

# Role of Microbial Flora and Probiotics in Host Immune Homeostasis

Pratibha Mishra<sup>1\*</sup>, Sunil Kumar Mishra<sup>2</sup>

<sup>1</sup>Department of Pharmacy, Dr B R Ambedkar University, Agra.

<sup>2</sup>Department of Pharmacy, S.N. Medical College, Agra.

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## ABSTRACT

Microbiota refers to an abundant and diverse population of bacteria, archaea, fungi and other microbial eukaryotic species reside in the gastrointestinal tracts and other body sites exposed to the environment. A Microbial symbiosis was established between the host and the microbial flora. The host provides a favorable environment for the colonization of microbiota and in respect to this microbial flora actively participates in the metabolic process of the host, synthesis of vitamins, production of nutrients, enhancement of mucosal barrier functions, and also inhibits the colonization of pathogens. In addition to this Microbial flora have the fundamental role in the development and establishment of host immune homeostasis. Microbiota induces the protective responses against the pathogens and modulates the regulatory pathways in such way to develop the tolerance against the innocuous antigens. Alteration in the composition of microbial flora results in many gastrointestinal diseases including obesity, inflammatory bowel diseases, systemic infections, autoimmune disorders, and even colon cancers. These features of microbiota focus the research on their composition and a wide range of their functional aspects. The objective of this review is to explore the symbiotic relationship between intestinal microbiota and host, the contribution of the microbial flora in host immune homeostasis as well as the concept of 'probiotics' as a possible therapeutic approach to restore the normal gut microbial flora and host immune homeostasis.

## GENERAL ACCEPTANCE AND INTEREST

The gut-associated lymphoid tissue is the largest immunity component, which serves as the major site of lymphocyte to contact with antigens in the daily course of life. Approximately, trillions of bacteria are associated with host gastrointestinal tract. This wide range of the microbial community (gut microbiota) was coevolved in a symbiotic manner with the host intestinal mucosa in such a way that the indigenous microbiota is essential for the gut homeostasis and establishment of innate and adaptive immunity of the host.

Current literature survey reveals that the gut microbial flora participates in a number of important functions including, exchange of the nutrients and metabolic waste, metabolism of the carbohydrates to generate the short-chain fatty acids (SCFAs), produce anti-inflammatory proteins, antioxidants, vitamins and as immunomodulatory. In addition, gut microbiota act as a safeguard

by developing a barrier to prevent direct interaction of pathogens with gut mucosal cells and also compete with pathogens for nutrients and site for their colonization. Gut microbiota by interacting with the intestinal epithelial cells induces the secretion of the antimicrobial peptides and potentially contributes to the development and establishment of the immune system (Vieira *et al.*, 2013).

The composition of the balanced microbial flora disrupted from various factors including diet climate, aging, medication (particularly antibiotic consumption), illness, stress, and lifestyle can upset this balance, leading to diarrhea, mucosal inflammation, or other serious illnesses. Predominantly the consumption of antimicrobials and antibiotics are the foremost issue since their oral administration significantly disrupt the ecology of the beneficial microbial flora and create an opportunity for the pathogens to invade to increase the severity previous infections and cause a new infection. Since, antibiotics were designed with an objective to produce a broad spectrum of activity, so as they can be used to treat a wide range of microbial infections. Because of the broad-spectrum impact of antibiotics, their antimicrobial effect is not selective towards the specific pathogenic population, the

\*Corresponding Author

Pratibha Mishra, Department of Pharmacy, Dr B R Ambedkar University, Agra, Uttar Pradesh - 282002, India.

E-mail: [mishra\\_prati@yahoo.co.in](mailto:mishra_prati@yahoo.co.in)

other related members of microbiota are also targeted, and they often leaving a lethal imprint on the gut community long after the antibiotic therapy removed. Long-term therapy with antibiotics also promotes the development of antibiotic-resistant microbial flora. The antibiotic-resistant strains of microbes also serve as a lasting reservoir for resistance genes in the gut microenvironment (De La Cochetière *et al.*, 2008; Brandl *et al.*, 2008). Consequences result by antibiotic therapy includes: (i) Loss of bacterial ligands responsible for the induction of immune responses in the host, (ii) Alterations in the fate of metabolism of metabolites produced by the microbiota and host, (iii) loss of specific bacterial signals involved in the host immune homeostasis, (iv) Imbalance of Ecology of Microbial Flora, (v) Transformation in Immunity (vi) Emergence of Resistance, (vii) Antibiotic-Associated Diarrhea, and (viii) Antibiotic Associated Genitourinary Tract Infection. In the view of above reported serious consequences, medical practitioners advise probiotics (*live microorganism* - Pharmaceutical Preparations) as an adjuvant with antimicrobial and antibiotic therapies. However, over last few years, the concept of “Probiotics” becomes a new line of therapy in supporting the health benefits and to restore the normal gut microbiota. Nowadays, probiotics are recommended as nutritional adjunct therapies to aid digestion, absorption of nutrients, and restore the dysbiosis of microbial flora. Animal studies data and clinical evidence suggested the effectiveness of the probiotic in the treatment and prevention of acute viral gastroenteritis, post-antibiotic associated diarrhea, and inflammatory bowel disease including Crohn’s disease, enterocolitis and certain allergic disorders (Table 2) (Vieira *et al.*, 2013).

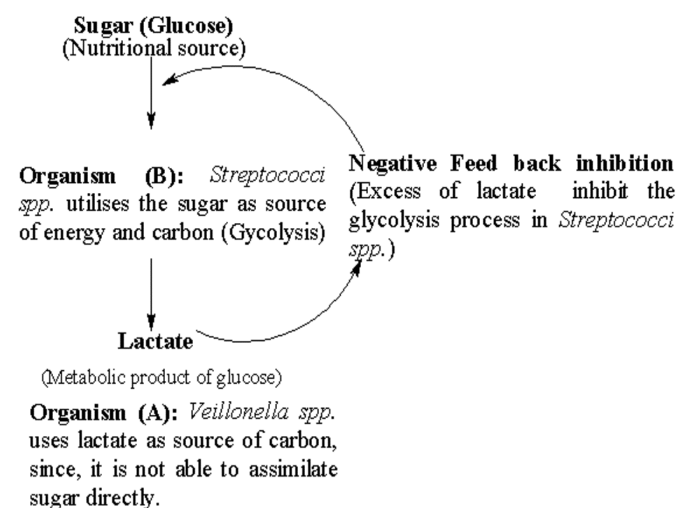
In this review, authors attempt to explore the symbiotic relationship between gut microbiota and host, with evident scientific literature and express the role of the gut microbiota in the development, establishment, and regulation of the various components of the host immune system, supported with recently well-established scientific findings regarding their direct and indirect mechanisms. Furthermore, authors also discuss the major consequences which may result on the dysbiosis of the microbial flora and the concept of ‘probiotics’ as a possible therapeutic approach to restore the normal gut microbial flora and host immune homeostasis.

## MICROBIAL FLORA

The gastrointestinal tract of a fetus is sterile. After birth, primarily large numbers of *E. coli* and *Streptococci* from the environment as well as mother’s oral and cutaneous bacterial flora colonize the gut of an infant. Subsequently, bifidobacteria dominated in Breastfed babies possibly breast milk contains growth factors for these anaerobes (Mishra *et al.*, 2012). While the composition of the microbial flora of formula-fed infants is more diversified and dominated by *Enterobacteriaceae*, *Enterococci*, *Bacteroids* etc. (Rodríguez *et al.*, 2015). Ingestion of solid food, the microbial flora of breastfed infant becomes similar to a formula-fed infant. Subsequent to end of the second year of life, the fecal flora resembles that of normal adults (Mishra *et al.*, 2012; Guarner *et al.*, 2003). In general, the ecology of the microbial flora of gut consists of Anaerobes: *Bifidobacterium*, *Clostridium*, *Bacteroides*, *Eubacterium*, and Aerobes: *Escherichia*, *Enterococcus*, *Streptococcus*, *Klebsiella* (Shanahan, 2002; Vasiljevic and Shah, 2008).

