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Symbiotic microbiota: A class of potent immunomodulators

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ABSTRACT: Microbiome is known to exist as symbiotic commensals in humans, domestic and wild animals, birds, fishes, reptiles, insects etc. DNA sequencing and metagenomic platforms have deciphered the complex role played by communities of microbiota (bacteria, fungi, viruses, protozoa and other eukaryotic species) in survival and regulation of host physiology, metabolism and regulation of host immune system. Any alteration in the microbial population or breach in the symbiotic alliance with the host may ultimately lead to development of different kinds of pathologies. Realization of the enormous role played by the microbiome in health and diseases of human and domestic livestock led researchers to find ways to modulate these resident microbiomes for improvement in health and management of diseases. Theoretically there are several ways that can be employed for manipulating the composition and functional capacity of the resident microbiome, which may lead to improvements in human and livestock health. Though studies have shown therapeutic potential of the microbiome, considerable challenges exist in the actual implementation of these strategies in clinical settings. This review discusses the symbiotic relationship between microbiome and host and strategies to modulate host immune responses by manipulating microbiome profile. Paper also highlights how to overcome existing obstacles for successful implementation of microbiome manipulation techniques. In this era of COVID-19, it would be worth analysing the role of resident microbiome in the magnitude of COVID-19 severity which may have occurred through immunomodulation.

KEYWORDS: immuno-modulation, microbiota, mutualism, symbiotic, virobiota

INTRODUCTION

Mainly, domestic livestock and human harbour large varieties of microbiota. This microbial population may even out-number the host cells in the body [1]. The complex resident microbiome comprises bacteria, fungi, viruses, protozoa and other eukaryotic species and lives in the host by establishing symbiotic relationship. Resident microbiome exerts major beneficial impact on host physiological, metabolic, immunological, developmental and evolutionary aspects. It is now believed that the animals and plants are no longer self-governing bodies, instead exist as a biological network which comprises both the host and its associated networks denoted by the term "holobiont". Collective genome of the host and microbiome is known as hologenome [2].

Interestingly besides bacteria and fungi, animal body is conquered with diversified resident microflora that also includes viruses. These resident viruses or more precisely known as "virobiota" are mainly bacteriophages and eukaryotic viruses. Resident viruses in the host are also contributed by acute, chronic or latent viral infections [3–5], they impact the immunity of the host [6]. Host experiences multiple infections throughout their lifetime and priorimmunity to the viral infections can influence the responses to related (homologous immunity) or unrelated pathogens (heterologous immunity) [7]. Thus resident virobiota and microbiota impact immunity through various mechanisms [8]. Several anti-inflammatory mechanisms, such as galectin-9and T cell Ig domain and mucin domain 3 signalling, have been shown to potentially modulate the virus-specific responses [9] to subsequent infections. For example, following viral infection, the host up-regulates immunoinhibitory receptors on CD8 T cells to limit immunopathology [9].

Resident microbiome helps in maintaining immune homeostasis and well-being of the host. However certain therapeutic interventions may alter microbiome and compromise the health of the host. For instance, in the medical and veterinary practice, antimicrobial substances are used as prophylaxis, as adjunct to operative treatment, and as therapy. Antibiotics are beneficial in limiting infection are frequently used for killing pathogenic bacteria through their antimicrobial activity. However, there are several harmful effects of these antibacterial agents on host health [10]. Use of antibiotics invariably leads to alterations in the composition of microbiota [11]. Antibiotics induce changes in microbiota and in turn impact immunity of the host [1]]. Therefore, antibiotics should be administered carefully to deplete the undesired microbiota and modulate the immune responses to generate beneficial

effects. Similarly, probiotics, prebiotics, virotherapy, fecal microbiota transplantation (FMT), and administration of microbial metabolites are some intervention strategies that could be exploited for regulating the microbial populations in order to harness health benefits.

Antibiotics are very commonly used despite the fact that they may negatively impact host health. Antibiotics can also alter the composition of host microbiome which plays a very important role in shaping host immunity. Intact immune responses are keys to good health. Furthermore, following infections such as COVID-19, variable outcomes of infections have been recorded in COVID-19 positive individuals, which could be due to their differential composition of resident microbiome that influences the host immunity.

In this review, we discuss that host microbiome shapes host immunity and thus various strategies could be exploited to modulate resident microbiome and in turn improve the immunity and the health of the host. We also discuss the challenges that might interfere with these microbiome modulations.

