microviridae*, are credited for heterogeneity in the gut virome due to their high mutation rates (Minot et al., 2013). Within these phyla, species such as *Bacteroides fragilis* and *Faecalibacterium Prausnitzii* play pivotal roles in maintaining gut health (Qin et al., 2010).

These microorganisms perform critical functions such as digesting complex polysaccharides, synthesizing short-chain fatty acids (SCFAs), and producing essential vitamins like B12 and K (Rinninella et al., 2019). SCFAs, such as butyrate, acetate, and propionate, serve as energy sources for colonic epithelial cells and have anti-inflammatory properties (Louis et al., 2014). The microbiome's composition varies significantly among individuals due to genetic and environmental factors, yet its diversity is essential for resilience against pathogenic invasions and metabolic disorders (Turnbaugh et al., 2007). A stable and diverse microbiome could be key to a healthy and disease-free life. Similarly, disturbances in this community will lead to dysbiosis and many maladies (Flores et al., 2011).

### 2.2. Development and Modulation

The gut microbiome begins colonization at birth, influenced by delivery mode (vaginal or caesarean), feeding methods (breastfeeding or formula), and environmental exposures (Dominguez-Bello et al., 2010). Studies have confirmed that prior to delivery, no virome or microbiome was established. The neonate is protected from microbial invasion due to the amniotic fluid's protective effect, which provides a sterile environment (de Goffau, M. C. et al.). However, post-delivery after ~37h, early colonizers of viral families like *Myoviridae, Podoviridae, and Siphoviridae* are reported (Liang, G. et al.). Pioneer colonies of bacteria like *Escherichia coli, Enterococcus, Staphylococcus, Streptococcus, and Klebsiella* were too reported (Bäckhed, F. et al.). Lytic cycle Phages are seldom present in infants up to 4 months of early life. All present are prophage-inducing sequences found in the bacterial genomes (Sausset, R. et al.). After the 4-month mark, there is a shift from *Caudovirales* to *Microviridae* domination. (Liang, G. et al; Lim, E. S. et al; Reyes, A. et al.). Mode of delivery also plays a major role in the determination of viral diversity, as depicted by a Metagenomic analysis of 20 infants.

Those born with a spontaneous vaginal delivery are found to be more diverse (in the viral population) than their caesarean section delivery counterparts (McCann, A. et al.). In early life, viral pathogens causing gastroenteritis have been detected in the metagenomic analysis of the faecal samples. These pathogens infect and replicate in human cells and prove fatal to infants as they prove to be one of the leading causes of childhood mortality. According to reports, Astrovirus, Calicivirus, Picornavirus, Polyomavirus, and Adenovirus are all associated with childhood diarrhoea (Hug, L. et al.; Oude Munnink. et al.). During infancy, *Bifidobacterium* species dominate, promoting immune system development and protecting against pathogens (Turroni et al., 2012). As to shape its composition dynamically (Zmora et al., 2019). For example, high-fibre diets are associated with increased diversity and SCFA production. At the same time, excessive antibiotic use can disrupt the microbial balance, reducing diversity and increasing susceptibility to infections (Jernberg et al., 2007). ). Hence, it can be inferred that infants are colonized sequentially. Pioneering bacteria provide prophage induction, which helps the initial population. Followed are the lytic Phages and viral pathogens that replicate in the human cells.