## Cross-feeding Synergism (Syntrophism)

The diversified ecology of the gut microbial flora is mutually dependent on each other for their nutrition (secondary metabolites), colonization, and removal of their waste materials (Jandhyala *et al.*, 2015). This complex phenomenon of co-dependence existence among the microbiota referred as ‘Syntrophism’ (Cross-feeding Synergism). Cross-feeding Synergism is a relationship in which both members are benefited in aspects of their nutrition. It is best exemplified with the cross-feeding phenomenon (Figure 1), suppose an organism (A) unable to utilize the complex form of nutrients directly but on its metabolism by other microorganisms (B) can be easily assimilated by the first one (A). In this manner microorganism (A) is dependent on microorganism (B) for its nutrients but simultaneously microorganism (A) helps (B) by blocking the negative feedback-inhibition from its own metabolite (Martín *et al.*, 2013).



## Cross-feeding synergism

**Fig. 1:** Cross-feeding synergism. *Veillonella* spp. is not able to assimilate sugar (glucose) directly as a source of energy and carbon so, it is dependent on the glycolysis process carried out by *streptococci* (*streptococci* utilize the glucose as a source of carbon and energy and produce lactate as metabolite). On the other hand, an increase in the level of lactate inhibits the glycolysis is a process in *streptococci*, but further utilization of metabolite lactate by *Veillonella* spp. prevents this negative feedback inhibition (Wilson, 2004).

For example,

- Production of biomolecules such as amino acids and Vitamins by one flora served as nutrition for other flora. *Veillonella* spp. produces Vitamin K, which serves as a nutrient for the *Prevotella intermedia* in the oral cavity. Similarly of *Strep. sanguis* which provides p-aminobenzoate for species *Streptococcus mutans* in the oral cavity (Martín *et al.*, 2013).
- Neutralization of substances those are toxic to other flora. For example, plentiful of Lauric acid on the skin is toxic to *Propionibacterium acnes* but it was metabolized by *Malassezia* spp., thus enabling the survival of latter organism (Martín *et al.*, 2013).

- Mucin is an important source of nutrient utilized by the number of bacteria but because of its complex nature (glycoprotein), its breakdown required a wide range of enzymes i.e. sulphatase, sialidase, glycosidase, and protease. Only a few microorganisms possess this set of enzymes, so, that collective cooperation of microorganism is required for the complete degradation of mucin. In gastrointestinal tract *Bacteroides spp.* by degrading Mucin liberates Sulphate which further serves as a source of energy for the growth of *Desulphovibrio spp.* (Martín *et al.*, 2013; Rodríguez *et al.*, 2015).

The composition of the microbial flora of the GI tract varies during the life of the individual and also individuals to individuals (Sujatha *et al.*, 2010). Factors can perturb microbial balance includes diet, climate, aging, medication (particularly consumption of the antibiotics/antimicrobials), illness, stress and lifestyle of the individuals. Imbalance in the ecology of microbial flora results diarrhea, mucosal inflammatory disorders, colon cancers or other serious complications (Mishra *et al.*, 2012). Maintenance of an optimal balance of gut microbial flora needed a barrier, created by the friendly bacteria such as gram-positive lactobacilli and bifidobacteria (>85% of total bacteria flora), which inhibit the invasion and colonization of the pathogenic bacteria (Mishra *et al.*, 2012; Sujatha *et al.*, 2010).

## MICROBIAL SYMBIOSIS

The human microbiota participates in the physiology of the host. Trillions of microbes, including bacteria, viruses, archaea, and eukaryotic microbes, colonize the human body (Wang *et al.*, 2017). There is a symbiotic relationship between the host and the gut microbial flora. Microbial flora actively participates in the metabolic process, gut protection and immunological functions of the host. These features of microbiota focus the research on their composition and a wide range of their functional aspects.

### Importance of microbial flora

#### Synthesis of vitamins

One of the beneficial effects of the microbial flora is synthesis and release of the vitamins, which further absorbed and utilized as nutrients by their host. For example, in humans, enteric bacteria produce Vitamin K and Vitamin B12, and lactic acid producing bacteria secretes other B-vitamins (Todar *et al.*, 2012). Vitamins produced by colonic bacteria include biotin, Vitamin K, nicotinic acid, folate, riboflavin, pyridoxine, Vitamin B12, and thiamine. Vitamin K is a coenzyme that is essential for the synthesis of several clotting factors, including Prothrombin-a, deficiency results in delayed clotting and excessive bleeding. A variety of microbes found in the small and large intestines synthesize Menaquinones (Vit. K2), such as *Bacteroides spp.*, e.g. *Lenta*, *Propionibacterium spp.*, *Veillonella spp.*, *Staphylococci*, *enterobacteria*, and *enterococci* (Table 1).

**Table 1:** Vitamins produced by the human microbial flora (Wilson *et al.*, 2004).

Vitamins	Produced by organism
Vit. K	<i>Bacteroides spp.</i> , Eg. <i>Lenta</i> , <i>propionibacterium spp.</i> , <i>Veillonella spp.</i> , <i>staphylococci</i> , <i>enterococci</i> , <i>enterobacteria</i> .
Vitamin B12	<i>E. coli</i> , <i>Bifidobacterium spp.</i> , <i>Klebsiella spp.</i> , <i>Veillonella spp.</i> , <i>Fusobacterium spp.</i> , <i>Eubacterium spp.</i> , <i>Clostridium spp.</i>
Folic acid	<i>Bif. Bifidum</i> , <i>Bif. Infantis</i> , <i>bif. Breve</i> , <i>Bif Longum</i> , <i>Ent. Faecalis</i> , <i>E. coli</i> , <i>streptococci</i> .
Thiamine	<i>Bifidobacterium spp.</i>
Nicotinic acid	<i>Bifidobacterium spp.</i>
Pyridoxine	<i>Bifidobacterium spp.</i>
Vitamin B2	<i>E coli</i> , <i>Cit. freundii</i> , <i>K pneumoniae</i> ,
Biotin	<i>E coli</i> , <i>Bifidobacterium spp.</i>

#### Nutrient metabolism

Microbial flora drives their nutrients from the dietary carbohydrates of the host. Colonic flora such as *Bacteroides*, *Roseburia*, *Bifidobacterium*, *Fecalibacterium*, and *Enterobacteria*, utilizes the remain undigested host carbohydrate and ferment them to synthesize the short chain fatty acids (SCFA) including butyrate, propionate, and acetate, which further served as a source of energy (Macfarlane and Macfarlane, 2003). The molar ratios of acetate: propionate: butyrate are 71: 21: 8 (compared with 3: 1: 1 in the colon), demonstrating that considerable quantities of butyrate are utilized by colonocytes. In addition, butyrate can also prevent the building up of the toxic metabolites (by-products) such as D-lactate (Bourriaud *et al.*, 2002).

- Acetates are an important source of energy for a variety of tissues, particularly for cardiac and skeletal muscles

and for the brain. The fate of propionate in humans is uncertain.

- The member of microbial flora such as *Oxalobacter formigenes*, *Lactobacillus species*, and *Bifidobacterium species* having the ability to metabolize the oxalate obtained from the carbohydrate metabolism, in this manner decreasing the risk of formation of oxalate stone in the kidney (Sidhu *et al.*, 1998; Magwira *et al.*, 2012).
- The microbiota of genus *Bacteroides* has been shown to synthesize conjugated linoleic acid (CLA) that is known for antidiabetic, antiatherogenic, antiobesogenic, hypolipidemic and immunomodulatory properties (Baddini Feitoza *et al.*, 2009; Devillard *et al.*, 2007; Devillard *et al.*, 2009).

### Xenobiotic and drug metabolism

The gut microbiomes are capable to metabolize xenobiotics and drugs. In a recent study carried by Clayton *et al.*, reported that gut microbial metabolite p-cresol can decrease the ability of the liver to metabolize acetaminophen because of competitive inhibition of hepatic sulfotransferase (Clayton *et al.*, 2009). Another interesting example of microbiota involvement in drug metabolism is the deconjugation of the anticancer drug Irinotecan, induced by microbial  $\beta$ -glucuronidase (Wallace *et al.*, 2010).