HUMAN AND ANIMALS ESPECIALLY DOMESTIC LIVESTOCK HARBOUR MICROBIOME

Microbiome term is used to describe the genome of all symbiotic/mutualistic and pathogenic/harmful microorganisms, living in all the vertebrates. Gut microbiome encompasses the concerted genome of microbes such as bacteria, archaea, viruses, fungi and protozoa (Fig. 1A). Microbiome may comprise single pathogen and microbial communities. Composition of microbiome in an individual is very unique and is more or less like a genetic signature of an individual, although around one-third of the microbial species are found to be similar across most humans. Status of hygiene, dietary factors/feeding practices, management system, geographical region and genotype of host impact greatly on the composition intestinal microbiome. Sex hormones and age of host have also been found to be crucial in determining the composition of intestinal microbial flora [13]. It may thus be regarded as host factors and manipulations of these host factors i.e. gut microbiome can become a target for host directed therapies [14].

In recentyears due to revolutionization in sequencing technology, it is a lot easier to explore and characterise composition and functions of host resident microbiome of nearly all species. Recently, microbiome of a number of vertebrate non-human species including livestock species has been sequenced [15]. Studying the evolution and ecological kinetics of resident microbiome may help in confirming their role in host health and disease management.

MICROBIOME ESTABLISHES MUTUALISM

According to some researchers, humans have coevolved with commensal microbes and have established a symbiotic relationship [16]. This concept is under critical review, since not all animals need microbiome [17]. Longitudinal studies suggest long-term stability of the gut microbiome [18]. Strong pieces of evidence support the fact that gastrointestinal tract of animals, is a home for complex ecosystem of microbiome, which is crucial for maintenance of health and digestion of crude fibre which form major part of their diet. Bacterial species in microbiome are known to inhibit growth of pathogenic bacteria by several ways such as direct inhibition through release of inhibitory metabolites, such as acetate, butyrate and bacteriocins. Further slowdown of growth of pathogens is influenced through oxygen and nutrient depletion, competition for attachment sites on various receptors, and also by rejuvenating immune defences [19]. Interaction between microorganisms and the host direct the immune system locally and systemically [20].

The changes in environment, especially depletion of forests and non-availability of biomass in grazing area, and reduction of grazing resources (grassland/common pasture lands or forests etc.), have led to the shifting of management system from extensive to semi-intensive and intensive. Therefore access to friendly microbe-like (saprophytic mycobacteria e.g. *Mycobacterium pheli*) has led to an increase in concentration of pathogenic Mycobacteria (*Mycobacterium avium* subspecies *paratuberculosis*) in rumen of the domestic livestock. Saprophytic mycobacterial population is losing its' habitat (forest/grazing lands/pasture lands) due to environmental change, hence the potential barrier provided by saprophytes were providing to ruminants by sharing of proteins (antigens) through cross immunization is gradually lost with time and their space is taken by pathogenic mycobacteria. Regular dose of saprophytic mycobacteria, which livestock species receive along with forage are now slowly disappearing from environment. This change in microbiome is leading to an increase in population of pathogenic mycobacteria [21].

For example, incidence of respiratory disease in pigs is influenced by their microbiome [20]. Microbiome of the corals involved in reef building, has significant influence on the response of reef ecosystems towards overfishing, nutrient pollution, and global warming [22]. Increasing urbanization, has led to an increase in the incidence of allergies, asthma, and other chronic diseases in humans, which may perhaps be due to a decreased exposure to a variety of microorganisms. Thus the changes in environment have brought the changes in microbiome which in turn results in altered host immunity and establishment of pathogenic microorganisms. Furthermore initial understanding of the gut microbiome composition in wild and captive animals may be useful in captive management and future reintroduction programs [23].

