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A REVIEW ON PROBIOTICS AS IMMUNOMODULATORS

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Abstract:
The present study focuses on various aspects of probiotics and their effects on immune system. Probiotics are live organisms which when taken in adequate amount imparts therapeutic effects on the host. The probiotics are obtained from various sources of microbes mainly from Lactobacilli and Bifidobacteria species. Probiotics acts on both types of immunity i.e adaptive and innate immunity and are responsible to activate T-cells, B- cells, NK cells which protects the body from various infections. Overall probiotics can be used in prevention/treatment of various types of diseases such as diarrhoea, ulcerative colitis, Irritable bowel syndrome etc. without disturbing the microbial balance of the body.

Keywords: *Probiotics, Immune system, Immunomodulators.*

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INTRODUCTION:

The term "probiotic" is obtained from Greek which means "for life". The history of probiotics started with the consumption of the fermented foods by Greeks and Romans. As far as it is known that first food containing living microorganisms was the fermented milk it has been written in an old literature. [1] The term probiotics was first used in 1950s as opposite of antibiotics. [2,3]. Introduction of antibiotics has initiated the revolution in the field of medicines. After introduction of antibiotics, life expectancy has been increased and it also improved the quality of human life by decreasing the mortality rate throughout the world. Major drawback of antibiotics is besides killing the bad bacteria it also kills the good bacteria and thus it disturbs the ecosystem of the body. In the era of advanced technology, the introduction of probiotics has widened the scope of medicines. In "Probiotics" the mechanisms are employed selectively to remove only the pathogens while leaving the remaining ecosystem of the body intact. [4] Immune system is a remarkably sophisticated defence system within vertebrates which protects them from foreign agents. It is possible to produce variety of cells and molecules capable of identifying and removing unlimited varieties of foreign and undesirable agents. Modulation of immune system means change in the immune response that can involve induction, expression, amplification or inhibition of any part or phase of the immune response. Thus immunomodulators are the agents which are used for their effect on the immune system. There are generally two types of immunomodulators based on their effects as immunosuppressant's and immunostimulators. Immunosuppressants are the agents which suppress or prevent the immune response. Immunosuppressants are used to prevent the rejection of transplanted organ and to treat the autoimmune diseases such as psoriasis, rheumatoid arthritis, and Crohn's disease. Immunostimulants are the substances which stimulates the immune system by inducing activation or increasing activity of any of its components. [5,6] Safety aspect is very important for every probiotic strain which mainly includes *in vitro* studies, animal studies, and human clinical studies have been used for the safety assessment of probiotics. [7,8] Bacterial spore formers, mostly of genus *Bacillus* are major probiotic product in use today. They have to be properly characterized for content stability and health effects to be categorized as probiotics. *Bacilli* being generally found everywhere consistently enter the gastrointestinal and respiratory tracts of healthy people through food water and air. *Bacillus* strains offers some advantages over the more common *Lactobaccilus* products in that they can be stored for a long time in a desiccated form without any effect on viability. In addition, they can also survive at low pH of gastric barrier. *Bacillus* species acts by increasing level of cytokines, competitive exclusion of gastrointestinal pathogen by competing for adhesion sites and secretion of antimicrobial compounds. Therapeutic benefits of *bacillus* species include improved nutrition and growth enhanced immunity and prevention of various gastrointestinal disorders such as diarrhoea, irritable bowel disease (IBD), Crohns disease, ulcerative colitis, respiratory disorders, allergies, skin disorders, bacterial vaginosis and cancer. [9] The production and use of probiotics is being worldwide. As probiotics are strain specific, toxicity studies need to be carried out in order to establish safety. Even though probiotics are generally regarded as safe (GRAS), their safety should not be taken for granted and evaluation of every product should be done on case basis. Assessment of acute and repeated dose toxicity must be carried out for all potential strain in order to establish safety. Lack of probiotics safety assessment can lead to probiotics being a source of food borne infectious diseases. As in one case the food poisoning was observed in a study for *Bacillus cereus* in China. The FAO/WHO report has laid down a set of guidelines for a product to be used as probiotic or novel supplement that must be judicially followed. A few animal studies include acute and sub chronic toxicity testing as well as *in vitro* studies have been carried out on some of the *bacillus* strains *B. subtilis, B. indicus, B. coagulans* and *B. licheniformis.* No adverse effects have been reported in any of the studies. However, there are some *bacillus* species which are found to be pathogenic such as *Bacillus anthracis* which is being involved in systemic and hospital acquired infections and *Bacillus cereus* is involved in diarrhoea and food poisoning. Worldwide use of probiotic bacteria joined in close association with antibiotic use or rather misuse, can over the time establish a reservoir of antibiotic resistant genes in probiotic bacteria. While intrinsic antibiotic resistance can be desirable trait as probiotic help restores host gut microflora during the course of antibiotic, the transfer of resistant genes to humans offers serious clinical threats. It is estimated that the risk of developing bacterinemia from ingested *Lactobaccilus* probiotics is less than 1 per 1 million users. [9,10] whereas the risk of developing funginemia from *Saccharomyces boulardi* is estimated at 1 per 5.6 million users and is estimated to be lower in healthy individuals. [10,11]

PROBIOTICS:

1.Probiotics: What Are They?

The word probiotic comes from the Greek word 'pro bios' which indicates 'for life'. The history of probiotics began with the history of man. Cheese and fermented milk were the well-known food products to Greeks and Romans, who recommended their consumption, especially for children and the patients who are recovering from illness. Probiotics are defined as the living bacteria which are

administered in sufficient number to survive in the intestinal ecosystem. They must have a positive effect on the host. [12] The term 'probiotic' was first used by Lilly and Stillwell in 1965 to describe the substances secreted by one microorganism that stimulate the growth of another. [12] A powerful evolution of this definition was coined by Parker in 1974, who proposed that probiotics are 'organisms or the substances which contribute mainly for microbial balance in intestine. [13] In more modern definitions, the concept of an action on the gut microflora, and even that of live microorganisms disappeared. Salminen et. al (1996) defined probiotics as the 'food which contains live bacteria is beneficial to health'. [14] whereas Marteau et al(2001) defined them as 'microbial cell preparations or components of microbial cells that have a beneficial effect on the health and wellbeing'. [15] Some modern definitions include more precisely a preventive or therapeutic action of probiotics. For example Charteris et al.(1997) defined probiotics as 'microorganisms, when taken, may show a good action in prevention and treatment of a specific pathologic condition'.[18] Finally, since probiotics have been found to be effective in the treatment of some gastrointestinal diseases. [17] They can be considered to be therapeutic agents.[16] It is clear that a number of definitions of the term 'probiotic' have been used over the years but the one derived by the Food and Agriculture Organization of the United Nations / World Health Organization and approved by the International Scientific Association for Probiotics and Prebiotics are: 'live microorganisms which, when administered in sufficient amounts, imparts a health benefit on the host'.[19] This definition retains historical elements of the use of living organisms for health purposes but does not set limits for the application of term only to oral probiotics with intestinal outcomes. [20] Despite these numerous theoretical definitions, however, the practical question arises whether a given microorganism can be considered to be a probiotic or not. Some strict criteria have been proposed.