### Antimicrobial protection

The commensals microbial flora prevents the multiplication and colonization of pathogenic microorganism by producing toxic metabolites including fatty acids, peroxides, and bacteriocins. In addition, they make a competition with the

pathogenic microbes for the attachment sites and for the essential nutrient provided by the host.

Microbial flora and its metabolites have been shown to stimulate pattern recognition receptor (PRR) mediated mechanism for the synthesis of antimicrobial protein (AMP) such as cathelicidins, C-type lectins, and (pro) defensins by the paneth cells of the host (Hooper, 2009; Salzman *et al.*, 2007). The pattern recognition receptor (PRR) family includes the membrane-associated TLRs, C-type lectin receptors (CLRs) such as Dectin-1 and cytosolic nucleotide binding ligand oligomerization domains (NOD) like receptors (NLRs) (Salzman *et al.*, 2007). Microorganism-associated molecular pattern includes various microbial components such as peptidoglycan, LPS, lipid A, flagella and bacterial RNA/DNA, fungal cell wall  $\beta$ -glucans are mainly responsible for the activation of pattern recognition receptors (PRR) to produce AMP (Takeuchi and Akira, 2010; Carvalho *et al.*, 2012).

**Table 2:** Major Pharmaceutical probiotics and their clinical benefits (Vieira *et al.*, 2013).

Probiotic	Clinical benefits	Mechanisms of action
Culturelle®	<ul style="list-style-type: none"> <li>Prevents rotavirus-related diarrhea in children.</li> <li>Reduces the risk of respiratory tract infections in children.</li> <li>Useful in the prevention of atopic dermatitis in children at high risk of allergy.</li> </ul>	<ul style="list-style-type: none"> <li>Prevention of systemic bacteremia.</li> <li>Improvement of intestinal epithelial homeostasis.</li> <li>Attenuation of local &amp; systemic inflammatory responses.</li> <li>Reduction in lipid accumulation.</li> <li>Secretion of anti-inflammatory substances.</li> <li>Local induction of reactive oxygen species.</li> <li>Production of bacteriocin.</li> <li>Interference in bacterium-induced signaling pathways.</li> </ul>
Enterogermina®	<ul style="list-style-type: none"> <li>Reduces adverse effect and increases tolerability of <i>Helicobacter pylori</i> eradication therapy.</li> <li>Allergic rhinitis in children.</li> </ul>	<ul style="list-style-type: none"> <li>Improvement of growth performance and immune response.</li> <li>The diminishment of intestinal bacterial overgrowth.</li> <li>Antimicrobial and immuno-modulatory activities.</li> </ul>
Florastor®	<ul style="list-style-type: none"> <li>Acute diarrhea.</li> <li>Recurrent <i>Clostridium difficile</i> infection.</li> <li>Antibiotic-associated diarrhea.</li> <li>Travelers' diarrhea.</li> <li>Inflammatory bowel disease.</li> <li>Irritable bowel syndrome.</li> <li>HIV/AIDS-associated diarrhea.</li> <li>Reduction of side effects of <i>H. pylori</i> treatment.</li> </ul>	<ul style="list-style-type: none"> <li>Antitoxin effects.</li> <li>Trophic effects on enterocytes.</li> <li>Anti-inflammatory effects.</li> <li>Enhancement of immune response.</li> <li>Enhancement of levels of disaccharidases.</li> <li>Detoxification of bacterial toxins.</li> <li>Binding to and elimination of pathogenic bacteria.</li> <li>Interference in bacterium-induced signaling pathways.</li> <li>Actions on bacterial virulence factors.</li> <li>Interference in bacterial motility.</li> </ul>
Miyarisan®	<ul style="list-style-type: none"> <li>Antibiotic-associated diarrhea.</li> <li>Reduction of side effects of <i>H. pylori</i> treatment.</li> </ul>	<ul style="list-style-type: none"> <li>Normalization of intestinal microbiota.</li> <li>Antimicrobial effects.</li> <li>Butyrate production.</li> </ul>
Mutaflor®	<ul style="list-style-type: none"> <li>Inflammatory bowel disease.</li> <li>Acute diarrhea.</li> <li>Chronic constipation.</li> <li>Irritable bowel syndrome.</li> </ul>	<ul style="list-style-type: none"> <li>Production of colicines.</li> <li>Adhesin associated with colonization.</li> <li>Resistance to colonization by pathogens.</li> <li>Effects on permeability.</li> </ul>
VSL#3®	<ul style="list-style-type: none"> <li>Inflammatory bowel disease.</li> <li>Pouchitis.</li> <li>Irritable bowel syndrome.</li> </ul>	<ul style="list-style-type: none"> <li>Enhancement of immune response/anti-inflammatory effects.</li> <li>Enhancement of mucosal host defense.</li> <li>Reinforcement of barrier function.</li> <li>Production of angiogenesis-promoting growth factors.</li> <li>Reduces inflammation in Food allergy.</li> </ul>

Interaction of PRR-MAMP (pattern recognition receptor-Microbe Associated Molecular Patterns) results in the activation of a number of signaling pathways which are needed for development and functioning of the mucosal barrier, and production of AMPs, mucin, glycoproteins and IgA (Kim and Ho, 2010). The members of the microbial flora such as *Bacteroides thetaiotaomicron* and *Lactobacillus innocua* are involved as a key role to increase the production of the AMP (Cash *et al.*, 2006;

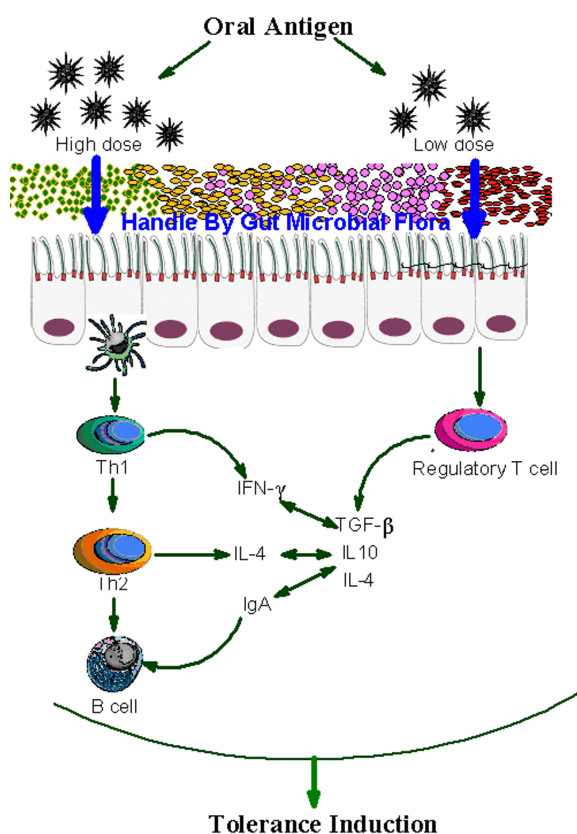
Hooper *et al.*, 2003).

*Bacteroides thetaiotaomicron* induces the expression in Paneth cells for the matrix metalloproteinase, which consequently cleaves prodefensin into active defensins (López-Boado *et al.*, 2000). *Lactobacillus* species produces lactic acid, which by disrupting the outer membrane of the bacterial cell wall facilitate the antimicrobial activity of host lysozyme (Bourlioux *et al.*, 2002).



## ROLE OF MICROBIOTA IN IMMUNITY

The fundamental role of the microbiota in host immunity is to induce, establish and regulating the functioning of the immune system. In response to this immune system helps in the establishment of symbiosis between the host and highly diverse and evolving microbes (Belkaid and Hand, 2014). The host immune system-microbiota symbiosis induces the protective response against the pathogens and modulates the regulatory pathways in such way to develop the tolerance against the innocuous antigens (Figure 2). However, overuse of antibiotics for the quick response against pathogens and changes in the diet results in the shifting of the host immune system-microbiota symbiosis, which may further precipitate as a drastic rise in autoimmune responses and inflammatory disorders.