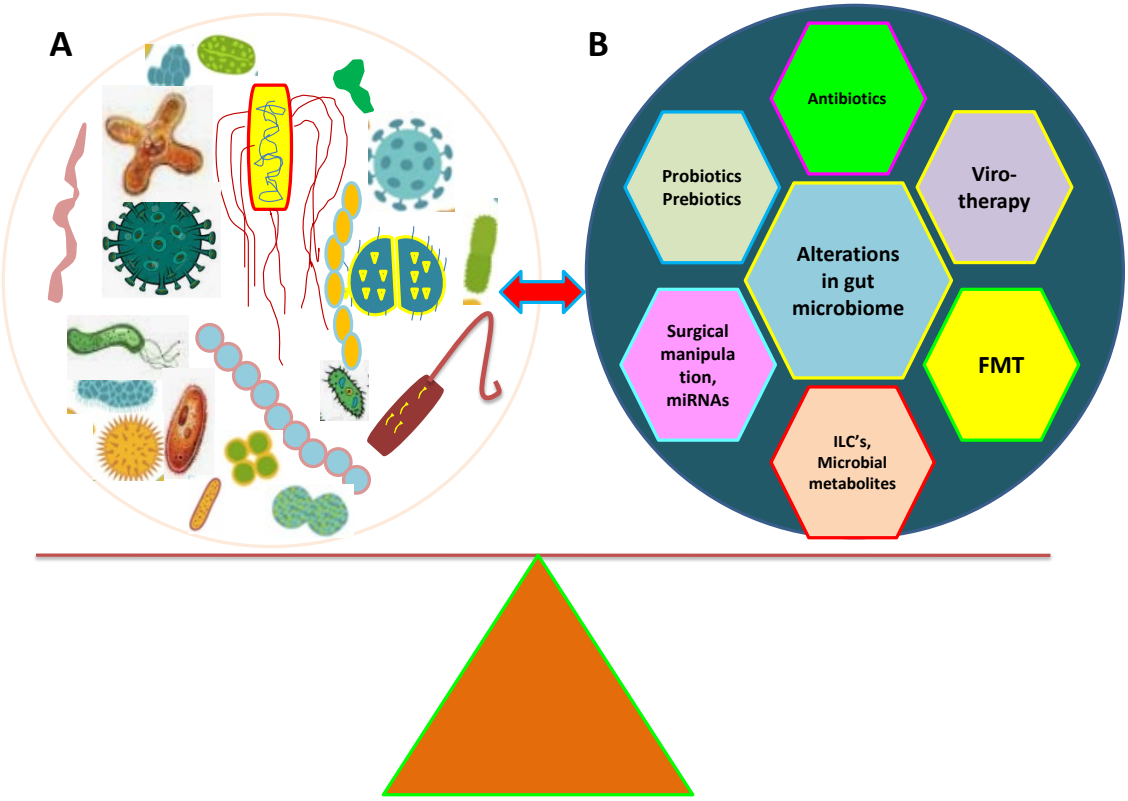

Fig. 1 Resident microbiome confers symbiotic mutualism to its host. (A) Gut microbiome encompass the concerted genome of microbes such as bacteria, archaea, viruses, and fungi. Resident microbiome influences hosts metabolism, physiology and immune functions, resistance and susceptibility against infection, and even behavioural modification. Resident microbiome is thus a tempting target for therapeutic manipulation in clinical settings. (B) Microbiome manipulations in the host could be achieved by probiotics, prebiotics, antibiotics, virotherapy, Fecalmicrobiota transplantation, surgical manipulations, microRNA, microbial metabolites and innate lymphoid cells.

Microbiome influences host physiology

Multiple components of the host body system; metabolic, physiological and immunological functions, drug metabolism [24], synthesis of essential vitamins [25], defence against pathogens [26], secondary processing of host bile acids [27], immune modulation [28, 29], resistance and susceptibility against infection [30], and modification of behaviour [31] influence the response to pathogenic microbes.

Gut microflora influences several metabolic and physiological functions of the host [32]. A variety of hormones such as cholecystokinin (CCK), peptide tyrosine tyrosine(PYY), glucagon-like peptide-1 (GLP-1), Gastric inhibitory polypeptide (GIP), and 5-hydroxy tryptamine (5-HT), have shown to be synthesized by endocrine cells residing in the mucosal lining of the gut. These hormones regulate carbohydrate and fat metabolism. Gut microflora and their metabolic by-products may influence gut mediated hormone release and thus in turn influence host metabolism. Role of gut microbiota in pathogen colonization, immune responses and inflammatory disease has been reviewed in detail previously [33]. Therapeutic interventions such as use of antimicrobials and dietary manipulations may help in the treatment/management of metabolic disorders. However, adverse consequences may also occur as a result of such manipulations.

By using DNA sequencing platforms, we have been able to decipher hundreds of distinct species of bacterial populations existing in the gut. Major populations include Firmicutes and Bacteroidetes phyla while Proteobacteria, Actinobacteria, Verrucomicrobia, and Fusobacteria phyla are minor populations. Gut microbiota, not only helps in digestion, but also regulates the host immune responses by establishing a symbiotic relationship with the host. Any alteration in gut microbial populations ultimately leads to many types of pathologies or digestive disorders.