For example, Havenaar et al.(1992), proposed the following parameters to select a probiotic

(i) Total safety for the host.

(ii) Resistance to gastric acidity and pancreatic secretions.

(iii) Adhesion to epithelial cells, antimicrobial activity.

(iv) Inhibition of adhesion of pathogenic bacteria.

(v) Evaluation of resistance to antibiotics, tolerance to food excipients and stability in the food matrix.

The probiotics in use today have not been selected on the basis of all these criteria, but the most commonly used probiotics are the strains of lactic acid bacteria such as *Lactobacillus, Bifidobacterium* and *Streptococcus* (*S. thermophilus*); the first two are known to resist gastric acid, bile salts and pancreatic enzymes, to adhere to colonic mucosa and readily colonize the intestinal tract. [21]

History of probiotics:

The origin of cultured dairy products dates back to the dawn of civilization. They are mentioned in the Bible and the sacred books of Hinduism. Climatic conditions favoured the development of many of the traditional soured milk or cultured dairy products such as kefir, koumiss, leben and yogurt. [22] These products, many of which are still widely consumed, had often been used therapeutically before the existence of bacteria was found. [20] At the beginning of the 20th century the major functions of gut flora were not known. I.I. Metchnikoff, the winner of Nobel Prize in Medicine in 1908, at the Pasteur Institute linked the health and longevity to ingestion of bacteria present in yogurt. [23] He believed that the composition of the human body presented several disharmonies inherited from primitive mammals, such as body hair, wisdom teeth, stomach, vermiform appendix, caecum, and large intestine. In 1907, he postulated that the bacteria involved in yogurt fermentation, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, blocks the putrefactive type fermentations of the intestinal flora and that consumption of these yogurt played a role in maintaining health. Indeed, he attributed the long life of Bulgarian peasants to their intake of yogurt containing *Lactobacillus* species. [24] In particular, he reported that the large intestine, probiotics are useful to mammals in management of rough food containing bulky vegetables which is useless in humans. Moreover, it is the site of dangerous intestinal putrefaction processes which can be opposed by introducing *lactobacilli* into the body, displacing toxins producing bacteria, promoting health, and prolonging life. [25] Tissier's discovery of *bifidobacteria* in breast fed infants also played a key role in establishing the concept that specific bacteria take part in maintaining health. In 1906, Tissier reported clinical benefits by improving the intestinal flora in infants with intestinal infections. [26] At the time, many others were having doubts about the concept of microbial therapy and were questioned about whether the yogurt bacteria (*L. bulgaricus*) are able to survive intestinal transit, colonize and convey benefits. [27] In the early 1920s, *L. acidophilus* milk was documented to have therapeutic effects, in particular, a settling effect on digestion. [28] It was believed that colonization and growth of these microorganisms in the gut are important for their effectiveness, and thus, the use of intestinal isolates was advocated. In Japan in the early 1930s, Shirota focused his research on selecting the strains of intestinal bacteria which could survive the passage through the gastrointestinal tract and use of such strains to develop fermented milk for distribution in his clinic.

His first product was containing *L. acidophilus* Shirota (subsequently named *L. casei* Shirota) was the basis for the establishment of the Yakult Honsha company. The world health organization defines probiotics as the living microorganisms which when administered in adequate amount confers the health benefit on the host.

Five conditions must be fulfilled for a probiotic to be effective: it must

(i) Have a proven beneficial effect on the host.
(ii) Not be toxic or pathogenic.
(iii) Contain sufficiently large number of viable microorganisms per unit.
(iv) Must be capable of surviving in the intestine, reproducing and maintaining itself and having intraluminal metabolic activity.
(v) Should remain viable during storage and use. [29]

Probiotics and their sources:

The probiotics bacteria generally belong to the *Lactobacillus* and *Bifidobacterium* genera. However, other bacteria and some yeast also have probiotic properties. Common bacteria include the following.

- Lactic acid bacteria (LAB): Genus *Lactobacilli* spp.;
  Species: *L.acidophilus, L.amylovorous, L.brevis, L.bulgaricus, L.casei, L.cellobiosus, L.crispatus, L.curvatus, L.delbrueckii spp. L.Bulgaris , L.fermentum, L.gallinarum, L.helveticus, L. johnsonii, L. lactis, L.paracasei, L.plantarum, L. reutri, L.rhamnosus;* Genus: *Streptococcus spp.* Species: *Streptococcus salivaris* spp. *Thermophiles*; Genus:*Lactococcus* ssp, Species:*L.lactis cremoris*; Genus: *Leuconostoc* ; Species: *Lc. Mesenteroides;* and Genus: *Pediococcus* spp., Species: *P.pentosaceus, P.acidilactici.*
- *Bifidobacteria:* Genus: *Bifidobacterium* spp., Species: *B. adolescentis , B.animalis, B.bifidum,B.breve, B.essensis, B.infantis, B.laterosporum, B.thermophilum, B.longum.*
- *Propionibacteria:* Genus: *Propionibacterium* spp., Species: *P.acidipropionici, P.jensenii, P.thoenii.*
- *Enterobacteria:* Genus: *Enterococcus* spp., Species: *E.fecalis, E. faecium.*
- *Sporulated bacteria:* Genus*: Bacillus spp.,* Species: *B.alcophilus, B.cereus, B.clausii, B.coagulans,B.subtilis.*
- Other bacteria: Genus:*Escherichia coli,* Species: *E-coli;* Genus: *Sporolactobacillus* spp. Species: *S.innulinus.*
- *Yeasts:* Genus: *Saccharomyces* spp., Species: *S.cerevisae (boulardii);* that isolated from litchi fruit in Indonesia have also been accepted and used as probiotics.