### 2.3. Parameters that influence gut virome

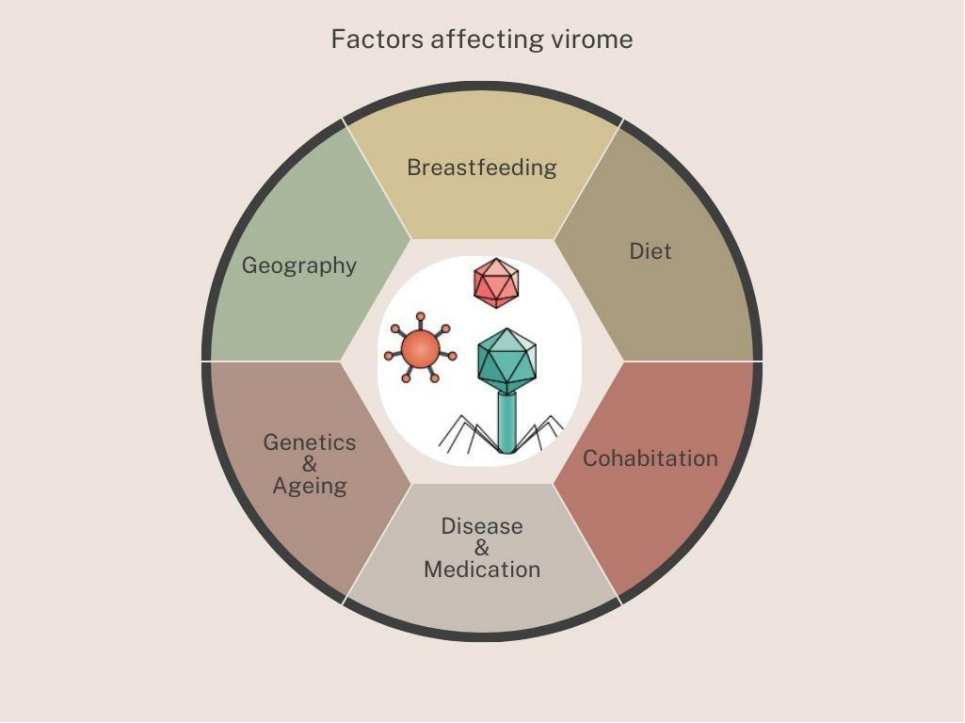


**Fig 2. Parameters that shape the human virome.** Major factors include the diet, breast milk or formula feeding, medications (including antibiotics and immunosuppressants), host genetics, cohabitation, geography, presence of disease and ageing (Liang, G. et al.)

**Diet** is one of the foremost and crucial factors that determines the early microbial flora of the gut. There are many reports

of breastmilk introducing the first pioneering colonies as well as providing protection to newborns by reducing the chances of viral gastroenteritis and infant mortality (Turin et al.,2014; Lamberti et al.,2011). Affected viruses are from the families of Adenoviridae, Picornaviridae, and Caliciviridae. Human breast milk contains specific components responsible for its protective effect, i.e. maternal antibodies, oligosaccharides, and lactoferrin (Turin et al., 2014). These components are reported to inhibit viruses like Rotavirus, Norovirus, Enterovirus, Influenza virus, and SARS-CoV (Turin et al.,2014; Lamberti et al.,2011; Conesa, C. et al.)

**Host Genetics and immunity** have often been linked to the microbial flora as they, just like diet, also play a crucial role in determining the diversity of the same. A study reported that monozygotic twins share a similar gut virome in infancy, which later on changes in adulthood. Emphasizing the role of environment over human genetic composition (Goodrich, J. K. et al.) However, in hereditary disorders such as primary immunodeficiencies, the profound implications of genetic factors on the virome have been unequivocally substantiated. The intricate relationship between genotype and virome manifests particularly in elucidating phenotypic expressions contingent upon the interplay between the host's genetic makeup and the viral ecosystem. One striking illustration is Epidermodysplasia verruciformis, a rare autosomal recessive cutaneous condition, colloquially termed the Treeman syndrome. The pathophysiology of this disorder is intricately linked to the dysregulated interaction between the host and papillomaviruses, which are typically benign. In individuals afflicted with Treeman syndrome, mutations within the TMC6 (EVER1) or TMC8 (EVER2) genes are of paramount significance. These genetic aberrations, which encode proteins crucial in regulating intracellular zinc homeostasis and DNA repair mechanisms, confer an increased susceptibility to the aggressive replication of cutaneous human papillomaviruses (HPV). The mutations in question compromise the host's immune surveillance system, facilitating the aberrant proliferation of HPV-infected skin cells. This results in the characteristic cutaneous phenotype, marked by extensive verrucous growths that mimic the texture of tree bark. The pathological phenotype associated with Treeman syndrome underscores the critical interdependence between the host genome and the virome. The nuanced interplay between these entities is essential to comprehensively understanding disease aetiology and progression. Consequently, the study of such genetic disorders not only advances our knowledge of the specific conditions but also elucidates broader principles governing the complex dynamics of host-virus interactions, which may hold the key to the development of innovative therapeutic strategies and preventive measures against various viral infections (Orth, G.,2006)

