ROLE OF GUT MICROBIOTA IN VIRAL INFECTIONS

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Abstract

Microbiome is a complex network of thousands of microorganisms including bacteria, viruses, fungi and other microorganisms that remain as commensals. These microorganisms maintain an intricate balance with hosts' immune system to stop overexploitation of hosts' resources while shaping the host immune system by priming the adaptive immune system for optimal development. Viruses are the obligatory intracellular parasites that depend on the host-cell machinery for their survival, genome replication, and producing progeny virions. The first key step in the virus life cycle is the entry during which the virus comes in contact with hosts' skin or mucous membrane where hundreds of thousands of microorganisms colonize. As such, there is a possibility of complex interaction of invading viruses and other microorganisms within the microbiome, for example symbionts bacteria may interact with viruses and also immune system maintaining the homeostasis within hosts. Although the interaction between viruses and the microbiome are not very clear it is possible that the microbiota, especially the bacteria prime the immune system and confer a resistance to the invading viruses to initiate an infection or a disease. It may also be possible that the bacterial components of the microbiome actually initiate an immunosuppressive reaction that may enhance viral infection or pathogenesis. In this review, we have highlighted the various outcomes that may happen due to the complex interaction of the microbiota and viruses and have also discussed how the viral entry, replication and/or pathogenesis are modulated by microbiota.

Key words: Host-pathogen, Intestinal microbiota, Microbiome, Virome, Virus

Introduction

The human as well as the animal body surfaces such as skin and body cavities are colonized by a humongous number of symbiotic commensal microorganisms, collectively known as microbiota. The human body comprises of about 100 trillion cells of which these microorganisms outnumber their host cells at least by a factor of 10 and also express at least 10-fold more unique genes compared to their hosts (Ley *et al.*, 2006). These large number of microorganisms which include bacteria, viruses, fungi, and other eukaryotic species maintain a homeostatic relationship

through the complex interaction with their host cells. These enormous microbiomes provide the host with tremendous enzymatic capabilities regulating many aspects of hosts physiology (Belkaid and Hand, 2014). In fact, with the advent of modern technology, advanced bioinformatics and culture-independent genomic techniques, microbiome research has been revolutionized over the past few years and it is now evident that these microorganisms just do not colonize hosts as passive bystanders rather actively playing fundamental roles in shaping host health and immunity, maintaining

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multiple aspects of host physiology, but not limited to digestion, metabolism, nutritional responses, circadian rhythm, and disease pathogenesis (Hacquard *et al.*, 2015; Lynch and Hsiao, 2019).

Viruses are the obligatory parasites that require host cellular machinery for their own replication and survival. Although most of the viruses are identified on the basis of their mechanism of pathogenesis, many more viruses are found within a healthy individual that actually do not cause disease (Domínguez-Díaz *et al.*, 2019). These viruses that reside within the body cavities as part of microbiome are collectively known as virome. These consist of all viruses of the prokaryotic and the eukaryotic organisms found in humans and animals. Although, at this moment the role of the virome in health and disease is very poorly understood, it is clear that the non-pathogenic viruses within microbiome remain mainly as commensals with their hosts along with bacteria and other microorganisms. They have co-evolved with their host for years maintaining a homeostasis, (Dethlefsen *et al.*, 2007). These intricate, perfectly balanced homeostatic relationships, require an optimally functioning immune system which is a complex network of innate and adaptive components that prevents the microorganisms from over-exploitation of their host resources (Zheng *et al.*, 2020). On the other hand, perturbations in the balance of the microbiome communities by different stimuli such as environmental factor including use of antibiotics, changes in diet or geography, and alterations of the immune function may lead to the systemic dissemination of the apparently harmless commensal microorganisms within hosts, enhanced susceptibility to pathogenic organisms resulting in alteration in disease severity and overall health status of the hosts (Zheng *et al.*, 2020). The alteration of microbiome however, may also provide resistance to some microorganisms including

viruses. The first key step in the virus life cycle is the entry into the host, during which the virus comes in contact with hosts' skin or mucous membrane where numbers of microorganisms colonize. As such, there is a possibility of complex interaction of invading viruses and other microorganisms, for example, commensal bacteria may interact with viruses and also immune system resulting in the alteration of the intricate homeostatic balance. Although the impact of this interaction between viruses and the microbiome are not very clear, however, it is possible that the microbiota, especially the bacteria primes the immune system and confers resistance to the invading viruses to initiate an infection or a disease. It may also be possible that the bacterial components of the microbiome actually initiate an immunosuppressive reaction that may enhance viral infection or pathogenesis. In fact, multiple recent studies have reported that disease pathogenesis caused by various human and animal viruses can be modulated by the normal intestinal microbiota of the hosts (Wilks and Golovkina, 2012; Karst, 2016). In this review we have discussed about the role of microbiota in the development of immune system, general health status of humans and animals and role of microbiota in the viral infections and their pathogenesis. We have also discussed the various outcomes that may happen due to the complex interaction of the microbiome, host and viruses and have pointed out how the viral infections and/or pathogenesis are modulated by microbiota.