Different cell types such asinnate lymphoid cells (ILCs), intestinal epithelial cells (IECs) and antimicrobial effectors such as antimicrobial peptides (AMPs), secretory IgA (sIgA) are in close contact with commensal microbes and restrict them to gut lumenand thus regulatehealth at the intestinal-lumen interface. AMPs are secreted by IECs and Paneth cells, which are specialized epithelial cell population localized at the base of intestinal crypts. IECs and Paneth cells have been shown to express TLRs (toll like receptors).These epithelial TLRs play an important role in immune responses to commensal or pathogenic microbes [34]. This TLR expression is compartmentalized with almost negligible expression of TLR2, 4, 5, 7, and 9 takes place in the epithelium of small intestines, but significantly higher expression of TLR2, 4, and 5 occurs in colon. TLR5 is exclusively expressed on the small intestinal epithelial Paneth cells. Paneth cells are located in small intestinal crypts and TLR5 senses the bacteria located at the crypt during infection or inflammation. TLR polymorphisms have been found to be associated with Crohn's disease and ulcerative colitis [35].

NOD1 and NOD2 are yet other innate sensors that identify bacterial components such as peptidoglycan. NOD1 senses diaminopimelate-containing N-acetyl glucosamine-N-acetylmuramic acid (GlcNAc-MurNAc) tripeptide that is present in peptidoglycan of gram negative bacteria and activates the transcription factor Nuclear Factor-kappaB (NF-κB). NOD1 is constitutively expressed by IECs and is essential for activating IKK(IκB kinase), NF-κB, and NF-κB target genes in colon epithelial cells that are infected with gram-negative bacteria which does not activate NF-κB through TLR activation. Thus signalling through these NOD-like receptors leads to neutrophil activation and subsequent antibacterial action [36].

ILCs are the resident cells mainly present in the intestinal and lung mucosal surfaces [37]. These ILCs, natural killer (NK)-cells (a type of ILC)and lymphoid tissue inducer (LTi) cells produce IL-22 (interleukin-22) that regulates immune defenses of gut. γδ T cells present in the intraepithelial compartment of the intestine aids in limiting the intestinal mucosal destruction by bacteria. Detailed role of ILCs in mucosal immunity has also been reviewed [37].

CD4+ TCRαβ T helper cells are also affected by the microbiota. A single bacterial species, for example as shown for *Bacteroides fragilis*, is efficient to re-establish the imbalanced Th1/Th2 cell ratio in Germ Free mice [38]. Autophagy and regulatory T cell differentiation is also influenced by microbiota. Further defects in Treg development leads to disruption of follicular T cells and regulatory T cell interactions which leads to improper IL-21 and antimicrobial IgA responses [39]. IgA is important for mediating mucosal barrier and intestinal tolerance. For example, *Mucispirillum* spp. and segmented filamentous bacteriaare shown to be directly involved in generation of intestinal IgA. Regulatory T cells promote IgA secretion, and also support diverse and balanced microbiota and thus prevent apparent inflammation [40]. RORγt$^+$ Th17 cells and the cytokine IL-17A produced by TH17 cells as well as other cell types also regulates IgA expression in intestine and thus add to sustain intestinal homeostasis [41].

Microbiome and resident microflora also include viruses

Microbiome mostly comprises of bacteria, however other microbes such as archaea, fungi, protists and viruses are also included [42]. Presence of viruses has been implicated mostly in context of pathogenic consequences. They are mostly known for causing disease and decreasing fitness of the host. Nonetheless previous studies have shown that several viruses are neutral (that do not influence fitness/health status of host) or are even mutualistic (that help in improving health status of the host) [43, 44]. In a mutualistic association, virus gains advantage by residing in the host cell and multiplies efficiently but the dilemma is that how the virus could be profitable to the host. Examples are functional studies which revealed that endogenous retrovirus derived proteins influence development of placenta by mediating cell-cell fusion, and protecting fetus from exogenous viruses [45]. The phenomenon has been reported in sheep and mice which is commonly observed in human as well as other mammals.

Viruses that cause chronic infections are also an important but little-perceived part of our metagenome known as the virome. The immunologic signatures of responses generated against our virome should be investigated during development of therapeutic and preventative vaccines. Despite the generation of detectable immune responses to target antigens by the adenovirus-based HIV vaccine, vaccination has proved to be a failure and it point out towards the challenges that need to be addressed.