**Geography** has always been a strong influencer on gut microbial flora. Largescale virome studies have elucidated the significant influences of geographic factors and stochastic colonization processes on the variability observed within the human virome. A comprehensive analysis of faecal samples derived from diverse regions throughout China has demonstrated that the spatial distribution of bacteriophage populations exhibits substantial heterogeneity, with geography exerting the most profound influence on this structure relative to other potential confounding variables such as dietary habits, ethnic background, and pharmaceutical interventions (Zuo, T. et al., 2020). This geographical dimension of viral ecology is not confined to bacteriophages alone but extends to eukaryotic viruses. An investigation into the eukaryotic viromes of children suffering from diarrheal illness in different Australian locations uncovered notable discrepancies in the prevalence of Adenoviridae and Picornaviridae species (Holtz, L. R. et al., 2014). This underscores the intricate relationship between geography and eukaryotic viral distribution patterns.

A study focusing on the blood viromes of individuals residing in the southern region of China revealed distinctive patterns not observed in other Chinese populations, thereby highlighting the potential role of geographic factors in shaping viral community structures (Liu S. et al., 2018). Another intriguing observation stemming from examining the infant virome indicated a higher prevalence of viruses capable of infecting human cells among cohorts of African descent compared to their counterparts from the United States (Liang, G. et al. 2020). This disparity suggests that the interplay between human genetic diversity and geography may contribute to the differential viral burdens experienced by various human populations.

The global perspective has been further expanded by recent research efforts, which have amassed and analyzed public metagenomic data sets to construct a comprehensive virome database encompassing over 30,000 viral genomes (Gregory, A. C. et al, 2020). The findings from these studies collectively point to a pronounced difference in viral diversity between non-western and Western populations, with the former exhibiting a greater richness of viral species. These results are corroborated by additional studies that have independently reported analogous trends (Rampelli S. et al., 2017). Consequently, the significance of geographic influences on human virome composition emerges as a salient theme, emphasizing the need for a more nuanced understanding of the complex interplay between human hosts, their associated viral communities, and the environments in which they reside.

### 2.4. Dysbiosis and Health Implications

Dysbiosis, an imbalance in the gut microbial ecosystem of the resident commensal communities, is linked to several health conditions (Petersen & Round, 2014). Dysbiosis can be observed when an increased level of pathogenic bacteria (like Gammaproteobacteria) and reduced level of advantageous species like *Bifidobacterium*, *Akkermansia* or *Faecalibacterium* along with subsequent differences in the alpha diversity (evenness and richness of species in a given microbiome). A greater alpha diversity can be considered a double-edged sword since it is a significant marker of health in the GI tract but also a marker of dysbiosis in the vaginal microbiome (Borgdorff et al., 2016). For instance, shifts in bacteriophage composition have been linked to inflammatory bowel diseases (IBD), where an increase in Caudovirales phages and a decrease in Microviridae phages have been observed (Norman et al., 2015). Similarly, viral dysbiosis has been implicated in systemic diseases such as metabolic disorders and neurological conditions, potentially through disruptions in microbial stability and immune modulation (Rasmussen et al., 2020). Emerging research highlights the gut

virome's potential role in cancer progression, autoimmune diseases, and responses to viral infections, emphasizing the need for a deeper understanding of its systemic impacts (Hannigan et al., 2018). Inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis, are associated with reduced levels of anti-inflammatory bacteria like *Faecalibacterium Prausnitzii* and an increase in pro- inflammatory species (Gevers et al., 2014). Similarly, obesity and metabolic disorders have been correlated with altered *Firmicutes* to *Bacteroidetes* ratios, which may impact energy extraction from food (Turnbaugh et al., 2006). Dysbiosis is also implicated in mental health conditions, as the gut-brain axis mediates communication between the microbiome and the central nervous system through neuroactive substances like serotonin and gamma- aminobutyric acid (Cryan et al., 2019). Understanding and addressing dysbiosis through targeted therapies, such as probiotics, prebiotics, and dietary interventions, is essential for improving health outcomes. As advanced metagenomic techniques continue to uncover the complexities of the virome, new therapeutic strategies targeting viral components may offer novel approaches to disease prevention and management.