**Fig. 2:** Mechanism of oral tolerance in relation to the dose of antigen. IFN- interferon; IG-immunoglobulin; IL-interleukin; TGF-transforming growth factor (Isolaauri, 2001).

The microbial flora plays a vital role in development as well as the function of the gastrointestinal immune system. But the immune system in response to this regulates the colonization, composition and/or the action of the microbiota. In the healthy individual, this bi-directional responses between the microbial flora and immune system were well balanced. Imbalance in this symbiotic relationship may lead GI diseases, such as inflammatory bowel diseases and extraintestinal disorders including metabolic problems. In this section, the author mainly focuses on the role of the microbial flora in the regulation of the immune responses in perspective to gastro-intestinal homeostasis, host defense, and pathogenesis of GI disease.

## Structural development of Gut-Associated Lymphoid Tissues (GALTs)

The mucosal-associated lymphoid tissue (MALT) in GIT develops an immunological barrier and is also termed as gut-associated lymphoid tissue (GALT). The GALT can be categorized into:

- **Organized lymphoid tissue.** It is composed of mesenteric lymph nodes, Peyer's patches, microfold (M) cells, dendritic cells (DC) and B cells. The dendritic cells of organized lymphoid work as antigen-presenting cells (APC) that interact with the antigens and communicate with other immune cells such as Native T cells to initiate the immune responses.
- **Diffuse lymphoid tissue.** It presents in the connective tissue of the lamina propria of the gastrointestinal tract. It is composed of the CD4+ T cells, CD8+ T cells also referred as Intraepithelial lymphocytes (IEL), B-lymphocytes (memory and plasma cells that produce type A-immunoglobins (IgA)), and natural killer cells (NK cells) (Erickson *et al.*, 2000).

The mucosal barrier of the intestinal tract is highly selective and intelligent to distinguish the pathogens and commensal bacteria thereby it preventing the entry of toxic metabolites, antigens and pathogens into systemic circulation (Di Giacinto, 2005). Its hypo-responsiveness towards commensal bacteria and their beneficial nutrients as well as the ingested material is because of the predominant response of the GALT. This hypo-responsiveness is termed as 'tolerance' mediated by both T- and B-cells. In general development of oral tolerance prevents the immunogenic responses but in case of suboptimal oral tolerance hypersensitivity reactions may result against the ingested antigens. For instance, if an individual is not able to tolerate the milk proteins, then a hypersensitivity reaction may result to dairy products. This hypersensitivity reaction often commences with the polarization of either T helper cells-1(Th1) or T helper cells-2(Th2) cell (Di Giacinto, 2005; Isolaauri *et al.*, 2001).

## Development of humoral immune system

Immune responses are due to the release of specific cytokines to stimulate the native T-helper (Th) cells to turn on either Th1 domination to promote cellular immunity or Th2 domination for humoral immunity (Figure 3). However, if there is an imbalance in release and level of cytokines sustained, then the higher level cytokines induce their respective T-cell pathways to produce immune pathological conditions like atopy, hypersensitivity reactions and chronic inflammation (Leah *et al.*, 2009).

This has been recently accepted that supplementation with probiotic preparations helps to modulate the immune system by restoring microbial flora (Neurath *et al.*, 2002). Probiotic preparations have an ability to modulate the components of an immune response including humoral, cellular or innate immunity (Smith *et al.*, 2007). Recently this has been accepted that specific probiotic strains induce the secretion of specific cytokines thereby they facilitate the development of native T-cells towards a particular immune pathway (Smith *et al.*, 2007).

Germ-free (GF) model was used to evaluate the importance of the microbiota for the establishment of both innate

and adaptive immunity (Rakoff-Nahoum *et al.*, 2004). Alternate approaches to validate the importance of the microbial flora in immune homeostasis are either with antibiotic treatment or reconstitution of microbiota. These approaches are also implacable for the study of the role of microbiota in autoimmunity since the gut microbial flora not only participates in the local intestinal immune system but also actively involved to influence the systemic immune responses (Mazmanian *et al.*, 2005; Gordon *et al.*, 1966).

Furthermore, the GF animals have the imbalanced development of the GALTs, such as Peyer's patches (PPs) and isolated lymphoid follicles (Bouskra *et al.*, 2008; Kamada *et al.*, 2013). This has been noticed that there are increased number of the immunoglobulin (Ig) E+ B cells and decreased numbers of IgA+ B cells were found in the PPs of GF mice. Colonization of commensals microbiota in GF mice induces an immune reaction in lymphoid cells clusters referred as germinal centers and increase and development of IgA.

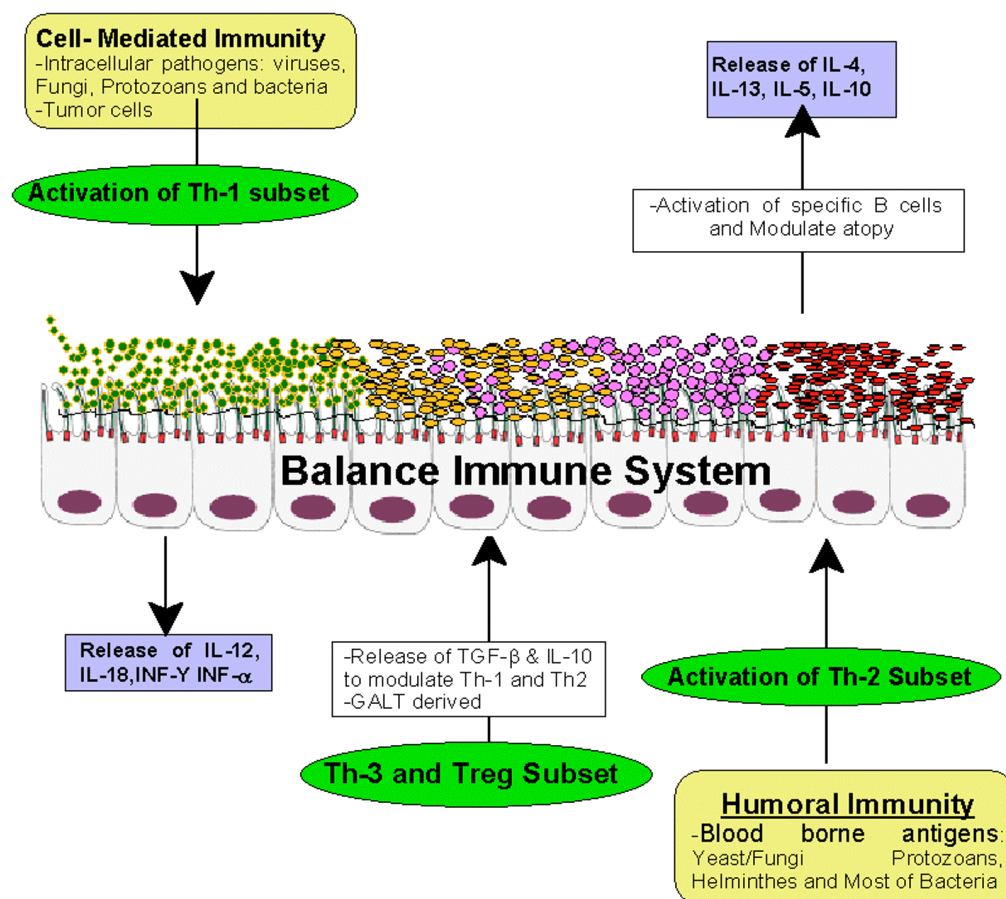


Fig. 3: This picture represents the three subsets Th1, Th2, and TH3/T (reg.) of the humoral immune system (Belkaid and Hand; 2014).