Many viruses in the microbiome fraction improve the health and the physical fitness of the host in which they reside. Resident viruses are now convincingly shown to secure their host against pathogenic microbes such as *Listeria monocytogenes* and *Yersinia pestis* [16]. Furthermore, chronic virus infections invariably stimulate the immune system which in turn responds efficiently to various pathogenic microorganisms [46].

Metagenomic studies have deciphered the questions regarding composition of human virobiota and associated genes, which opened the gates of host-virobiota interactions [47]. Studies also revealed that every living organism has a characteristic virome of its own with specific proportion of species. However geographical positions, age, style of living, climate, are the factors that may influence an individual's encounter

with viruses. Susceptibility of an individual to viral or other infections may be influenced by pre-existing immunity and by viral and human genetics.

Studies with human intestinal virome revealed that bacterial viruses are abundant compared to the eukaryotic viruses [48, 49]. However genetic imprints of retroviruses, single-stranded DNA viruses, double-stranded DNA viruses, single-stranded RNA viruses have been identified in the fecal virome fractions of healthy individuals [48].

Similar to gut microbiota, resident viruses impact host immune responses [6, 50]. Study of resident viruses in detail is now the developing field and research is targeted on understanding whether these resident microbiota crosstalk with the host immune system and if they do, then how does they interact? There is sufficient understanding of microbial interactions with immune system and thus useful skeleton for initial studies on virobiota-immune system interactions is already known.

In the actual clinical scenario, co-infections are more common compared to the single infections. Co-infections with viruses and their influence on the disease patterns compared to those of single infection are now very well characterized. Therapeutic strategies in the clinics are based on the understanding that clinical outcome is an aftermath of single virus infection identified in clinical samples, however, such approach may be biased as it does not take into account other additional agents that might be accounting for clinical outcome. For example, SIV infection in monkeys leads to entropathy and thus promotes AIDS disease progression in these non-human primates. Metagenomic studies have revealed that the intestinal virome of SIV infected monkeys expands drastically [51]. Furthermore, in cystic fibrosis patients, complex and variable resident lung virome is associated with poor clinical outcome, whereas in healthy human lungs, virome is comparatively constant [52].

In the natural environment, every individual experiences sequential infections and the immunity developed against previously experienced viruses can influence the responses to unrelated pathogens. Prior immunity to a related pathogen provides complete protection against a homologous agent and forms the basis of vaccination strategy. However, prior infections could also result in immune-pathology to new incoming infection with homologous or heterologous agents. The mechanisms of immunity and immunopathology in homologous and heterologous coinfections [53] have already been reviewed. Resident/latent viruses such as HSV-2 may alter epithelial integrity which in turn is advantageous for establishment and transmission of HIV infection [54]. Furthermore by infecting the immune cells (HSV infects T-lymphocytes, and monocytes/macrophages, human cytomegalovirus (HCMV) infects monocytes/macrophages and T-lymphocytes, and Epstein-Barr virus type 1 (EBV-1) infects B-lymphocytes), resident/latent viruses impair immune responses and favouring bacterial infections.

MICROBIOME MANIPULATIONS IN THE HOST

Divergent characteristics of the resident microbiome and the complex relationship it establishes with the host and its' immunity makes it a tempting target for therapeutic manipulation in clinical settings. For example, resident microbiome by virtue of their metabolites or direct immune modulation has huge impact on development of tumors. By understanding microbiome mediated modulations of host immunity, it would be further advantageous in designing microbiome-based therapeutics [55].

Probiotics

Following surgery, use of probiotics for modulation of the intestinal microbiota seems to be an effective method of reducing infectious complications in surgical patients. Translocation of endogenous bacteria triggers severe complications in patients following major abdominal surgeries. With advancements in technologies, we now understand that almost all kinds of medical interventions (antibiotics, opioids, bowel preparation, etc., in addition to stress-released hormones), are important regulators of richness and diversity of the enteral microbiome [56]. A liaison exists between the gut flora and the development of postoperative complications [57]. Better understanding of the role of microbiome in surgical disease is warranted to pave the path for preventive therapy [32].

Probiotics have been shown to exert anti-inflammatory effect in inflammatory bowel diseases (IBDs), necrotizing enterocolitis, and malabsorption syndromes. ulcerative colitis, Crohn's disease [58], and multiple sclerosis both in experimental animal models as well as human subjects [59]. Various mechanisms of immune modulation exerted by probiotic bacteria have been discussed [60].