### 2.5. Functions and Roles

The primary function of the gut virome is the regulation of bacterial populations through predation by bacteriophages, maintaining microbial diversity and preventing the overgrowth of any single bacterial species (Virgin, 2014). This regulation promotes ecological stability within the gut and protects against pathogenic invasions by creating competitive microbial niches (Minot et al., 2011). Bacteriophages also contribute to the genetic diversity of the gut microbiota by facilitating horizontal gene transfer, which can enhance bacterial adaptability and functionality (Barr et al., 2013). Health conditions such as cancer and neurodegenerative disorders have been the subject of extensive research, and phage therapy has emerged as a promising treatment modality. Phage therapy involves the utilization of bacteriophages, which are bacterial viruses, to target and eliminate pathogenic bacteria, thereby ameliorating the symptoms associated with these diseases. Notable among these conditions are Alzheimer's and Parkinson's diseases, which are neurodegenerative disorders characterized by the accumulation of β-amyloid and α-synuclein proteins in the brain. Research conducted by Ksendzovsky et al. (2012) has demonstrated the significant binding potential of the M13 bacteriophage to these aberrant proteins within the cerebral tissue of Macca mulatta, non-human primates, thereby suggesting its applicability in the treatment paradigms for these debilitating conditions.

In the realm of oncology, phage therapy has shown efficacy in treating cancer cells through the employment of engineered phages capable of delivering anticancer proteins directly into malignant tumours. This targeted delivery system has been instrumental in inducing tumour atrophy and facilitating cancer gene therapy, ultimately resulting in the programmed death of cancer cells. This approach enhances the specificity of treatment and minimizes the collateral damage to adjacent healthy cells.

Furthermore, phages have been found to exert immunomodulatory effects, which are of particular interest in cancer treatment and managing various pathologies. For instance, Dabrowska et al. (2004) reported that phages can mitigate the synthesis of reactive oxygen species (ROS) during endotoxin-induced oxidative stress. This is crucial as ROS are known to play a pivotal role in the initiation and progression of various diseases, including cancer and neurodegenerative conditions. By modulating the immune response and reducing oxidative stress, phages may serve as a complementary strategy to bolster the innate immune system and improve treatment outcomes.

The therapeutic potential of phages has been the focus of numerous studies and reviews, with Abedon et al. (2017) providing an in-depth analysis of their clinical and preclinical applications. This body of work underscores the importance of exploring phage-based therapies as a viable alternative to conventional treatment options, particularly in the face of growing antibiotic resistance and the unmet medical needs posed by complex and multifactorial diseases such as cancer and neurodegeneration. The scientific community continues to scrutinize the mechanisms underlying the therapeutic actions of phages and their potential synergies with existing treatments, thereby advancing our understanding and expanding the therapeutic arsenal available to combat these challenging health issues.

Moreover, the virome influences gut ecology by interacting with the immune system, triggering innate and adaptive immune responses that maintain mucosal immunity and tolerance (Cadwell, 2015). These interactions underline the vital role of the gut virome in sustaining the balance between the host and its microbial inhabitants.

## 3. Interactions Between the Microbiome, Virome, and Immune System

### 3.1. Cross-talk Between Microbiome and Virome

The microbiome and virome engage in dynamic interactions that profoundly influence immune responses. Bacteriophages, the predominant members of the virome, modulate bacterial populations through lytic and lysogenic cycles, indirectly shaping microbial diversity and immune signalling (Norman et al., 2015). For instance, lysogenic bacteriophages can transfer genes that enhance bacterial resistance to immune responses, while lytic phages can release bacterial components that activate innate immunity (Hsu et al., 2019). Conversely, bacteria produce metabolites, such as short-chain fatty acids (SCFAs), which regulate immune functions, including cytokine production and T-cell differentiation (Arpaia et al., 2013). These interactions highlight the mutualistic relationship between the microbiome and virome in maintaining immune homeostasis and resilience against infections The immune system recognizes and interacts with the gut microbiome and virome through a complex network of mechanisms that maintain tolerance and prevent inflammation. Pattern Recognition Receptors (PRRs), such as Toll-Like Receptors (TLRs) and NOD-like receptors (NLRs), play a pivotal role in detecting microbial-associated molecular patterns (MAMPs) and viral-associated molecular patterns (VAMPs) (Medzhitov, 2007). For example, TLR4 recognizes lipopolysaccharides (LPS) from gram-negative bacteria, while TLR3 detects viral double-stranded RNA,