To be considered as probiotics, the different strains should be normal inhabitants of a healthy intestinal tract, they must survive the upper digestive tract, must be capable of surviving and growing in intestine (acid and bile resistant), be safe for human consumption, produce antimicrobial substances (i.e.bacteriocins), and have the ability of adherence to human intestine cell lines and colonization. [30,31]

Tomasik and Tomasik (2003) established the criteria for microorganisms to be included in the probiotic group as follows:

(i) Surviving on passing through the GIT at low pH and on contact with bile;
(ii) Adhesion to intestinal epithelial;
(iii) Stabilization of the intestinal microflora;
(iv) Nonpathogenic
(v) Survival in foodstuffs and possibility for the production of pharmacopoeia lyophilized preparation;
(vi) Fast multiplication with either permanent or temporary colonization of GIT; and
(vii) Generic specificity of probiotics. [30,32]

Mechanism of Action of Probiotics:

Major probiotic mechanisms of action include

(i) Enhancement of the epithelial barrier,
(ii) Increased adhesion to intestinal mucosa, and
(iii) Inhibition of pathogen adhesion,
(iv)Competitive exclusion of pathogenic microorganisms,
(v) Production of anti-microorganism substances and
(vi) Modulation of the immune system (Fig.1).

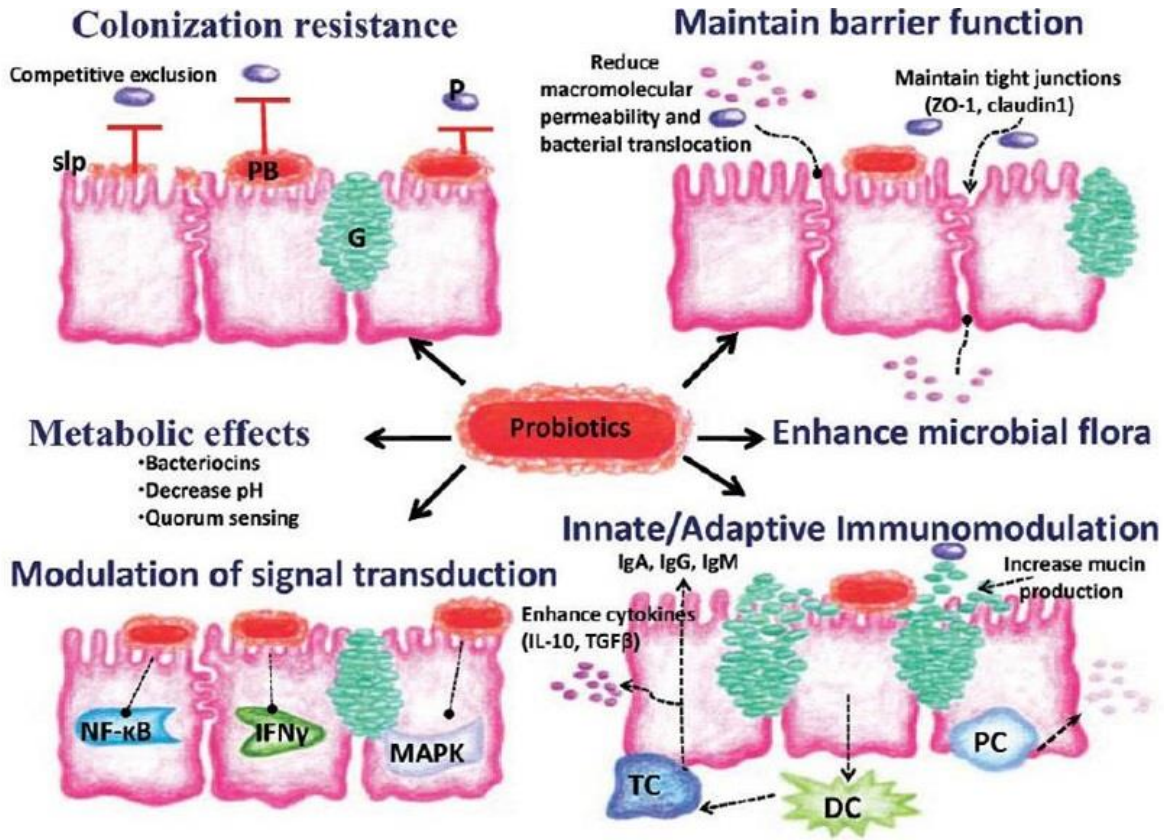

Figure. 1. Major mechanisms of action of probiotics. [33]

PROBIOTICS AND THE IMMUNE SYSTEM:

We know that probiotic bacteria can show modulatory effect on immune system. These bacteria have the ability to interact with epithelial and dendritic cells (DCs) and with monocytes/macrophages and lymphocytes. The immune system can be divided between the innate and adaptive systems. The adaptive immune response is based on B and T lymphocytes, which are specific for particular antigens. Also, the innate immune system shows response to common structures called pathogen associated molecular patterns (PAMPs) which are shared by most of the pathogens. The primary response to pathogens is triggered by pattern recognition receptors (PPRs), which bind PAMPs. The best studied PPRs are toll like receptors (TLRs). In addition, extracellular C type lectin receptors (CLRs) and intracellular nucleotide binding oligomerization domain containing protein (NOD) like receptors (NLRs) are known to transmit signals upon interaction with bacteria. It is well known that the host cells that interact most extensively with probiotics are IECs. In addition, probiotics can encounter DCs, which have an important role in innate and adaptive immunity. Both IECs and DCs can interact with and respond to gut microorganisms through their PPRs. Figure 2 shows a summary of how probiotics may interact and modulate the immune system.