Prebiotics

Along with probiotics, prebiotics can also regulate gut microbiota. Prebiotics resist degradation and absorption in the upper digestive tract and selectively promote the growth of beneficial resident microbiota. Honey, inulin, laminarin, and fucoidan supplementation have been shown to regulate microbial composition and associated with several beneficial effects such as diminished inflammation and plasma triglyceride levels, improved glucose tolerance, and increased bacterial de-conjugation of bile acids.

Antibiotics, immunity, and disease

In medical and veterinary practice, antimicrobial agents are administered as prophylactic agents, as an adjunct to operative treatment, and as therapeutic

agents. Though antibiotics are administered to kill the pathogenic microorganisms however, they may be harmful to the host also. One adverse effect of antibiotic therapy is development of antimicrobial resistance by repeated use of same antimicrobial and is a natural phenomenon. When an antibiotic is used, bacteria that can resist the antibiotic have a greater chance of survival than those that are 'susceptible'. Susceptible bacteria are killed or inhibited by an antibiotic, resulting in a selective pressure for the survival of resistant strains of bacteria [61].

Another adverse effect of antimicrobial therapy is the fact that it leads to alteration in the composition of microbiota [62]. Antibiotic therapy results in decreased diversity, richness and evenness of fecal microbiota. Resident microbiome of an individual reacts differently to antibiotic therapy. Our intention is to encourage the growth of potentially favourable microorganisms and to reduce the number of pathogenic microorganisms thus improving the health of humans and livestock by preventing dysbiosis. To achieve this target is cumbersome since use of antibiotics impairs both pathogenic and beneficial microorganisms at random leading to dysbiosis [63].

Data on experimental animals suggest that antibiotic administration disrupts intestinal motility by affecting resident microbial composition (Fig. 1B) which in turn disturbs secondary bile acid and serotonin metabolism in the colon [64]. Competitive exclusion principle governs the fact that two species that thrive on exactly the same niche cannot coexist stably and antibacterial therapy causes derangements in competitive exclusion machinery, a key property by which microbiome erases pathogenic microorganisms [65]. Intestinal pathogens primarily enter the gut through contaminated food and water. Once inside the gut, these pathogenic microorganisms multiply and cause disease. Here resident microbiota is very useful in hampering colonisation of pathogens and pathogen clearance after resolution of pathogen induced inflammation [66]. Disruption of resident microbiome sets a favourable stage for growth of pathogens, for example, *Clostridium difficile*. Clindamycin [67], clarithromycin, metronidazole [68] and ciprofloxin [69] manifest long term effects on resident microbiome. Development of intestinal lymphoid tissues is also affected by antibiotic treatment [70].

Antibiotics are imperative in the treatment of bacterial infections and thus enhance the life span of human and livestock. However, antibiotic usage has been linked with the development of a range of immune-mediated disorders such as allergies and IBD. Use of broad spectrum antibiotics also disrupts antibody mediated protective immune responses against Influenza. Changes in plasma metabolomic profile and several inflammatory imprints of the circulatory system have also been observed following broad spectrum antibiotic therapy in humans with already existing immune system disturbances [12]. Lab animals such as rats and mice provide a useful model system where antibiotic administration disturbed microbial homeostasis, establishes a pro-inflammatory milieu and further susceptibility to infection. Antibiotic therapy mediated microbial disruption leads to the over growth of enteric fungi and polarization of M2 macrophage. This in turn supports allergic airway inflammation [71].

Due to the lifestyle changes (hygiene hypothesis), we now have reduced exposure to various microorganisms. On one hand reduced exposure to microorganisms prevents us from various diseases. However certain level of microbial exposure during developmental stages of an individual is recommended for the development of immune-regulatory mechanisms. For instance, prevalence of *Helicobacter pylori*, a bacterial species that is commonly found in the stomach in humans, has decreased in developed countries due to hygienic protocol. *H. pylori* activates the immune system in a way that prevents immune disorders such as asthma. Presence of *H. pylori* is inversely associated with development of childhood asthma. In line with this, antibiotic cocktail containing ampicillin, gentamicin, metronidazole, neomycin and vancomycin (that may disrupt microbes such as *H. pylori*) leads to TH2 biased responses and allergic inflammations in mice [72].

It is suggested that antibiotic enforced negative effects on immunity could be counter-balanced by probiotics, usually live bacteria, which would supplement antibiotic-induced deficiencies in the microbiota fraction. Other strategies include oral administration of anaerobic microbiota cultures. Bacterial ligands could also be administered as an alternative to live probiotics to boost the immune tone during antibiotic therapy.