initiating immune signalling cascades (Kawai & Akira, 2010). These receptors are essential for distinguishing between commensals and pathogens, ensuring that beneficial microbes and viruses are tolerated while harmful ones are neutralized. Furthermore, secretory immunoglobulin A (IgA) production contributes to mucosal immunity by coating gut microbes and preventing their translocation into the systemic circulation (Macpherson et al., 2001). Recent in vitro and in vivo experimental analyses have elucidated the potential for bacteriophages to engage directly with the host immune system, independent of bacterial mediation. These viruses are known to induce immune responses through mechanisms involving TLR signalling pathways. A noteworthy study demonstrated that phages derived from pathogenic bacteria can be internalized by murine immune cells, including dendritic cells, B cells, and monocytes, leading to the activation of type I interferon (IFN) signalling cascades via the TLR3 pathway. This interaction suggests that phages may serve as innate immune adjuncts capable of modulating the host's initial defensive responses. (Sweere, J. M. et al, 2019).
Additionally, research conducted on germ-free mice has revealed that phages originating from human gut microbiota, such as Escherichia coli, can significantly enhance the frequency of interferon-gamma (IFNγ)-producing CD4+ T helper cells and CD8+ cytotoxic T cells within mucosal compartments. This intriguing phenomenon implies that phages could be instrumental in regulating adaptive immunity. Moreover, the study also disclosed that phages associated with Lactobacillus, Escherichia, and Bacteroides species can stimulate the secretion of various pro-inflammatory cytokines, namely Interleukin-12 (IL-12), Interleukin-6 (IL-6), and Interleukin-10 (IL-10), as well as IFNγ, via the nucleotide-sensing TLR9. These findings underscore the complex interplay between phages, commensal bacteria, and the host's immune apparatus. (Gogokhia, L. et al, 2019).

### 3.2. Role in Maintaining Gut Immune Homeostasis

The gut microbiota and viral community, collectively termed the gut virome, exert profound influences on regulating pro-inflammatory and anti-inflammatory processes within the host. These complex microbial ecosystems interplay with the gut-associated lymphoid tissue (GALT), the pivotal immunological interface within the intestinal tract (Mowat & Agace, 2014). The equilibrium between pro- and anti-inflammatory signalling is modulated through a delicate interplay with the commensal microbiota and their derived metabolites, which have been shown to promote the development and function of regulatory T lymphocytes (Tregs). These specialized immune cells attenuate inflammatory responses (Round & Mazmanian, 2009). Conversely, pathobionts or an altered viral composition can precipitate the activation of pattern recognition receptors (PRRs), thereby eliciting pro-inflammatory pathways.

Bacteriophages, as integral components of the gut virome, contribute significantly to preserving microbial homeostasis by imposing a regulatory mechanism on bacterial populations. Through the lytic destruction of bacteria, these viruses control microbial overgrowth and mitigate the potential for aberrant immune system activation (Manrique et al., 2016). This tripartite interaction between the microbiome, virome, and the host immune system is essential in maintaining a stable intestinal environment. Disruptions in this equilibrium have been implicated in the pathogenesis of various disorders, including inflammatory bowel disease (IBD) and systemic autoimmune conditions (Norman et al., 2015). Thus, the study of these microbial networks and their interrelations with the GALT holds significant promise in elucidating novel therapeutic strategies to restore and preserve gut health.

## 4. Virome and Immune-Related Disorders

### 4.1. Inflammatory and Autoimmune Diseases

The gut microbiome and virome are intricately involved in the pathogenesis of inflammatory and autoimmune diseases, including Crohn's disease, Ulcerative colitis, and Rheumatoid arthritis. Dysbiosis in the gut microbiome, characterized by reduced bacterial diversity and an imbalance of pro-inflammatory and anti-inflammatory species, is a hallmark of inflammatory bowel diseases (IBD). For instance, decreased levels of *Faecalibacterium prausnitzii* and increased *Escherichia coli* have been linked to Crohn's disease, highlighting the role of microbial metabolites in regulating inflammation (Gevers et al., 2014). Similarly, in ulcerative colitis, an altered virome with an increased abundance of bacteriophages has been observed, suggesting a direct impact on microbial balance and immune activation (Norman et al., 2015). In rheumatoid arthritis, gut microbial dysbiosis is thought to contribute to systemic autoimmunity by triggering molecular mimicry and T-cell activation (Scher et al., 2013).