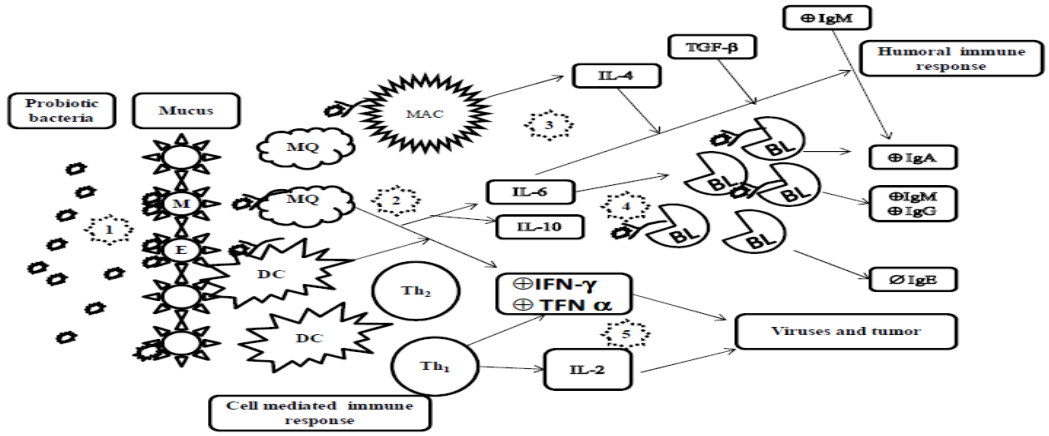

Figure 2. Hypothesized mechanism of immunomodulation by probiotics. [34]

(i) Interaction of probiotic bacteria with epithelial cells (E) or Mucus cells (M) or the Dendritic cells (DC) results in the internalization of the bacteria or its components.
(ii) This interaction stimulates the release of IL-6 by epithelial cells and stimulates macrophages (MQ) and dendritic cells to produce TNF-α and IFN-γ.
(iii) Mast cells (MAC) or also stimulated to produce the cytokine IL-4, which together with IL-6 and TGF-b induce the T independent switch from IgM to IgA on the surface of B lymphocytes (BL), thereby enhancing the production of Ig A.
(iv) IL-6 favours the clonal expansion of B lymphocytes. There is also an associated increase in the production of antibodies such as IgA,IgM, IgG and reduced secretion of Ig E.
(v) Th1 cells produce pro-inflammatory cytokines such as IFNγ, TNFα and IL-2, which stimulate the phagocytosis and destruction of microbial pathogens and induce macrophages, natural killer cells and cytotoxic T-lymphocytes to kill viruses and tumors. [34]

THERAPEUTIC USES OF PROBIOTICS:

Probiotics are used in prevention or treatment of various diseases. The therapeutic benefits of probiotics are shown in figure 3.

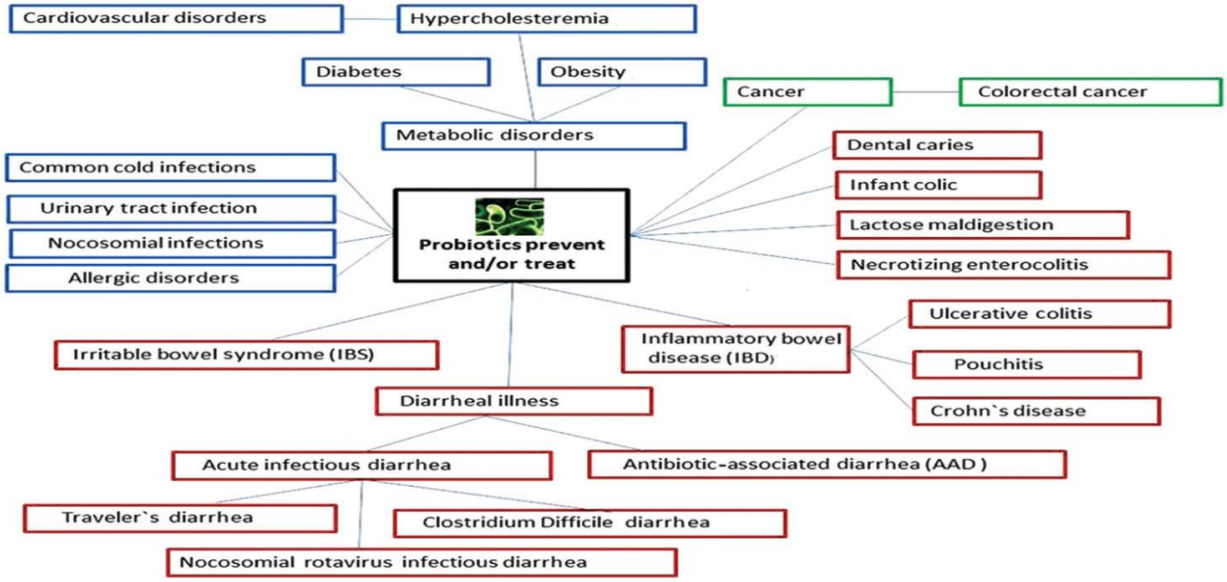

Figure 3: Therapeutic uses of probiotics. [36]

IMMUNITY:

Immunity is sum of all naturally occurring defense mechanism that protect humans from infectious diseases. Immunology is the science related with study of the organs, cells, and chemical composition of the immune system. The immune system creates both innate and adaptive immune responses. The innate response exists in many lower species and it acts through relatively crude means against large classes of pathogens. The adaptive response is unique to vertebrates reacting to the foreign invaders with specificity and selectivity. The immune system must maintain a delicate balance with potent defensive responses. [35,37]

Immune system:

Immune system composed of various components as shown in figure 4 also many interdependent cell types that collectively protects the body from the bacterial, fungal, viral infections and from the growth of tumor cells. Many of these cells have specialized functions. The cells of immune system can engulf bacteria, kill parasites or tumor cells or kill viral infected cells. Also, these cells depends on T helper subset for the activation signals formally known as cytokines, lymphokines or interleukins. [35,37]

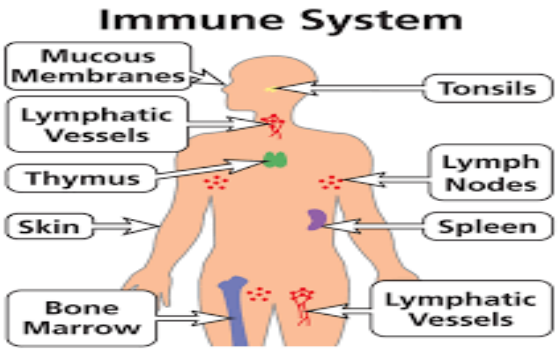

Figure 4. Human Immune system. [38]

1.Innate immune system:

Innate immunity comprises a series of host defences as shown in figure 5. It also includes barrier functions such as cytokines, phagocytes, natural killer (NK) cells and T cells to provide initial nonspecific response to pathogen or injury. These

responses are phylogenetically ancient and have been developed to cope with the pathogens that are encountered regularly but that rarely caused disease. Unlike the adaptive immune system, responses are generic and leave no memory. Innate immune system functions effectively to keep the microorganisms healthy. Indeed failing in innate immunity is hypothesized to contribute secondary infections in critical illness and death in sepsis. Stimulation of active components of innate immune system occurs by way of pathogen associated molecular pattern (PAMP) receptors or damaged associated molecular pattern (DAMP) receptors. PAMPs are identified by membrane bound or vesicular pathogen recognition receptors (PRRs) which includes the toll like receptors (TLRs), nucleotide binding oligomerization domain (NOD) like receptors and RIG-I like receptors. Bacteria stimulate these PRRs to activate various intracellular signaling cascades which leads to pro inflammatory response. For example, gram negative bacteria endotoxin lipopolysaccharide binds to TLR4, whereas the gram positive murine binds to Toll Like Receptor 2 in the setting of tissue damage from an infection, DAMPs stimulates the innate immune system through these pattern recognition receptors. Indeed, there is significant overlap in mechanism stimulated by PAMPs and DAMPs. As sedatives are mostly administered during infection and surgery, systemic examination of their immune effects on these mechanism of immune stimulation would be seen carefully. [34]

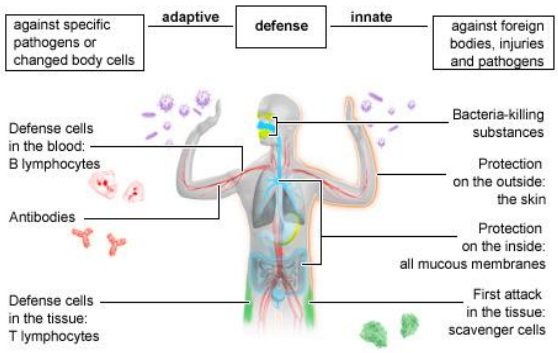

Figure 5. Types and functions of immune system. [39]

2.The adaptive immune system:

Adaptive or acquired immunity response is different from the innate immunity response as it is specific, it is also having part of memory, and is specific to vertebrates. The humoral immunity includes the rapid increase in number of antigen stimulated B lymphocytes into plasma cells which secretes antibodies. The components of cells shows physiological effects by T lymphocytes, the predominant cell types being helper T cells (Th) and cytotoxic T cells. Recently, regulatory T cells that likely dampen the immune response have been identified. T cells are responsible to identify antigens bound to major histocompatibility complex (MHC) proteins by way of T cell receptors which are specific to particular antigens. T lymphocytes act through secretion of cytokines to produce immune response. This action includes increasing immunoglobin levels such as IgA,IgG and IgM,switching of B cells, activation of T-cells, and makes the best use of bactericidal potential of phagocytes. Th lymphocytes are identified by expression of CD4 proteins and are activated when MHC type II molecules, expressed on professional antigen-presenting cells (dendritic cells, macrophages, and B cells), activate the specific T cell receptor. Th1 cells are regarded as"pro inflammatory'' secreting cytokines such as interferon-g and interleukin (IL)-12, and stimulate macrophage function and cytotoxic T cell function. Th2 cells have an "antiinflammatory" phenotype and secretes cytokine such as Interleukine-4 and Interleukine-10, acting cooperatively to activate B cells. Further, T- cells include the regulatory T cells that act to weaken the immune response and the Th17 class that modulates neutrophil function. A shift from Th1 to Th2 cells has been observed in the latter stages of sepsis, possibly induced by the apoptotic cell death of lymphocytes, and the subsequent anti-inflammatory phenotype has been associated with secondary infections in this patients. [34,35]

The Organs of the Immune System

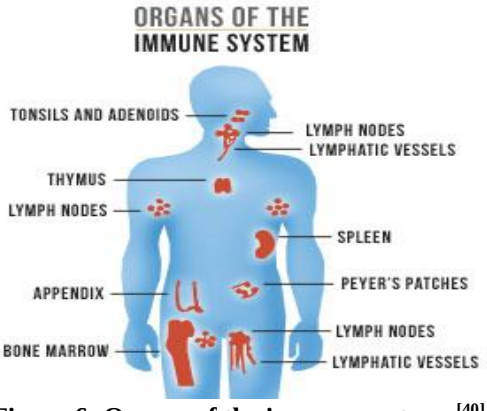

Figure 6: Organs of the immune system. [40]

1.Bone Marrow

As shown in figure 6. All the cells of the immune system are initially derived from the bone marrow. They are formed through a process called hematopoiesis. During the process of hematopoiesis, stem cells produced through bone marrow differentiate into either mature cells or precursors of cells that migrate out of the bone

marrow to continue their maturation elsewhere. The bone marrow secretes B-cells, natural killer cells, granulocytes and immature thymocytes, in addition to erythrocytes and thrombocytes. [34,37,41,43]

2.Thymus

The function of the thymus is to produce mature T cells. Immature thymocytes, also known as prothymocytes, leave the bone marrow and migrate into the thymus. Through a remarkable maturation process sometimes referred to as thymic education, T cells that are useful to the immune system are made available, while those T cells that might evoke a detrimental autoimmune response are eliminated. The mature T cells are then released into the bloodstream. [35,37,41,43]

3.Spleen

The spleen is an immunologic filter of the blood. It is made up of macrophages, B cells, T cells, dendritic cells, lymphocytes and erythrocytes. Spleen captures foreign materials (antigens) from the blood that passes through it, migratory macrophages and dendritic cells bring antigens to the spleen via the bloodstream. An immune response is generated when the macrophage or dendritic cells brings the antigen to the appropriate B or T cells. This organ can be regarded as an immunological center. Spleen, activates the B cells which produces large amounts of antibodies. Also, old red blood cells are destroyed in the spleen. [34,37,41,43]

4.Lymph nodes

The lymph nodes acts as an immunologic filter for the body fluid known as lymph. Lymph nodes are present throughout the body. Lymph nodes are mostly composed of T cells, B cells, dendritic cells and macrophages, the nodes removes the fluid from most of our tissues. Foreign materials are filtered out of the lymph in the lymph node before returning to the circulation. In a similar way as the spleen, the macrophages and dendritic cells that capture antigens present these foreign materials to T and B cells, results to produce an immune response. [34,35,37,41]

The Cells of the Immune System:

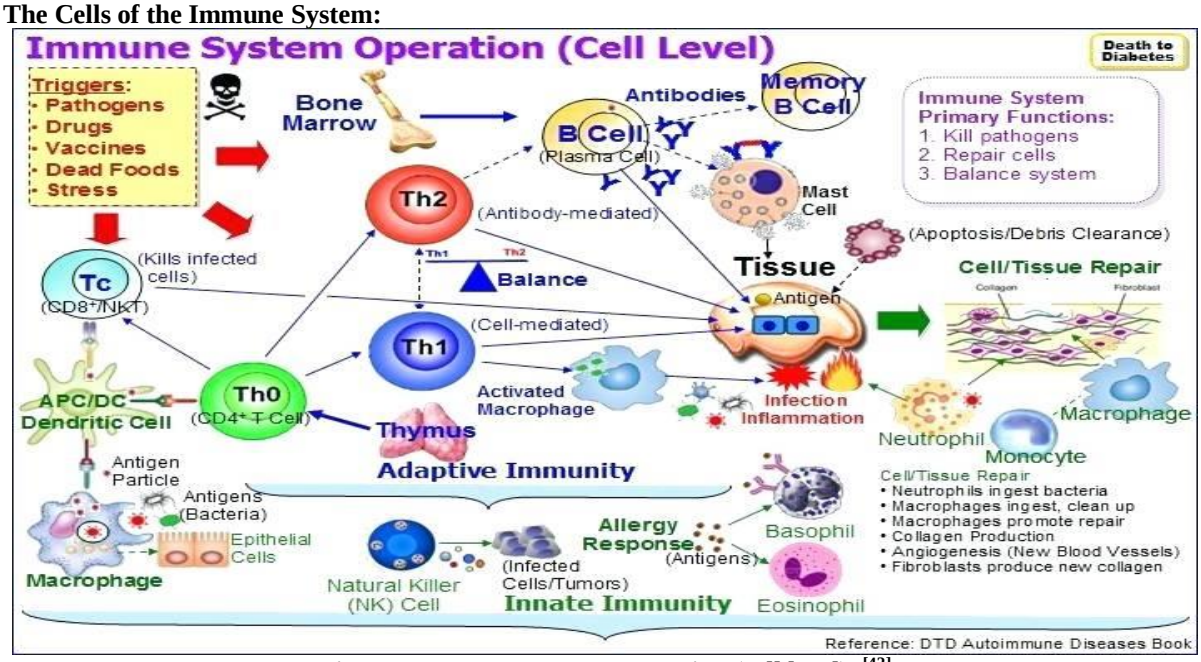

Figure 7: Immune system operation (cell level). [42]

1.T-Cells

T lymphocytes are mostly classified into two major subsets which are functionally and phenotypically (identifiably) different from each other as shown in figure 7. The T helper subset, also known as CD4+ T cell, is a pertinent coordinator of immune regulation. The main function of the T helper cell is to stimulate immune responses by the secretion of special factors which activate other white blood cells to fight off infection. Another important type of T cell is called the T killer/suppressor subset or CD8+ T cell. These cells mostly involved in directly killing certain tumor cells, viral-infected cells and sometimes parasites. The CD8+ T cells are also important in down-regulation of immune responses. T cells of both type are present throughout the body. Many a times T cells are depended on the secondary lymphoid organs i.e lymph nodes and spleen as sites where activation occurs, but they are also found in other tissues of the body, mostly in lungs, liver, blood, and reproductive and intestinal tracts. [34,43.44,45,46]

2.Natural Killer Cells

Natural killer cells, often referred to as NK cells, are similar to the killer T-cell subset (CD8+ T-cells). These cells functions as effector cells which are responsible for directly killing certain tumors such as malignant tumor associated with skin cancer (melanomas), viral infected cells, mostly herpes and cytomegalovirus infected cells and lymphoma. NK cells, unlike the CD8+ (killer) T-cells, kill their targets without a prior "conference" in the lymphoid organs. However, NK cells that have been activated by secretions from CD4+ T cells will kill their tumor or viral infected targets more effectively. [34,43.44,45,46]

3. B Cells

The main function of B lymphocytes is the production of antibodies in response to foreign agents such as viruses, bacteria, and cells of tumor. Antibodies are specialized proteins that specifically identifies and bind to one particular protein. Antibody production and binding to a foreign substance or antigen, many a times is critical as a means of signaling other cells to engulf, kill or remove that substance from the body. [34,43,45,46,47]

Granulocytes or Polymorphonuclear (PMN) Leukocytes:

Another group of white blood cells is collectively referred to as polymorphonuclear leukocytes (PMNs) or granulocytes. Granulocytes are composed of three cell types identified as neutrophil, eosinophils and basophils, based on their staining properties with certain dyes. These cells are mostly important in the elimination of parasites and bacteria from the body. They engulf these foreign bodies and degrade them using their powerful enzymes. [34,35,43,44,45,46]

1. Macrophages

Macrophages are important factor responsible for maintainance of immune response. They are also known as antigen presenting cells (APC) or scavengers because they ingest foreign materials and present these antigens to other cells of the immune system such as T cells and B cells. This is the first step in the initiation of an immune response. Stimulated macrophages shows increased levels of phagocytosis and are also secretory. [35,41.45,46,47]

2. Dendritic Cells

Another cell type, addressed only recently, is the dendritic cell. Origination of dendritic cells is in bone marrow, Dendritic cells acts as antigen presenting cells (APC). In fact, the dendritic cells are more effective APC as compared to macrophages. Dendritic cells are usually found in the lymphoid organs such as the thymus, lymph nodes and spleen. Also they are found in the bloodstream and other tissues of the body. It is said that DC captures antigen or bring it to the lymphoid organs where an immune response is initiated. Unfortunately, one reason we know so little about dendritic cells is that they are extremely difficult to isolate, which is many atimes a prerequirement for the study of the functional qualities of specific cell types. Of particular issue here is the recent finding that dendritic cells bind high amount of HIV, and may be a reservoir of virus that is transmitted to CD4+ T cells during an activation event. [34,43.44,45,46]

Immunomodulators:

Immunomodulators modulates and potentiate the weapons of our immune system to keep them in a highly prepared state for any harm it may experience. With this balancing effect, all subsequent immune responses improve. When immune system of body is in this highly balanced state, it is difficult for foreign agents to build up force and strength to weaken the immune system. Immunomodulation is the process of modification of an immune response either by stimulation or by suppression of system by administration of a drug or compound. Most of proteins, amino acids, and natural compounds are having significant potential to regulate immune responses, including interferon-γ (IFN-γ), steroids, DMG. These are biological or synthetic substances, which can stimulate, suppress or modulate any of the immune system including both adaptive and innate type of the immune response. Clinically immunomodulators can be classified into following three categories. [34,43]