### Development and balancing of effectors T cells immune responses

Microbial flora also involved in balancing the effector's T-cell immune responses in the GI tract (Figure 4) (Chung *et al.*, 2012). In germ-free mice, there is decreased number of T-helper (Th-1) and Th-17 cells since the intestinal T-cell immune response in GF animals is primarily controlled by Th-2 cells (Gaboriau-Routhiau *et al.*, 2009). The imbalance T-cell immune response in GF mice can be balanced by reconstitution with conventional microbiota, indicating that the microbial flora shapes the Th-1 cell-mediated immunity (Bouskra *et al.*, 2008). Recently this has been accepted that commensal Clostridia-related bacteria, called segmented filamentous bacteria (SFB), were involved in the induction of Th17-cell development in the small intestine (Ivanov *et al.*, 2009; Round *et al.*, 2010). Furthermore, SFB is not the only bacteria that can induce Th17 cells colonization of GF mice with Altered Schaedler Flora (ASF), a cocktail of 8 defined commensals,

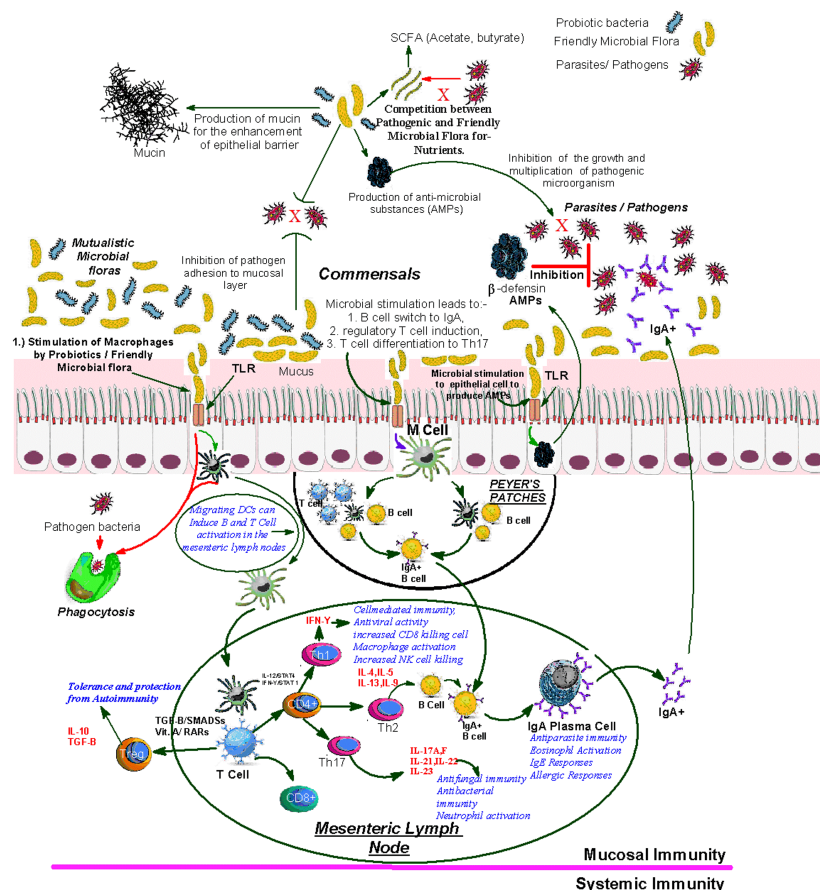
also significantly increased the numbers of Th17 cells, although the ASF is less effective than SFB (Round *et al.*, 2010).

### Establishment of T regulatory (T-reg) cells

Intestinal microbial floras were also essential for the establishment and function of the Foxp3<sup>+</sup> Treg cells in the intestine (Figure 4). In condition of the imbalanced microbial flora or in GF animals the numbers of Foxp3<sup>+</sup> Helios<sup>-</sup> Treg (iTreg) cells were significantly reduced in the colonic lamina propria (LP), but not the LP of the small intestine or mesenteric lymph nodes (Round *et al.*, 2011; Fagarasan *et al.*, 2010). Colonic T-reg cells can be restored with re-colonization of the microbial flora in GF animals. Bacterial species such as *Clostridia*, *Bacteroides spp.*, *Lactobacillus*, or SFB has been reported to possess Treg inducing activity. Colonization of mice with *Bacteroides fragilis*, a human commensal, robustly facilitates the differentiation of Treg cells and interleukin (IL) 10 production by Treg cells, whereas

The intestinal microbial flora also involved to influence the immunoglobulin responses within the intestinal mucosa,

such as IgA regulates the intestinal homeostasis. It means that commensal microbial flora participates in the development of the intestine-specific B-cell receptors representative. Most of the IgA producing mature B-cells in Peyer's of patches on the stimulation by resident microbial flora (Figure 4), the possible mechanism behind this is the participation of the microbiota in the development and organization of the GALTs. The immature formation of the germinal centers in the PPs and reduced generation of IgA-producing B-cells in the GF mice is because of the lack of the microbiota-derived signals for these events (Sonnenberg and Artis, 2012).



**Fig. 4:** Contribution of Microbial flora in the Mucosal Immune Homeostasis. T cells and CD4+ cell are a chief component of the adaptive immune system, located in the lamina propria of the intestine. On stimulation of native CD4+-T cell, they are differentiated into four major subtypes: T helper 1 (Th1), Th2, Th17, or regulatory T cell (Treg). Th1 cells are critical for the host defense against intracellular microbial infection, while Th2 cells play an important role in eliminating parasite infections. Th1 and Th17 responses have been linked to autoimmune diseases while the Th2 response has been associated with allergic reactions. Treg is a key mediator of immune tolerance; its dysfunction can lead to autoimmune disorders (Willing *et al.*, 2011; Ohland *et al.*, 2015).

The signals and metabolites produced by the microorganisms are sensed by the hematopoietic and non-hematopoietic cells of the innate immune system, which were further translated into physiological responses (Thaiss *et al.*, 2016). The innate lymphoid cells (ILCs) are the representative of the innate immune system and they function in association with the T-cells. ILC is originated from the lymphoid precursors, grouped into (Satoh-Takayama *et al.*, 2008): *Group 1*: T-bet+; *Group 2*: GATA-3+; and *Group 3*: RORγt+.

Recently this has been evidently established that group 3 (ROR $\gamma$ +) ILCs participating in the development of the intestinal immunity. These ILCs are similar to the Th-17 cells in the stimulation of the cytokine profile (production of IL22 and/or IL17 upon IL23 and IL1 $\beta$  stimulation) (Sato-Takayama *et al.*, 2008). Some Studies also reveals that commensal microbial flora is involved in the differentiation of group 3 ILCs, since the proportion of ROR $\gamma$ +NKP46+CD127+NK1.1- or ROR $\gamma$ +NKP46+CD127+NK1.1 into ILCs is consistently decreased in GF mice (Franchi *et al.*, 2012; Kamada *et al.*, 2005).



In recent studies this has been reported that in GF mice the production of the IL22 was significantly reduced, this indicates that the function of the ILCs is regulated by commensals (Franchi *et al.*, 2012).

### Development of lamina propria phagocytes

Mononuclear phagocytes such as macrophages and dendritic cells (DCs) and polymorphonuclear phagocytes such as neutrophils are responsible for the protection of the organs and GI tract against microbial infections. The resident microbiota potentiates the production of pro-IL1 $\beta$  (the precursor of IL1 $\beta$ ) in resident macrophages. Nevertheless, full processing and secretion of pro-IL $\beta$  do not take place in phagocytes until they encounter the pathogenic microorganism. For example, Pathogens such as *Salmonella* and *Pseudomonas aeruginosa* can promptly induce secretion of the mature form of IL1 $\beta$  from intestinal macrophages, which contributes to the elimination of infectious pathogens (Kamada *et al.*, 2005).

Unlike to microbial flora, this pathogen by injecting flagellin into the cytosol through type III secretion systems, activate the Nod-like receptor (NLR) C4 inflammation to induce caspase-1-dependent processing of pro-IL1 $\beta$  (Kamada *et al.*, 2005).

The resident microbial floras also potentiate the anti-inflammatory responses of intestinal macrophages by stimulating them to produce anti-inflammatory cytokine IL10, which is needed to maintain the GI homeostasis (Rivollier *et al.*, 2012; Zhou *et al.*, 2007). Intestinal microbial flora also modulates the function of neutrophils, during intestinal infection with pathogen *Clostridium difficile*, the commensals translocated into the intestinal tissue, where they induce expression of pro-IL1 $\beta$  in recruited neutrophils. The resident microbial flora is therefore involved in the regulation and function of intestinal phagocytes.