Small animal model to study gut microbiome

For the infectious disease research and the preclinical evaluation of vaccines and therapies against many human pathogens, small animal models such as mice are one of the most important tools (Sarkar and Heise, 2019). Similarly, mouse models serve as invaluable tool for studying microbiome, especially intestinal microbiome. Scientists have adopted two important strategies to study the effect of

gut microbiota on disease pathogenesis including metabolic diseases, cancer, bacterial diseases or viral diseases. One strategy is the infection of germ-free or sterile mouse with the pathogens in question and the other strategy is the infection of mouse that are treated with a cocktail of oral antibiotics/antimicrobials to deplete of their natural microbiota in the intestine. Both of the models have pros and cons. For biomedical research purposes majority of the mouse that are used, are specific pathogen free (SPF) but not completely devoid of all microorganisms. On the other hand, germ-free mice are bred and raised in a sterile condition; hence, are free from all microorganisms, including the commensal intestinal microbiota (Karst, 2016). As such, these germ-free mice can be easily used to investigate the effect of the commensal gut microbiota on the pathogenesis of a particular virus, bacteria or any other pathogens. These mice can also be used to study the effect of gut microbiota on the homeostatic condition of the host. However, because of complete absence of all commensal microbiota in the intestine, that otherwise play an important role in the development of mucosal immunity, the germ-free mice have many abnormalities both in the morphology of the intestine as well as in the mucosal immunity (Karst, 2016).

In the antibiotic-depletion method, mice are treated with oral gavage containing either broad-spectrum antibiotics or a cocktail of antibiotics for example ampicillin, vancomycin, neomycin, metronidazole and amphotericin B daily for a few consecutive days (time depends on the study) targeting both Gram-positive and Gram-negative bacteria (Karst, 2016; Sun *et al.*, 2019). After a certain time of post-gavage treatment, antibiotics are added in the drinking water and then the efficiency of the microbiota-depletion is assessed by culturing the feces for microorganisms in anaerobic followed by aerobic conditions (Karst, 2016). The use of

antibiotic depletion method has advantage over the use of germ-free mice, as in the antibiotic-depletion methods, mice do not require a special handling in sterile condition such as using sterile isolators. Further, it also caused to increase the cost of mouse-breeding, maintenance and overall research as well as mice with antibiotic treatment will also have normal mucosal immune system. Although majority of the commensal microbiota from intestine are removed, antibiotic-depletion cannot remove all intestinal commensal microbiota; hence, researchers must account for the effect of the residual microbiota on the questions they ask (Karst, 2016).

Modulation of the viral infections by microbiota

It has now been shown that the fate of the many pathogenic viral infections depends on the nature of the microbiota present in the hosts and especially microbiota present in the intestine which is the richest source of microbial community among others (Karst, 2016). Generally, microbiota can influence viral infections in three different ways. Firstly, the commensal microbiota provides protective immunity against invading pathogenic viruses either by priming immune system or working as a physical barrier to viruses for entry to host cells (Kasubuchi *et al.*, 2015; Winkler *et al.*, 2020). Secondly, the microbiota can directly enhance the invading viral infections (Kane *et al.*, 2011; Karst, 2016). Thirdly, the commensal microbiota can indirectly promote viral infections (Isaak *et al.*, 1988).

Gut microbiome promoting resistance to viral infections: There is no doubt that intestinal microbiota interacts with invading pathogens whether it is virus or bacteria, modulates the host's immune system, and thus modulates the disease pathogenesis. In fact, multiple evidence suggests that gut microbiota maintains a mutualistic relationship that benefits their