1.Immunoadjuvant

These agents are used for enhancing vaccines efficacy and therefore, could be considered specific immune stimulants for example in this regard is of Freud's adjuvant. The immunoadjuvant acts as a true modulator of immune response. It has potential to act on both cellular and humoral immunity, Th1 (helper T1 cells) and Th2, (helper T2 cells) immunoprotective and immunodestructive, and reagenic (IgE) versus immunoglobin G (IgG) type of immune responses, which poses to be a real challenge to vaccine designers. [34,41]

2.Immunostimulant

These agents are inherently nonspecific in nature as they envisaged enhancing body's resistance against infection. Immunostimulants can stimulate both innate immune response and adaptive immune response. In healthy individuals the Immunostimulants serves as long term promoter agents i.e. as immunopotentiators by enhancing basic level of immune response, and in the individual with impairment of immune response as immunotherapeutic agents. [34,44]

3.Immunosuppressants

These are group of drugs, which are often administered in combination pattern to treat various types of organ transplant rejection and autoimmune diseases. [34,44]

Methods for Testing Immunological Factors:
The routine process for screening is to identify single active ingredient or single distilled fraction from herbal drugs and to determine its bioactivity by the classic pharmacological methods. The whole animal model is very important at the aspect of medicine evaluation because it can respond to the efficacy, side effect and toxicity of medicines in whole. Although this method requires high cost and having less efficiency, till now it is still preferred for drug discovery and evaluation process. Several *in vitro, in vivo* methods of pharmacological screening of medicines having immunomodulatory activity have been listed.

***In vitro* methods:**
1. Inhibition of histamine release from mast cells
2. Mitogens induced lymphocyte proliferation
3. Inhibition of T cell proliferation
4. Chemiluminescence in macrophages
5. PFC (plaque forming colony) test *in vitro*
6. Inhibition of dihydro-orotate dehydrogenase

***In vivo* methods:**
1. Spontaneous autoimmune diseases in animals
2. Acute systemic anaphylaxis in rats
3. Anti-anaphylactic activity (Schultz-Dale reaction)
4. Passive cutaneous anaphylaxis
5. Arthus type immediate hypersensitivity
6. Delayed type hypersensitivity
7. Reversed passive arthus reaction
8. Adjuvant arthritis in rats
9. Collagen type II induced arthritis in rats
10. Proteoglycan-induced progressive polyarthritis in mice
11. Experimental autoimmune thyroiditis
12. Coxsackievirus B3-induced myocarditis
13. Porcine cardiac myosin-induced autoimmune myocarditis in rats
14. Experimental allergic encephalomyelitis
15. Acute graft versus host disease (GVHD) in rats
16. Influence on SLE-like disorder in MRL/lpr mice
17. Prevention of experimentally induced myasthenia gravis in rats
18. Glomerulonephritis induced by antibasement membrane antibody in rats
19. Auto-immune uveitis in rats
20. Inhibition of allogenic transplant rejection. [34,45]

Effects of Probiotics on immunity:
It is clear that probiotics might have immunomodulatory effects, but it is still unknown how these effects are achieved. There have been several reports recently describing the effects of probiotics on IgA in both rodents and humans. Generally, an enhanced IgA production was observed during probiotic treatment. *Lactobacillus casei* and *Lactobacillus acidophilus* enhanced the number of IgA-producing plasma cells in a dose-dependent manner. In general IgA production was enhanced whereas IgE production was decreased. [46]

Effects of probiotics on cytokines:
Most of studies have shown that cytokine production by cells of the immune system can be increased by use of probiotics. This accounts for cells cultured *in vitro*, where the production of IL6 and to a lesser extent TNFα was increased after the cells were stimulated with a mitogen. Murine studies have been performed to determine the effect of *Lactobacillus casei* strain *Shirota* on resistance and immune parameters. This has been studied in host resistance models to the *Trichinella spiralis* parasite in different mouse strains. These studies showed no effect on severity of the infection when the lactobacilli were orally administered in different mouse strains, but another study showed that there was an effect when the administration was performed parenterally. [46] The studies did show an effect on several immune parameters. The Th1/Th2 balance was shifted more towards the Th1 side. [48] In this study the effects of orally administered viable *Lactobacillus casei Shirota* on the immune system in two rat strains was studied. The *T. spiralis* specific DTH (delayed type hypersensitivity) response was enhanced in both strains compared to the control groups. In both rat strains fed *L. casei*, serum *T. spiralis* specific IgG2b concentrations were also significantly increased. Other serum specific antibodies were not altered. Orally administered *Bifidobacterium breve* or *Bifidobacterium bifidum* had no effects on the immune system in both rat strains. This clearly demonstrates differences in mechanisms, and hence in outcome of various probiotics. Since the rat DTH response is considered to be a manifestation of Th1 cell-mediated immunity and the IgG2b isotype has been associated with Th1 activity, it was concluded that Th1 cells could play an active role in the immunomodulatory effects of orally administered *L. casei.* [47] Many more studies have been performed and they are summarised in Table 1 and 2. From these results can be concluded that probiotics can increase the production of serum IgA and IFNγ and enhance phagocytosis. [46]

Table no.1: Effect of probiotics on modulation of humoral immunity. [46]

	Species	Assessment	Effect
Lactobacillus casei Shirota, oral (heat killed)	Rodent	Systemic antibody response to ovalbumin	Inhibited splenocyte IgE in vitro and serum IgE.
L. casei, oral (live)	Rodent	*L. casei*, oral (live) Rodent Infection and antibody production in malnourished animals.	Increased sIgA and reduced enteric infection.
L. acidophilus + *Peptostreptococcus*, oral (live)	Rodent	Translocation of *E. Coli* and serum total anti-*E. Coli* IgG, IgE and IgM.	Decreased translocation and increased IgM and IgE.
Bifidobacterium bifidus, oral (live)	Rodent	Total IgA and response to polio virus	Increased sIgA.