In response to the pathogen invasion the LP mononuclear phagocytes, including macrophages, express pro-IL1 $\beta$  for the rapid production of mature IL1 $\beta$  (Kamada *et al.*, 2005; Rivollier *et al.*, 2012). The building up of pro-IL1 $\beta$  in LP macrophages are required MyD88 signaling produced or induced by microbial flora (Rivollier *et al.*, 2012). Similarly, the anti-inflammatory function of intestinal macrophages such as the impulsive production of IL-10 is also controlled by the microbial flora.

## ROLE OF MICROBIAL FLORA IN THE REGULATION OF IMMUNE SYSTEM

### Induction and regulation of intestinal Th17 cells

*In-vitro* studies reported that the differentiation of the Th-17 cell was regulated by transforming growth factor (TGF) $\beta$  and IL6 or IL21 while their expansion is mediated by IL-23 (Shaw *et al.*, 2012; Hasegawa *et al.*, 2010). There is reduced numbers of intestinal Th-17 cell in mice deficient with IL1 $\beta$  or the IL1 receptors. This has been reported that in GF mice the production of IL1 $\beta$  decreased in resident intestinal phagocytes. Administration of IL1 $\beta$  is sufficient to induce development of intestinal Th17 cells in GF mice. The intestinal microbiota, therefore, appears to promote the development of Th17 cells, by inducing the IL1 $\beta$  but not IL6 (Hasegawa *et al.*, 2010).

Mice deficient in MyD88 adaptor have a low level of IL1 $\beta$  and decrease in the number of TH17 cells. MyD88 an

adaptor required for the Toll-like receptor (TLR), IL1-like, and IL18 receptor signaling, involved in to increase the level of IL1 $\beta$  and development to TH17 cells in the intestine. Microbial flora might, therefore, induce IL1 $\beta$  production via TLR-MyD88 mediated signaling in the innate immune response pathway (Smith *et al.*, 2013). Studies carried by Atarashi *et al.* reported that luminal ATP produced by the microbiota activate the DCs in the Lamina Propria (LP) which further promotes the development of the TH17 cell. Nevertheless, the microbial flora maintains the balance of the effectors-T cell populations-mainly in concern of Th17 cells. However, more research is required to established and understand the mechanism by which microbial flora induce the Th17 cell in the intestine (Atarashi *et al.*, 2015).

### Induction and regulation of Foxp3+-inducible Treg cells

Metabolites produced by the commensals microbial flora were reported to induce the development of Treg cells. Short-chain fatty acids (SCFAs) have been proposed to regulate development and function of colonic Treg cells. Fall down in the concentration of SCFAs such as acetate, propionate, isobutyrate, and butyrate in GF mice connected with the non-functional development of intestinal Treg cells. Furthermore, again restoring of the microbial flora or supplementation with SCFAs in GF mice increase the number of Treg cell, indicating the role of the bacterial metabolite in Treg cell development. SCFAs affect Treg cell function via epigenetic regulation of the Foxp3 gene, particular butyrate, can act directly on T cells, increasing acetylation of the Foxp3 locus (Atarashi *et al.*, 2015; Arpaia *et al.*, 2013). Likewise, dietary folic acids regulate the survival of Foxp3+ Treg cells in the colon (Rossi *et al.*, 2011). Animals having impaired or non-functioning synthetic pathways for de novo production of folic acids, then folic acids obtained from the microbial flora of intestine are likely to be involved in the induction of intestinal Treg cells (Coombes *et al.*, 2007).

Although this has been need to establish that LP DCs may appear to be involved in the mechanism by which SCFAs, via transforming growth factor- $\beta$  (TGF- $\beta$ ), promote the development of Treg cell. CD103-expressing subsets of DCs in the LP (including CD103+CD11b+CD11c+ and CD103+CD11b-CD11c+) induce differentiation of Treg cell from native CD4+ T cells via TGF $\beta$  and retinoic acid (RA) (Mazmanian *et al.*, 2008). Treg cell induction by the microbial flora protects the mice from-colitis, infection with enteric pathogens, and allergic diarrhea. For instance, *Clostridium*-induced Treg cells suppress colitis and allergic diarrhea in mice. Similarly, Treg cell induced by *B. fragilis*, reduce the intestinal inflammation caused by *Helicobacter hepaticus* (Suzuki *et al.*, 2010). These studies indicate that generation, maturation, and function of Treg cell were induced by the intestinal microbial flora and their metabolite, which were further needed to maintain the GI homeostasis.

### Induction and regulation of IgA-producing B cells

Commensals microbial flora regulates the development of IgA producing cell in the intestine. Commensals microbiota activates MyD88 signaling in LP DCs and follicular DCs-Cells that promotes the generation of IgA+B cells. In response to microbial stimulation, follicular DCs in Peyer's Patches (PPs) secrete-TGF $\beta$ , CXCL13 and B-cell activating factor (BAFF, a member



of the TNF family), which further stimulate the induction and IgA production (Rossi *et al.*, 2011). Similarly, the microbial flora activates the MyD88 signaling in the subsets of LP DCs, which further leads to their expression of RA, TGF $\beta$ , TNF $\alpha$ , inducible nitric oxide synthetase, BAFF, and proliferation-inducing ligands to promote the generation of IgA+B cells (Umesaki *et al.*, 1999). However, the microbial flora is involved in the development and maturation of intestinal B cells, but the specific bacteria have not been identified which mediate these process. Mono-colonization of GF mice with SFB, in addition to TH17 cells, can induce IgA-11 production (Umesaki *et al.*, 1999). However, the amount of IgA in SFB mono-colonized mice is low compared to mice colonized with conventional microbiota, indicates that other bacterial species, or mixtures of commensals microbial flora, are needed for maximal induction of intestinal IgA-11.

## MAJOR CONSEQUENCE OF IMBALANCE OF THE MICROBIAL FLORA

In general, the gut microbial flora and the host survive in a symbiotic manner. Dysbiosis of this balance results many diseases for example:

- Pseudomembranous colitis because of toxin produced by *Clostridium difficile* during antibiotic therapy and surgery.
- Sepsis because of the colonization of *Escherichia coli*, *Enterococcus faecalis*, and *Enterococcus faecium*, and
- Intra-abdominal abscesses due to *Bacteroides fragilis* (Elliott *et al.*, 2003).

In addition, many other diseases were also resulted because of the perturbation of the microbial floral balance in GIT, includes inflammatory bowel diseases, obesity, diabetes, liver diseases, chronic heart diseases, cancers, HIV, and autism (Zhang *et al.*, 2015). In general, the microbiota of the healthy adults was dominated with four major bacterial phyla-about 90% of the microbial flora is constituted with obligate anaerobes i.e. Firmicutes and Bacteroidetes, and to a lesser extent by Proteobacteria as well as Actinobacteria (Gevers *et al.*, 2012). However, the composition of the gut microbiota of IBD subjects undergoes marked alterations at both taxonomical and functional level (Kostic *et al.*, 2014; Manichanh *et al.*, 2012). The abundance of both *Bacteroidetes* and *Firmicutes* are significantly decreased, while those of *Actinobacteria* and *Proteobacteria* are significantly increased in IBD. Concurrently, fall down in the level of the protective anaerobic microbiota generally Firmicutes (e.g. *F. prausnitzii*, *Clostridium spp.*) and Bacteroidetes (e.g. *B. fragilis*), and with an increase in colonization of facultative aerobes such as enterobacterium (Phylla: *Proteobacteria*), cause imbalance of the microbial flora and results in mucosal intestinal inflammation.