human host in many ways (Karst, 2016). These commensal microbiota for example would degrade the otherwise indigestible dietary fibers resulting in the production of short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate (Flint *et al.*, 2008; Kasubuchi *et al.*, 2015). These SCFAs are then used for the de novo synthesis of the end products like lipids and glucose which serve as the energy source for hosts' cellular metabolism and regulators of gut motility (Tremaroli and Bäckhed, 2012). The gut microbiota is also involved in the development of the mucosal immune system, as evident from the inability of the germ-free mice to mount an optimal immune response including the reduction in the secretion of immunoglobulin A and intestinal T cells because of the impaired development of mature lymphoid organs within the gastrointestinal tract (Kamada and Núñez, 2014). Certain intestinal microorganisms especially bacteria can induce the secretion of antimicrobial proteins like angiogenin and the C-type lectin RegIII γ by intestinal epithelial cells which in turn protect the hosts from invading pathogenic bacteria (Hooper *et al.*, 2012). However, gut microbiota can also provide hosts protection against invading pathogenic viruses including influenza, dengue, human immunodeficiency virus (HIV), and chikungunya virus among others. Intestinal microbiota not only regulates immune homeostasis in intestine but also regulate immune response in other mucosal surfaces such as in the respiratory tract. It has been observed that the gut microbiome critically regulates the development of influenza-specific CD4⁺ and CD8⁺ T cell responses as well as the virus-specific antibody-mediated immune responses (Ichinohe *et al.*, 2011). In their mouse model, they have shown that there is impaired generation of influenza-specific CD4⁺, CD8⁺ T cell responses and also antibody (IgA) mediated immune responses when natural commensal microbiota especially neomycin-sensitive bacteria were depleted by the use of

oral antibiotic treatment (Ichinohe *et al.*, 2011). The neomycin-sensitive commensal bacteria in the intestine provided signals for robust priming of pro-IL-1 β and pro-IL-18 expression at steady state which provided immuno-competence in the lung (Ichinohe *et al.*, 2011). Either local or distal injection of Toll-like receptor ligands rescued the impaired virus-specific immune responses (Ichinohe *et al.*, 2011). In another study, Abt and associates have documented an exacerbation of influenza disease pathogenesis both in antibiotic-treated commensal microbiota-depleted SPF as well as germ-free or gnotobiotic mice (Abt *et al.*, 2012). In the microbiota-depleted influenza-infected mice, the viral titers were significantly higher with an increased mortality rate compared to control SPF mice with normal intestinal microbiota. The histological analyses found morphological features of bronchiole epithelial degeneration and necrosis in the microbiota-depleted mice correlating the disease severity (Abt *et al.*, 2012). Similar findings such as increased viral titers in the lung, exacerbated pathology and decreased survival rate were found in the germ-free mice when infected with influenza virus suggesting that intestinal commensal microbiota provides resistance against influenza virus infection in experimental animals (Abt *et al.*, 2012). However, the increased disease severity in the sterile or microbiota-depleted mice may also be occurred due to the impaired protective mucosal immunity in these mice.

Chikungunya virus (CHIKV), which is an arthropod-borne (Arbo) emerging alphavirus, has infected millions of people around the globe posing a threat worldwide along with other emerging alphaviruses (Gunn *et al.*, 2018; Broeckel *et al.*, 2019; Nguyen *et al.*, 2019). In a recent study, scientists have shown that gut microbiota plays a crucial role in the disease pathogenesis and protection against CHIKV. When infected with CHIKV, both germ-free

mice and antibiotics treated, microbiota-depleted SPF mice either completely or partially devoid of intestinal microbiota, have shown an exacerbated disease pathogenesis producing early clinical signs, higher viral burdens in serum, spleen and contralateral ankle tissues, and also enhanced dissemination compared to SPF mice with normal intestinal microbiome (Winkler *et al.*, 2020). The absence of microbiota or alteration of gut microbiota in mice impairs the TLR7-MyD88 signaling in plasmacytoid dendritic cells (pDCs) and suppresses systemic production of type I interferons (IFNs) resulting in the expression of fewer IFN-stimulated genes (ISGs) in the circulating monocytes making them more permissive for CHIKV infection (Winkler *et al.*, 2020). Furthermore, they have demonstrated that the reconstitution of mice gut with a single species of bacteria, *Clostridium scindens*, or its derived metabolite, the secondary bile acid deoxycholic acid, can actually restore pDC- and MyD88-dependent type I IFN responses to protect mice from systemic CHIKV infection and transmission back to mosquito vectors (Winkler *et al.*, 2020).

Another important arbovirus is dengue which belongs to the family *Flaviviridae*, infects millions of people around the world every year with significant morbidity and mortality. Dengue virus (DENV) is transmitted predominantly by the mosquito, *Aedes aegypti* which takes up the virus in their blood meal from an infected host (Ramirez *et al.*, 2012). The virus infects the mid-gut of the mosquitoes, replicates there and migrates towards the salivary glands from where it can again be transmitted to a new host during another blood meal from an uninfected host. During this process of infecting a human host the virus actually comes in the vicinity of the microbiota present in the environment of mosquito mid-gut which has the possibility of modulating the Dengue fever disease pathogenesis. In fact, it

has been shown experimentally that depletion of commensal gut microbiota of *Aedes aegypti* mosquitoes with antibiotic treatment, caused enhanced DENV replication with significantly higher titers and when mosquito-microbiota were reconstituted with a single isolate of *Proteus* sp. it mitigated the virus replication in the mosquitoes (Ramirez *et al.*, 2012). Their studies indicated that gut microbiota of the mosquitoes promoted a basal level stimulation of the MyD88-dependent Toll pathway in response to DENV infection providing an anti-viral immunity to DENV (Xi *et al.*, 2008; Ramirez *et al.*, 2012).