### 4.2. Allergies and Immune Hypersensitivity

The gut microbiome is critical in modulating immune responses associated with allergies and hypersensitivity. Early-life exposure to a diverse microbiome promotes immune tolerance by facilitating the development of regulatory T cells (Tregs) and reducing the likelihood of allergic diseases (Fujimura et al., 2016). Conversely, reduced microbial diversity, as seen in urbanized lifestyles or after antibiotic use, has been linked to an increased prevalence of allergic diseases such as asthma, eczema, and food allergies (Strachan, 1989; Stiemsma et al., 2015). Specific gut microbes, such as *Clostridia* and *Bifidobacterium*, have been shown to regulate IgE responses and protect against allergic sensitization (Atarashi et al., 2011). Interactions between the virome and microbiome also contribute to immune regulation, as specific bacteriophages can modulate bacterial populations that influence allergy risk.

### 4.3. Viral Infections and Probiotics

Probiotics are typically categorized as microorganisms belonging to the broad taxonomic groups of Lactobacillus (sensu lato) and Bifidobacterium. These bacterial entities have garnered significant interest in the medical and scientific

communities due to their professed efficacy in addressing conditions characterized by an inflammatory nature, such as infectious diarrhoea, atopic dermatitis, and Inflammatory Bowel Disease (IBD). Nevertheless, it is imperative to recognize that the mechanistic basis of their action is more complex than previously thought, as they have been demonstrated to elicit not only anti-inflammatory responses, as reported by Plaza-Diaz et al. (2013), but also pro-inflammatory effects, as observed in the study conducted by Chiba et al. (2010).

Therapeutic interventions that modulate the human microbiome, including but not limited to probiotics, prebiotics, and postbiotics, represent a burgeoning area of research with many applications. These biotherapeutics encompass a spectrum of preparations, such as viable bacteria, non-living microbial components, and cell lysates, which have been historically employed across a diverse range of medical conditions (Aguilar-Toalá et al., 2018). The intricate interplay between these microbial agents and the host's immune system underscores the need for a deeper understanding of their molecular underpinnings to optimize their therapeutic potential.

The gut virome plays a dual role in systemic viral infections and immune dysregulation. On one hand, gut viruses can influence susceptibility to systemic infections by modulating host immune responses. For example, enteric viruses like noroviruses interact with the microbiome to enhance mucosal immunity, potentially providing cross-protection against other pathogens (Kernbauer et al., 2014). On the other hand, dysbiotic viromes, characterized by an overrepresentation of specific bacteriophages or eukaryotic viruses, can exacerbate immune suppression or chronic inflammation. For instance, in HIV infection, gut viral dysbiosis is linked to persistent immune activation and systemic inflammation (Monaco et al., 2016). These findings underscore the importance of maintaining a balanced virome for immune homeostasis and infection resistance.

### 4.4. Cancer and Immune Surveillance

The gut microbiota and virome play pivotal roles in modulating cancer immunity and the efficacy of therapeutic interventions. These microbial communities are implicated in regulating the immune microenvironment, where they exert influence over the complex interplay between cancer cells and the host's immune system. Research conducted by Louis et al. (2014) has elucidated the potential of microbial metabolites, notably butyrate and propionate, to confer anti-inflammatory and anti-carcinogenic effects, thereby bolstering the body's immune surveillance mechanisms and attenuating the progression of malignant neoplasms.

Dysbiosis, on the other hand, presents a significant challenge in this context, as it is known to precipitate carcinogenesis through the initiation of chronic inflammation and the dysregulation of epithelial cell metabolic processes. In colorectal cancer, distinct microbial signatures, such as the prevalence of Fusobacterium nucleatum, have been shown to correlate with tumorigenesis and the evasion of immune surveillance (Kostic et al., 2012). This underscores the intricate relationship between the intestinal microbiota and cancer development.