Virotherapy

Attenuated, inactivated or killed viruses are most commonly used for the purpose of vaccination. With the help of new generation sequencing platform, it is now clearly evident that the viruses are not entirely pathogenic but are also beneficial to their hosts. These beneficial viruses have been shown to play an important role in the host metabolism and function (Fig. 1B). Information on the existence of mutualistic viruses has opened the doors for therapeutic advancements, for example phage therapy. Here virulent phages are administered into a patient with an idea that the phage will lyse the pathogenic bacteria and alleviate suffering and clinical symptoms of infection. Phage therapy gained popularity in some countries however it was considered unsafe in certain countries. Due to the concerns related to antimicrobial therapy, the field of phage therapy is again receiving interest and the researchers are now interested in combining antimicrobial therapy and phage therapy to treat pathogens

that are resistant to antibiotics [46]. Phages exert immune-regulatory properties, they knock out bacteria and inhibit local immune-inflammatory reactions [73] as has been shown in *Staphylococcus aureus*-infected mice [74].

ILCs as potential therapeutic targets

As discussed earlier, ILCs are a heterogeneous group of cells that includes NK cells, ILC1, ILC2, ILC3 and LTi cells; they play an important role in gut immune defence and are thus a tempting target for therapeutic intervention. Presence of NK cells in intestinal cancer has been linked with better prognosis thus boosting NK cell function and increasing cell numbers in tumors could be a useful approach.

ILC1 has a protective role against bacterial infections by producing the cytokines IFN-γ and TNFα as shown in the mouse models. However, these cells also lead to development of chronic and excessive inflammation [75] and promoting chronic obstructive pulmonary disease and IBD. Thus, in such cases managing excessive cytokine production by these cells or deleting these cells using strategies like antibody directed depletion might be therapeutically meaningful. Further ILC2 cells mediate their action through the production of classical type 2 cytokines such as IL5, 9, 4, 13. Thus, these cells are important for immunity against parasites. But excessive type II responses are detrimental and may lead to allergies and asthma. Thus, targeting and depleting these ILC2 in such circumstances could be a promising target. Increasing the numbers of ILC2 and ILC3 has been shown to provide protection in Graft versus host disease, thus expanding their numbers during transplantation could be a useful therapeutic intervention to prevent graft rejection.

The idea of exploiting ILCs as a therapeutic target is very appealing; however, it seems that the functions of ILCs are quite redundant with the T cells. Therefore, careful experimental validation is required to understand the redundance and pleiotropism amongst T cells and the ILCs. Majority of the studies are done in mouse models that are RAG (recombination activation gene) knockout which lack adaptive immune arm. Human studies have been done but the data generated represent a single time point and a particular stage of disease. Therefore several subsets of ILCs along with multiple time points throughout the course of disease are warranted to understand the clear role and impact of ILCs in pathogenesis. Moreover, the paradoxical roles of ILC3, for example secretion of IL-17 and IFN-γ, aggravate the pathogenesis of IBD whereas IL-22 secretion by them may be advantageous. Thus determining the ratio of such cells that produce IL-17 and IFN-γ vs those that produce IL22 will further clarify the picture [37].

Fecal microbiota transplantation (FMT)

Advantages of FMT include enhancement of intestinal microbial diversity anti-inflammatory, anti-allergic, and immune-modulation such as maintenance of Th1/Th2 and Th17/Treg balance. FMT is one of the most direct approaches for regulating gut microbial composition (Fig. 1B). In this aspect, the entire gut microbiome is transferred instead of a single microbial species. Furthermore, FMT has been successfully used in alleviating the adverse effects of chemotherapy [76].

Microbial metabolite-mediated interventions

Microbiome and their derivatives are now known to play essential role in development and gut immune defence of the host, through bacterial metabolites (Fig. 1B). Microbiome and their derivatives are now known to play essential role in development and gut immune defence of the host, through bacterial metabolites (Fig. 1B). Administration of these microbiota derived metabolites impacts its host. *Bacillus fragilis*-derived polysaccharide A (SYMB-104) given to IBD patients has been explored with some encouraging results [77]. In another study with mouse model of autism, therapeutic administration of 5-aminopentanoic acid and taurine (microbial metabolites) diminished behavioural anomalies and hyperexcitability of brain [78].