Like gut microbiome, vaginal microbiome also plays an important role in providing immunity or resistance to viral infection as has been seen in the case of vaginal human immunodeficiency virus-1 (HIV-1) infection in women. Alteration of vaginal microbiota increases the risk of acquiring and transmitting HIV-1 infection. In women, the vaginal microbiota is dominated by the presence of *Lactobacilli* that generate a byproduct, L-lactic acid which acidifies the vagina serving as antimicrobial agent (Lai *et al.*, 2009; Ravel *et al.*, 2011). L-lactic acid has been shown to function as broad-spectrum HIV virucide (both anti-HIV-1 and anti-HIV-2) *in vitro* conditions (Lai *et al.*, 2009; Ravel *et al.*, 2011). L-lactic acid mediated acidified mucous of the cervix and vagina can slow down the diffusion of HIV particle by trapping them within the acidified mucous at acidic pH which in turn reduces the acquisition and transmission risk of HIV infection (Lai *et al.*, 2009).

Gut microbiome promoting viral infections:

Although commensal microbiota can provide resistance or antiviral immunity to many viral infections, it can also promote invading pathogenic viral infections both directly and indirectly. The first important study which demonstrated that gut microbiota enhances pathogenic viral infections included poliovirus,

reovirus and mouse mammary tumor virus (MMTV) (Kane *et al.*, 2011; Kuss *et al.*, 2011; Karst, 2016). Antibiotic-treated mice depleted of commensal gut microbiota showed an attenuation of poliovirus infection compared to mice with normal microbiome (Kuss *et al.*, 2011). Interestingly, reconstitution of the gut microbiota in the antibiotic-treated mice restored the pathogenesis of poliovirus indicating that intestinal microbiome enhances poliovirus infection (Kuss *et al.*, 2011). Similar findings were documented in the reovirus mouse model of IFN-deficient mice which are normally susceptible to the infection but when mice were treated with antibiotics to deplete its natural microbiota and infected with reovirus, the mice failed to produce the disease. Tissue pathologies and fecal abnormalities suggested a role of intestinal microbiome in reovirus infection and pathogenesis (Kuss *et al.*, 2011). Another group of scientists also conducted a study where they had taken either germ-free mice or SPF mice treated with antibiotics and infected with MMTV. These MMTV-infected mice failed to transmit the virus to their offspring through milk and a simple restoration of intestinal microbiota restored the transmission capabilities of the mother to their pups, corroborating the role of intestinal microorganisms enhancing the viral infections (Kane *et al.*, 2011). Very similar observations were made in rotavirus and murine norovirus infections where microbiome plays crucial role in the promotion of viral infection and pathogenesis of the diseases. When antibiotic treated microbiota-depleted mice were infected with rotavirus, the mice produced an attenuated disease signs with reduced level of viral antigen in feces, reduced levels of viral genomes in intestinal tissue and delayed viral shedding when compared with control mice (Uchiyama *et al.*, 2014). Moreover, antibiotic-treated rotavirus-infected suckling mice showed reduced incidence as well as duration of the diarrhea further establishing the role of gut

microbiome in enhancing the viral infections (Uchiyama *et al.*, 2014). Multiple studies have also found that intestinal microbiome promotes murine norovirus infections. Using either gnotobiotic mice or SPF-mice treated with oral antibiotics, it has been shown that the absence of gut microbiota attenuates norovirus pathogenesis by reducing *in vivo* virus replication, and virus shedding through feces (Jones *et al.*, 2014; Kernbauer *et al.*, 2014; Baldridge *et al.*, 2015). These studies further demonstrate that intestinal microbiota is not only maintaining homeostasis in the host, rather can actually enhance a diverse set of viral infections and disease pathogenesis.