Furthermore, the gut virome has been identified as an additional factor contributing to cancer immunity. For instance, specific bacteriophages can exert indirect effects on tumour immunity by sculpting bacterial populations that, in turn, influence the expression and function of immune checkpoints (Virgin, 2014). This suggests a multifaceted interaction between the viral and bacterial constituents of the gut microbiome in the context of cancer pathogenesis and the host's immune response.

Moreover, the gut microbiome has significantly impacted the efficacy of cutting-edge treatments such as immune checkpoint inhibitors. As presented by Routy et al. (2018), emerging data indicates that the gut microbiome plays a critical role in shaping systemic immunity, thereby affecting immunotherapy outcomes. This intricate interplay between the microbiome and cancer immunity underscores the need for a comprehensive understanding of these interactions' underlying mechanisms. This knowledge may ultimately inform the development of personalized therapeutic strategies that leverage the gut microbiota and virome to optimize cancer treatment efficacy and improve patient prognosis.

## 5. Therapeutic and Clinical Applications

### 5.1. Microbiome Modulation

Modulating the gut microbiome through probiotics, prebiotics, synbiotics, and dietary interventions has become a cornerstone of therapeutic strategies to restore microbial balance. Probiotics, live microorganisms such as *Lactobacillus* and *Bifidobacterium*, have been shown to improve gut health by enhancing barrier integrity, modulating immune responses, and outcompeting pathogenic bacteria (Ouwehand et al., 2002). Prebiotics, non- digestible food ingredients like inulin and fructooligosaccharides, selectively promote beneficial gut bacteria growth and support short-chain fatty acids (SCFAs) (Gibson & Roberfroid, 1995). Synbiotics, a combination of probiotics and prebiotics, offer synergistic benefits, enhancing microbial diversity and functional capacity. Dietary interventions, such as high-fibre diets and polyphenol-rich foods, further support a healthy microbiome, reducing the risk of dysbiosis and associated diseases (Conlon & Bird, 2015).

### 5.2. Virome-Based Therapies

The virome, particularly its constituent bacteriophages, presents an intriguing prospect for therapeutic interventions, most notably in antibiotic-resistant bacterial infections. Bacteriophages exhibit a high degree of specificity, selectively targeting pathogenic bacteria while preserving the integrity of the commensal microbiota, thereby mitigating the deleterious ecological consequences often associated with indiscriminate antibiotic treatment (Nobrega et al., 2015). Empirical evidence suggests that phage therapy has been successfully implemented in treating infections instigated by Escherichia coli and Clostridium difficile, thereby underscoring its potential as a complementary or standalone modality

to conventional antibiotic regimens (Chan et al., 2013). The advent of advanced phage engineering strategies, such as the CRISPR-Cas systems, has significantly bolstered the development of tailored phages capable of exerting precise antimicrobial effects against specific pathogenic targets, thereby enhancing the therapeutic arsenal against bacterial pathogens (Yosef et al., 2015).

### 5.3. Faecal Microbiota Transplantation (FMT)

Fecal Microbiota Transplantation (FMT) involves transferring stool from a healthy donor to a recipient to restore gut microbial diversity. It has proven highly effective in treating recurrent *Clostridioides difficile* infections (CDI), with success rates exceeding 90% in some studies (van Nood et al., 2013). Beyond CDI, FMT is being explored for conditions such as inflammatory bowel disease (IBD), metabolic disorders, and even neuropsychiatric conditions, leveraging the gut-brain axis (Moayyedi et al., 2015). However, FMT poses challenges, including donor screening, risk of pathogen transmission, and variability in efficacy due to differences in donor and recipient microbiomes. Efforts to develop standardized microbial consortia or synthetic stool products aim to address these limitations (Kellingray et al., 2018).