Surgical manipulation

Bariatric Roux-en-Y gastric bypass surgery performed for weight loss actually restructures the resident microbiome in such a way that it causes significant increase in certain bacterial genera which are beneficial for host metabolism such as attenuation of insulin resistance, endotoxemia, inflammation, and fat deposition [79].

microRNAs

Non coding RNAs such as microRNAs are known for regulating gene expression at the post-transcriptional level. Studies have now revealed that both the hosts as well as the dietary microRNA could regulate resident microbiome and intestinal immunity. Further, microRNA have immense role in intestinal mucosal barrier and development of intestinal epithelial cells and even intestinal adaptive immunity. Intestinal miR-146a, miR-29, miR-128, and miR-126 have a role in induction of Th1 in gut intestine. microRNA 155 promotes intestinal inflammation and knockdown of microRNA 155 reduces TH17 cells and in turn inflammation [80]. Therefore, microRNA may be targeted for therapeutic intervention in gut dysbiosis.

Challenges

Certain challenges prevail in the therapeutic potential of various immuno-modulatory strategies of resident microbiome. Major concern with the use of probiotics is the survivability of the bacterial species in the gut. A

possibility exists whereby the resident microbial population repel the presence of the bacterial strains in the probiotics and thus inhibiting the exertion of immune-modulatory effect of probiotics on the resident microbiome. In such circumstances antimicrobial therapy may facilitate contact of probiotics with the resident microbiome, however the pre antibiotic composition might be altered [81]. Several after-math effects of probiotic therapy has been observed such as adverse metabolic activity, chances of systemic infection, exaggerated immune responses and may even be a source of gene transfer in vulnerable individuals. Therefore, probiotic therapy may be useful as a personalized immune-modulatory strategy given the fact that different individuals may respond differently to probiotics. Further favourable outcomes of prebiotics are more or less short-lived and also bound to be outpaced by feed stuff.

Metabolites derived from microbiota have been shown to have therapeutic potential. Although microbial metabolite therapy might lead to undesirable side effects by accumulation or breakdown of these metabolites before they actually reach their predilection sites [77]. Virulent phages are administered into a patient with an idea that the phage will lyse the pathogenic bacterium and alleviate the suffering and clinical symptoms of infection. However, clinical implication of phage therapy is still in very naive stages. With anti-cancer therapy using oncolytic virus, the major issue occurs with the delivery systems and the choice of the virus type [82]. The immune responses may develop against the oncolytic viruses which may be positive or may have detrimental consequences. Further studies are warranted to identify the factors that are detrimental and the others which are favourable and exert anti-tumor efficacy. Alternatively, it may be beneficial to inhibit antiviral IFN-α/β response while simultaneously potentiating the expression of antitumor cytokines. This would favour the survival of oncolytic viruses and also enhancement of antitumor factors [83]. Bacteriophages could disturb the microbial composition, disseminate antibiotic resistance genes among bacteria and may form a pool of these genes in the microbial population [84]. No doubt viruses can preferentially kill cancer cells over normal cells they approach and insertion into tumor, and inappropriate conditions in the tumor tissues are some of the challenges that might interfere with their appropriate usage [85].

FMT is another strategy to modulate microbiome, however there remain some complexities such as selection of appropriate donor, the mode of transplantation, stool collection methods and the issues related to efficacy and safety of FMT transplantation are still unknown [86]. In addition, it has been reported that FMT has resulted in bacteraemia, thus strict monitoring of fecal material, intended for transplantation is required, so as to reduce the risk of infection [87]. Furthermore, FMT from unhealthy individuals may lead to chronic inflammation and inflammation induced cancer.

ILCs are mysterious cells and seem to possess immense therapeutic implications. However, there remains a probability of adverse effects of modulation of one subtype on another type of ILCs, immune cells or several other tissue types. Although around five types of ILCs have been identified, they exhibit plasticity thus it is possible that interfering with the functions of one type of ILCs may affect the functioning of other types of ILC subsets [88].

CONCLUSION

It is now clear that human and animals harbour benign and beneficial microorganisms that generally promote human/animal health through their effects on the nutrition, immune function and other physiological systems of the host. Improved understanding of the alterations in the gastro-intestinal tract microbiota during metabolic disorders (e.g., rumen acidosis, diarrhea, mastitis) and stressful conditions (e.g., weaning or lactation peak) are needed to manage the host-microbiome interactions. It is conceivable that resident microbiome may also influence immune response to various injectable and oral vaccines as well as the pathogenesis of infections such as novel SARS-COV-2 (COVID-19), and the fact that individuals differ in their resident microbiome compositions, they thus end up with different disease outcomes. Thus assessment of correlation in microbiome composition and infection sequel might be valuable in devising microbial manipulation strategies as an adjunct to antiviral therapies.

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