I. Indirect mechanism of enhancing microbiome-mediated viral infections:

The intestinal microbiota can enhance invading viral infections in multiple ways. One example is the enhancement of viral infections by stimulating the activation and/or proliferation of the target cells which has been reported for the retroviruses that infect proliferating cells such as murine leukemia virus (MuLV) (Isaak *et al.*, 1988). Germ-free mice infected with MuLV have failed to produce leukemia which was thought to be due to the absence of microbiota mediated lymphoid cell proliferation (Isaak *et al.*, 1988). The gut microbiota can also enhance viral infections indirectly, specifically of enteric viruses by modulating antiviral immune responses, either by inducing a tolerogenic microenvironment rendering viral evasion of the host immune system, or by suppressing the production of virus-specific antibodies or also by altering the IFN-mediated signaling (Karst, 2016).

II. Direct mechanism of enhancing microbiome-mediated viral infections:

The intestinal microbiome can directly enhance the infection of many invading viral pathogens in several ways including by virion stabilization further promoting viral transmissibility and by

promoting the virus attachment to host cells (Karst, 2016). It has been shown that intestinal commensal bacteria helps in the stabilization of poliovirus particle and enhances its infection of host cells (Robinson *et al.*, 2014). Interestingly, this stabilization by bacteria did not require the live bacteria, rather just the bacterial membrane component, lipopolysaccharides (LPS) and other N-acetylglucosamine-containing bacterial surface polysaccharides that were longer than six monosaccharides, were sufficient to provide this effect of virion stabilization (Robinson *et al.*, 2014). This phenomenon is thought to be mediated by a direct interaction of poliovirus virion and LPS or other N-acetylglucosamine (Robinson *et al.*, 2014). The poliovirus interaction with either bacteria or bacterial surface polysaccharides increases the thermostability and resistance of virus to inactivation by dilute chlorine bleach (Robinson *et al.*, 2014). In addition to the virion stability, gut microbiota also promotes virus attachment as has been shown for poliovirus (Robinson *et al.*, 2014). The bacterial LPS also enhances poliovirus attachment to the surface of target cells facilitating the viral binding to the poliovirus receptor resulting in the enhanced infection (Robinson *et al.*, 2014). The similar findings were documented in the human norovirus infection where an enhanced infection of B cells with norovirus was found in the presence of *Enterobacter cloacae* that express the H-type histo-blood group antigen (HBGA) but not H-type-negative, *Escherichia coli* strain (Tan and Jiang, 2005; Karst, 2015).

In summary, host microbiome can interact with the components of the immune system, actively shapes the immune system, and modulates the immune system either in the favor or against of the host depending on the interacting microbes. This in turn either protects the host from the invading pathogenic virus infections or enhances the severity of the viral infections directly or indirectly.

Conclusion

There have been significant discoveries in the field of microbiome within the last few years and a plethora of information are available describing the importance of microbiome in human and animal health. It is now clear that the tremendous number of microorganisms that reside within the bodies as commensals are not just passively present there, rather they are actively involved in the development of immune system, priming the immune system, modulating them to regulate host response to various pathogens. However, to date very little is known about the broad picture of the contribution of the microbiome in human and animal health. So far whatever the information is known primarily highlights the role of bacteria as commensals and their role in human and animal health. We now know that commensal bacteria both in intestine and other areas of the body cavities actively take part in modulating the pathogenesis of many pathogenic bacteria as well as viruses. It is very interesting to note that microbiota especially bacteria directly or indirectly interacts with viruses or host immune system and influences the outcome as either protective or detrimental for the host. While the commensal microbiota positively manipulates the host adaptive immune system in favor of hosts and thus provides a protective immunity against viruses like influenza, dengue, chikungunya viruses or HIV, microbiota also interacts with different components of immune system or directly with invading viruses like poliovirus, MuLV, norovirus, reovirus and others favoring the pathogens resulting an increased virus replication, dissemination and overall disease severity. It is thus imperative that researchers are focused to understand this complex microbiota-virus-host interaction in more details to identify a therapeutic approach. For example, by identifying the bacterial components required in virus infection as is shown for poliovirus requirement for bacterial surface polysaccharides, a therapeutic intervention may be targeted. As the dysregulation or ablation of commensal

organisms enhances disease severity as in the case of influenza infection, it is worth evaluating the consequences and benefits of administering antibiotic therapy in severe cases of influenza with bacterial co-infections and in general use of antibiotics should be done with more cautions. However, further research is required to reveal more about the complex host-microbiota-virus interactions which will undoubtedly provide more opportunities to design novel therapies against many pathogenic viruses and bacteria. It is also interesting to note that besides bacteria, host

microbiome also contains a very large number of commensal viruses and very little or no information is available about the importance of the virome if any in the pathogenesis of invading microorganisms. Probably, with the advent of modern technologies, next generation sequencing facilities and bioinformatics future studies will be conducted and more information will be available identifying the role of virome in the microbiome in the overall health of hosts.

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