The structural imbalance of the microbial flora produce further consequence includes malfunctioning in function of the microbiota such as bacterial amino acid biosynthesis and carbohydrate metabolism with enhanced uptake consumption of nutrients (Morgan *et al.*, 2012). In addition, the production of the bacterial metabolites SCFAs known for the immunosuppressive function was also suppressed (Morgan *et al.*, 2012; Singh *et al.*, 2014; Smith *et al.*, 2013). In a recent study by Rooks *et al.*, on

animal model *T-bet*  $\times$  *Rag2* ulcerative colitis (TRUC) mouse for ulcerative colitis reported (Rooks *et al.*, 2014):

- An increase in the bacterial motility.
- Tetrathionate respiration (metabolic pathway) which promote the colonization of the pathogenic *Salmonella enterica* subsp.
- *Typhimurium* in the inflamed gut (Winter *et al.*, 2010).
- Benzoate degradation (metabolic pathway) associated with growth and virulence of *Enterobacteriaceae* in active colitis (Freestone *et al.*, 2007; Lyte *et al.*, 2011).

## Inflammatory bowel disease

Inflammatory bowel disease (IBD) is characterized as ulcerative colitis (UC) and Crohn's disease (CD). This is because of the aggressive Th-1 mediated cytokine response against the luminal antigen of commensal bacteria. Besides this fault bacterial recognition by macrophages is strongly associated with the pathogenesis of IBD. Ulcerative colitis (UC) is one of the two major idiopathic IBDs. The pathogenesis of ulcerative colitis is associated with an imbalance in the composition of the microbial flora i.e. there is decreased in the number of beneficial bacteria such as *Lactobacilli* and *Bifidobacteria* with increased in the colonization of pro-inflammatory bacteria such as *Enterobacteriaceae*, and *Bacteroides fragilis* (Mishra *et al.*, 2012).

In an investigation by Macpherson AJ on animal colitis model, they reported that *E. coli* may be served as a biomarker for the severity of the colitis. They found that pathogenesis of colitis is associated with higher *E. coli* colonization and with a high load of bacterial TLR2 ligands. Bacterial TLR2 ligands and their products intensify the acute inflammation via TLR2 and TLR-4 signaling and potentially trigger the TLR-dependent accumulation of neutrophils and T cells (Macpherson *et al.*, 2007; Kwon *et al.*, 2010).

## Crohn's disease (CD)

Another type of IBD is Crohn's disease (CD). This has been accepted that Crohn's disease is an autoimmune disorder, involves the activation of the immune system against the normal flora of GIT and causes inflammation of Mucosa (Bhattacharjee, 2012). Seksik *et al.* found a significant increase in the enterobacteria in fecal microflora of both active and inactive Crohn's disease patients than the healthy individuals (Seksik *et al.*, 2003). In one more study, this has been reported in Crohn's disease patients that there is a decrease in the number of *Dialister invisus*, *Clostridium* cluster XIVa (an uncharacterized species), *Faecalibacterium prausnitzii* and *Bifidobacterium adolescentis* with an increase in the colonization of *Ruminococcus gnavus* (Joossens *et al.*, 2011).

Commensal *E. coli* strain in Crohn's disease induce the release of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-8 (IL-8) from the inflamed mucosa. On the other hand, some strains of *Lactobacilli* such as *L. casei* suppress the inflammatory responses and also downregulate the spontaneous release of TNF- $\alpha$  induced by *E. coli* (Tighe *et al.*, 2011). Therefore, the approach of the treatment of the CD may be based to eliminate some bacteria with antibiotics or to balance the gut flora with more beneficial bacteria, by the use of probiotics and prebiotics (Fujimori *et al.*, 2007). Proposed approaches to manage the CD include:

- by improving the short chain fatty acids (SCFA) production patterns (Rooks *et al.*, 2016),
- improving Th1/Th2 ratios, by decreasing the pro-inflammatory cytokine secretion,
- inhibiting the colonization of pathogens, and
- improving and enhancing mucosal barrier function.

In 1917, this has been accepted that *E. coli* Nissle is a nonpathogenic strain of *E. coli*, which suppresses the growth of enteropathogenic bacteria. It was proposed that *E. coli* Nissle, by inhibiting the colonization of enteropathogenic bacteria, may produce a long-term suppression of remission of CD. In an investigation by Malchow, on 28 patients suffering from active CD, were treated with a tapering dose of prednisolone and either placebo or *E. coli* Nissle. The *E. coli* Nissle was given for 24 days in an increasing dose to the last dose of  $5 \times 10^{10}$  bacteria per day. They assessed for the remission and reported that the patients in the placebo group have higher relapse rate about 63.6% as compared to the *E. coli* group which is 33.3% (Malchow, 1997).

### Consequences of obesity

Commensal microbial flora plays an important role in controlling the diet-induced obesity (Karimi *et al.*, 2015). This has been reported that Germ-free mice found to be thinner with a less adipose tissue even when subjected to a high-fat diet (Ding *et al.*, 2010). The high-fat diet promotes composition of microbial flora with a higher level of luminal *Firmicutes* and *Proteobacteria* and lower levels of *Bacteroidetes* (Hildebrandt *et al.*, 2009). This reveals that obesity may be associated with the ecological composition of the gut microbial flora. SCFAs such as Butyrate and propionate were reported to lower the incidence of the diet-induced obesity by activating the complementary mechanism for intestinal gluconeogenesis (IGN). Butyrate directly activated Intestinal gluconeogenesis gene expression via an increase the level of cAMP in enterocytes, while propionate acts as free fatty acid receptor-3 (FFAR-3) agonist and promotes the glucose metabolism (Morgen *et al.*, 2014). Conjugated linoleic acid (CLA) has also been reported for its anti-obesity effects. There were six strains of bacteria (four *Bifidobacterium breve* strains, a *Bifidobacterium bifidum* strain and a *Bifidobacterium pseudolongum* strain) from the GIT microbial flora have been reported for their capability to produce different CLA and conjugated  $\alpha$ -linolenic acid isomers from free linoleic acid and  $\alpha$ -linolenic acid available from diets, such as food products from natural, and processed cheeses, beef, milk fat, yogurt, and plant oil (Gorissen *et al.*, 2010). Dietary probiotic and probiotics preparations, namely synbiotics, can also be used to control the obesity (Mozaffarian *et al.*, 2011; Solis *et al.*, 2010). Several studies reveal that eating yogurt amazingly prevents age-associated weight gain (Safavi *et al.*, 2013).

### Consequences of cancers

The pathogenesis of colon cancer generally associated with the imbalance of the intestinal bacterial community or infestation pathogens. Gut bacteria by triggering macrophages produce diffusible clastogens or chromosome-breaking factors which may further mediate as DNA damage and chromosomal instability in neighboring cells (Zhang *et al.*, 2015). Intestinal

preoxidative stress may also contribute bacteria-associated intestinal cancers (Chu *et al.*, 2003).

Butyric acid is a vital nutrient for the colon cells since it reduces the incidence of proliferation and induction of apoptosis of human colon cancer cells. The level of butyric acid was significant decreases in the stool samples of colon cancer patients compared to healthy subjects since there is a decrease in the colonization of butyrate-producing bacteria such as *Ruminococcus spp.* and *Pseudobutyrvibrio ruminis* (Zhang *et al.*, 2015).

In an investigation this has been reported that cyclophosphamide distorted the balance of microbial flora in the small intestine of mouse models and it also promotes the translocation of some selected species of Gram-positive bacteria in the secondary lymphoid organs, which further stimulate the generation of a specific subset of “pathogenic-T-helper-17” (pTh17) cells and memory Th1 immune responses (94). Germ-free mice treated with antibiotics or bearing tumor, inhibit the colonization of the gram-positive bacteria which results in a fall in the pTh17 responses and their tumors were also resistant to cyclophosphamide. The results indicate that the gut flora may involve in the shape and regulation of the anticancer immune response (Viaud *et al.*, 2013). Dietary supplementation of *Bifidobacterium longum* significantly suppresses the incidence of the colon tumor, multiplicity, and volume of the tumor (Singh *et al.*, 1997). *Bifidobacterium longum* exhibit the inhibition of the azoxymethane-induced cell proliferation, ornithine decarboxylase activity and expression of ras-p21 oncoprotein activity significantly (Safavi *et al.*, 2013).