### 5.4. Emerging Therapeutic Approaches

Emerging therapeutic approaches in gut microbiome and virome interventions are centred on precision medicine and advanced biotechnological tools. CRISPR-based gene editing can precisely modify viromes by targeting specific bacteriophages or viruses, potentially addressing dysbiosis or eradicating harmful pathogens (Bikard et al., 2014). Personalized microbiome therapies, guided by individual microbial profiles, are being developed to tailor interventions such as customized probiotics or dietary recommendations to optimize therapeutic outcomes (Zeevi et al., 2015). Additionally, advances in microbiome-virome interaction studies are paving the way for integrated therapies that simultaneously modulate the gut ecosystem's microbial and viral components, promising a holistic approach to treating complex diseases.

## 6. Challenges and Future Directions

### 6.1. Limitations in Current Research

Despite significant advancements, several challenges remain in understanding the gut microbiome and virome. One of the primary limitations is the complexity of the virome. Unlike bacteria, most gut viruses lack reference genomes, making it challenging to identify and classify them using conventional methods (Handley et al., 2012). The high variability of virome composition between individuals further complicates the development of a universal model for gut virome function (Norman et al., 2015). Additionally, isolating and culturing gut viruses, particularly bacteriophages, remains technically challenging due to their dependency on specific bacterial hosts and environmental conditions (Roux et al., 2016). Another gap lies in understanding the dynamic interactions between the microbiome, virome, and host immune systems, which involve intricate and context-dependent mechanisms.

### 6.2. Technological Advances

Recent technological advances are transforming the gut microbiome and virome study, offering new opportunities to overcome existing limitations. Metagenomics, the sequencing of all genetic material in a sample, has enabled comprehensive profiling of microbial and viral communities without the need for culturing (Raveh-Sadka et al., 2015). Transcriptomics provides insights into the active functional roles of microbiota and virome by analyzing RNA transcripts, helping to identify pathways critical for host interactions (Franzosa et al., 2014). Proteomics and metabolomics further enhance our understanding by identifying proteins and metabolites involved in host-microbe-virome interactions, revealing their functional impact on health and disease (Lamichhane et al., 2018). Integrating these multi-omics approaches holds promise for elucidating the complex interplay within the gut ecosystem.

### 6.3. Future Research Areas

Future research should focus on comprehensively understanding the interactions between the host, microbiome, and virome. This includes exploring how these ecosystems collectively influence immune regulation, metabolism, and disease susceptibility. Artificial intelligence and machine learning advances can be leveraged to analyze large datasets generated by multi-omics approaches, uncovering hidden patterns and interactions (Zhou et al., 2019). Another promising area is the development of microbiome- and virome-targeted precision medicine, where interventions such as tailored probiotics, engineered bacteriophages, and personalized dietary recommendations are designed based on an individual's microbial and viral profiles (ElRakaiby et al., 2014). Additionally, studying the role of the virome in therapeutic responses, such as in immunotherapy and gut-brain axis modulation, could open new avenues for clinical applications.

Investments in standardized methodologies for virome isolation and analysis and interdisciplinary collaborations will be crucial for addressing these challenges and advancing our understanding of the gut ecosystem's role in health and disease.

## 7. Conclusion

The human gut microbiome and virome are integral to maintaining health, with their interactions playing a pivotal role in immune regulation, metabolism, and disease prevention. This review highlights the complexity and diversity of these ecosystems, emphasizing their dynamic interplay with the host immune system. The microbiome contributes to essential physiological processes, while the virome modulates microbial populations and influences immune responses. Dysbiosis in either component is linked to various diseases, from inflammatory and autoimmune disorders to cancer, underscoring

the importance of maintaining balance within these ecosystems.

The gut microbiome and virome impact local gut immunity and have systemic implications, influencing conditions such as allergies, metabolic disorders, and neuropsychiatric diseases. Emerging therapeutic strategies demonstrate significant potential, including microbiome modulation through probiotics and dietary interventions, virome-based therapies, and faecal microbiota transplantation. However, the field faces challenges, such as gaps in understanding virome complexity and limitations in isolating gut viruses. Advances in multi- omics technologies and computational tools offer promising avenues for unravelling these complexities and paving the way for precision medicine.

Integrated research approaches are imperative to fully harness the gut microbiome's and virome's therapeutic potential. Collaborative efforts across disciplines, standardized methodologies, and innovative technologies are necessary to deepen our understanding and develop targeted interventions. By bridging current knowledge gaps, future research can unlock the full potential of these ecosystems in promoting health and combating disease.

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