### CONCEPT OF “PROBIOTIC” (Live-Microorganism Pharmaceutical Preparations)

Probiotic is the preparation containing beneficial and nonpathogenic bacteria meant for the oral administration along with dietary supplements to improve and restore the microbial flora of gastrointestinal tract (Johnston *et al.*, 2012). Probiotics are defined by the World Health Organization as “live microorganisms that can provide benefits to human health when administered in adequate amounts, which confer a beneficial health effect on the host” (WHO/2001). Probiotic are often found as a better approach and effective preparations in the treatment of wide range of disease associated imbalance of the microbiota of gastrointestinal tract such as *C. difficile* infection, antibiotic-associated diarrhea, and acute infectious diarrhea (Johnston *et al.*, 2012; Videlock *et al.*, 2012). Probiotics establish the microbial balance through various mechanisms including preventing the colonization of pathogenic organism by means of their ability to compete for nutrients or producing antimicrobial proteins, modifying the immune responses, improving the mucosal barrier functions, lowering the pH of GIT and also by detoxifying the ingested carcinogens (Johnston *et al.*, 2012). Nowadays, microbial genera of *Lactobacillus* and *Bifidobacterium* are considered as safest for the probiotic preparations.

- *Lactobacillus* genera: *acidophilus*, *brevis*, *casei*, *fermentum*, *gasseri*, *johnsonii*, *paracasei*, *plantarum*, *delbrueckii*, *rhamnosus*, *reuteri* and *salivarius*.
- *Bifidobacterium* genera: *adolescentis*, *animalis*, *bifidum*, *breve*, and *longum* (Hill *et al.*, 2014).

In addition, microbial strains belonging to

Propionibacterium and Streptococcus (particularly *thermophilus*) were also considered for the probiotics preparations. This has been reported that dairy *propionibacterium* are able to restore the gut microbial balance, inhibit the colonization of the pathogenic microbes and also participate in the immunomodulatory activity (Zarate *et al.*, 2012), while *S. thermophilus* is able to produce excess of the enzyme lactase, hence, effective in the prevention of lactose intolerance (Rul *et al.*, 2012).

The world's most documented and recommended probiotics preparation includes, strains *Lactobacillus rhamnosus* GG (Valio®), *Lactobacillus paracasei* Shirota (Yakult®) and *Bifidobacterium lactis* BB12 (Chr. Hansen®), are reported effective in the treatment of infections including *Clostridium difficile* infection, protection against *Helicobacter pylori* infection, rotaviral diarrhea, antibiotic-associated diarrhea, Travelers' diarrhea, as well as some other bacterial diarrheas. They are also prescribed by the doctors in metabolic imbalance such as lactose intolerance and in immune response modulation (Hui, 2006). The major physiological mechanisms of probiotic strains are based on their ability to (Otlés, 2013) produce of inhibitory substances i.e., hydrogen peroxide or bacteriocins to inhibit the colonization gram-positive and gram-negative bacteria, compete with pathogenic microbes for their nutrients, attachment on the intestinal epithelial surface and by blocking attachment sites, as well as by modulating and activating the immunity system against pathogens.

### Probiotic: An approach to restore the microbial flora

Probiotic preparations influence the several pathways of immune responses including humoral, cellular and innate immunity. Recently, this has been accepted that probiotics preparations can influence the secretion of cytokines and also involved to direct native helper T cells for Th1 mediated Cellular immunity or Th2 mediated humoral immune responses.

#### Th1 cell-mediated immune response

Th1 pathway induces the cell-mediated immunity and prevents the infections associated with intracellular pathogens such as viruses, certain bacteria, yeast, fungi, and protozoans. Th1 cell-mediated immunity also plays an important role in preventing the incidence and development of the tumor. Continuous stimulation of the native T cell for Th1 cell pathway produces a large amount of pro-inflammatory cytokines including IFN- $\gamma$  and TNF- $\alpha$ . These cytokines further activate macrophages to produce additional pro-inflammatory mediators i.e. IL-12 and IL-18. A persistent Th1 cell-mediated inflammatory episode of the gastrointestinal tract is the main etiology behind the several pathological conditions such as Crohn's disease, *H. pylori* gastritis, cellular autoimmunity, chronic recurrent inflammation and possibly rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus (Leah *et al.*, 2009). Certain probiotic strains help to down-regulate over activation Th1 cell-mediated immunity responses and restore the balance between Th1 and Th2 mediated immune responses.

Overproduction of the TNF- $\alpha$  via Th1 cell-mediated immunity plays a key factor to induce the inflammation of gastrointestinal tract in the pathogenesis of the Cohn's disease involved in the In an investigation by Borruel *et al.* studied the effect of probiotic bacteria on the TNF- $\alpha$  production in the ileal specimens of 10 patients suffering from Crohn' disease. They found

a significant fall in the production to TNF- $\alpha$  by the inflamed mucosa when cultured with *L. casei* or *L. bulgaricus*, but not with *L. crispatus* or *E. coli* (Borruel *et al.*, 2002). They reported that probiotic strains have an ability to interact with the immune-competent cell and inhibit the release of the pro-inflammatory cytokines.

#### Th2 cell-mediated immune response

Th2 cell pathway regulates the humoral immunity responses. It acts by inducing the release of cytokines IL-4, IL-13, IL-5 and IL-10 that activate the formation and release of specific B cells, mast cell and/or eosinophils (Kidd, 2003). Persistent activation of Th2 cell pathway leads atopy, ulcerative colitis, and eosinophilic rhinosinusitis. This has been accepted that Extracellular proteins secreted by probiotic bacterial strains can able to diffuse into the mucus layer and interact with the epithelial and immune cells. The interaction of extracellular proteins with the epithelial and immune cells generate signals which were further communicated to the nucleus of these cells via several cascades of biochemical pathway including mitogen-activated protein kinases (MAPKs), phosphatidylinositol 3-kinase (PI-3K) and glycogen synthase kinase-3 (GSK-3) (Hoarau *et al.*, 2006).

Activation of these biochemical cascades initiate signaling pathways and cellular responses such as secretion of chemokines, cytokines, antibacterial proteins (AMP), secretion of mucus and also induces changes in the surface properties of mucosal barrier including rearrangement of tight junctions and modulation in the immune responses of gut-associated lymphoid tissue (GALT) cells (102). For example, 'Serpin' extracellular proteins secreted by the probiotic bacterial strain of Bifidobacteria species (*Bifidobacterium breve*, *Bifidobacterium dentium*, and *B. longum* subsp. *infantis*) improve and enhances the mucosal barrier functions and also able to modulate the GALT immune function, in animals and cellular models. In a recent study, this has been found that *B. longum* subsp. *Infantis* secretes uncharacterized extracellular proteins which improve and enhances the mucosal barrier functions by increasing the production of zonula occludens-1 and occludin, the two tight-junction proteins (TJPs) in epithelial cells (Ewaschuk *et al.*, 2008). A Probiotic strain *lactobacilli acidophilus* NCFM cell secretes S-layer protein A (SlpA) which induces the production of IL-10 in the dendritic cells by doing so it produces anti-inflammatory responses (Konstantinov *et al.*, 2008).

### Conclusions and prospects

The main focus of this review is to understand and explore the role of microbial flora in the regulation of the host immune system. Microbial flora also actively involved in the metabolic process of the host, synthesis of vitamins, production of nutrients, enhancement of mucosal barrier functions, guts protection and also inhibits the colonization of pathogenic microorganism. There is a symbiotic relationship between the need of both host and microbial flora, and even between the different strains of the microbial flora in respect of their nutrients or removal of their waste material. Furthermore, more studies were needed to investigate symbiotic relationship and functional aspects of millions of bacterial strain available in our gastrointestinal tract.

This has been accepted that alteration in the composition of gut microbiota perturbs the microbial ecology which may result in many diseases such as obesity, systemic infections,



IBD, autoimmune disorders, and even colon cancers. Nowadays, probiotics and prebiotics have been widely clinically practiced to restore the imbalanced microbial flora as an alternate strategy of treatment, but still, more studies and clinical trials were required to explore the exact basis of pathogenesis and how and which microbial flora strain can be a remedy for diseases, associated with the microbial flora of GIT.

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## CONFLICT OF INTERESTS

There are no conflicts of interest.

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