Table no 2: Effects of probiotics on nonspecific immunity. [46]

Probiotics	Species	Assessment	Effect
Lactobacillus casei Shirota, intravenous	Rodent	Peritoneal Macrophages	Increased Phagocytosis
L. acidophilus or Bifidobacterium bifidum, oral (live)	Rodent	Peritoneal or peripheral blood macrophages	Enhanced Phagocytosis
L. acidophilus or casei, oral (live)	Rodent	Resident peritoneal Macrophages	Enhanced Phagocytosis
L. casei Shirota, oral (live)	Human	Peripheral blood	No effect on natural killer cell cytolysis in vitro

Probiotics and Innate Immune System:

Dendritic and epithelial cells are the furthermost cell types of the innate immune system described in the probiotics literature; these cells are usually the earliest to be in contact with intestinal microbes and their metabolic products. [49]

1.Probiotics and Dendritic Cell

Intestinal dendritic cells occupy the gut associated lymphoid tissues (GALT), or are distributed throughout the intestinal lamina propria. [49] Upon contact with microbes, dendritic cells (DCs) act as 'sensors' of microbial ligands; bacterial-derived molecules bind to receptors on DCs and activate different signaling pathways that result in modifications of their phenotypes and secreted cytokines; example of such receptors on DCs are Toll-like receptors and c-type lectin receptors [48,50]. Probiotic strain such as *Bifidobacterium infantis* 35624 can modulate the activity of DCs resulting in CD103(+) DCs increase in the lamina propria. The mechanism is retinoic acid-dependent leading to a reduction in the severity of Dextran sulfate sodium-induced colitis. [48,51] Moreover, *Bifidobacterium infantis* 14.518 orally administered to BALBC/mice stimulates DCs maturation and the accumulation of CD103+ tolerogenic DCs in the GALT, which induce the differentiation of regulatory T cells and suppression of Th2-biased response. [48,52] *Bifidobacterium longum, B. infantis* CCUG 52486, *B. longum* SP 07/3, Lactobacillus rhamnosus GG and L. casei enhance the expression of CD40, CD80 and CCR7 in both young and old DCs donors while TGF-β, TNF-α secretion is increased only by old donors. In addition, *B. longum, B. infantis* CCUG 52486 favours IL-10 production. [48,53] Certain probiotics like *Lactobacillus rhamnosus* can induce heme oxygenase in dendritic cells resulting in mucosal T regulatory (Treg) cells within the GALT. [48,53,54] Furthermore, *Lactobacillus rhamnosus* JB-1 modulates the DCs response by involving DC-SIGN and TLR-2 pattern recognition receptors (PRRs). *Lactobacillus rhamnosus* JB-1 primed human monocyte derived dendritic cells (MDDCs) show a reduced induction of costimulatory molecule expression, cytokine production and TH1 or TH17 transcription factors. In addition, Lb. rhamnosus JB-1 primed MDDCs induce Foxp3 expression in autologous lymphocytes leading to an increase in IL-10 production. [48,55] The immunoregulation of DCs is also associated with bacterial cell wall components. For example, capsular polysaccharide A interacts with TLR-2 of plasmacytoid DCs which respond by limiting the expression of pro-inflammatory cytokines and stimulating IL-10 secretion by CD4+ T cells mediating an anti-inflammatory response in a mouse model of colitis. [48,56] Likewise, bacterial exopolysaccharides derived from *Bacillus subtilis* protect against intestinal inflammation by the

enteric mouse pathogen *Citrobacter rodentium* in a TLR4-dependent manner. [48,57] On the other hand, probiotics can modulate DCs stimulation by modulating bacterial populations While MHCII-dependent antigen presentation of segmented filamentous bacterial antigens by intestinal DCs is crucial for Th17 cell induction, administration of Lactobacillus plantarum modulates the population of segmented filamentous bacteria in the intestine of immunocompromised mice leading to changes in Th17 activation. [48,58,59]

2.Probiotics and Epithelial Cells

Epithelial cells are known for their important absorptive function. In terms of immunoprotection, epithelial cells protect the host from pathogenic microorganisms and toxic agents by forming a mucosal barrier. A complex relationship exists between the gut mucosal barrier and the underlying immune cells of the lamina propria and Peyer's patches as well as with the luminal contents e.g. commensal bacteria, bacterial metabolites, invaders, nutrients, etc). [48,60] Probiotics are known to maintain intestinal barrier integrity by different mechanisms; they compete with pathogens for nutrients and attachment sites on epithelial cells preventing pathogens' colonization and they can modulate the immune response resulting in an immunostimulation or immunoregulation (tolerogenic response). For example, *Bifidobacterium infantis* 35624 protects against *Salmonella* infection damage by a Treg-dependent mechanism and by reducing Peyer's patch macrophage inflammatory protein-1α (MIP)-1α and MIP-1β secretion. [48,60,61,62] In addition, probiotic bacteria can reinforce the mucosal barrier defences by inducing antimicrobial peptides, such as human β-defensin-2 (hBD-2); noting that defensins are broad spectrum anti-microbial peptides produced by epithelial cells, Paneth cells, neutrophils and macrophages contributing to innate immunity response. *Lactobacillus casei* strain Shirota by augmenting hBD-2 in Caco-2 colonic intestinal cells and subsequently enhances defensin mRNA expression. [48,60] Probiotics can also influence intestinal epithelial cells autophagy which is an essential mechanism for the maintenance of the epithelial barrier: *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium bifidum* and *Bifidobacterium adolescentis* can upregulate the autophagy response in intestinal epithelial cells. [48,63] In addition they can promote mucin production which plays a role as the first line of intestinal defence against infection and injury; *Lactobacillus rhamnosus* GG3 (LGG)3 up-regulates Muc2 gene expression in intestinal epithelial cells and activates epidermal growth factor receptor by p40 (a *Lactobacillus rhamnosus* GG-derived soluble protein) leading to mucin secretion [48,64]. Upon infection, intestinal epithelial cells secrete proinflammatory cytokines and chemokines as well as some monocyte chemoattractant protein activating an efficient immune response. An extensive range of probiotics can suppress epithelial cell proinflammatory chemokine production after infection: *Lactobacillus rhamnosus* L34 and *Lactobacillus casei* L39 reduce IL-8 production by colonic epithelial cells after infection with *Clostridium difficile*, *Lactobacilli* species diminish Caco-2 cells IL-8 secretion in response to TNF-a and *Salmonella* challenge and *Bifidobacterium longum subsp. infantis* BB-02 attenuates ulcerative colitis in an acute murine experimental model of inflammatory bowel disease by reducing KC/CXCL-1 levels. [48,65,66,67]

3.Probiotics and Natural Killer T cells

NK cells are influenced by probiotic lipid antigens. *B. polyfermenticus* increases CD56+ NK cells, *Bifidobacterium lactus* HN019 supplementation improves NK tumoricidal activity in elderly adults and *Lactobacillus casei* Shirota (LcS) induces the surface expression of CD69 and CD25 on both CD8+ and CD56+ subsets without any other stimulus present thus activating NK cell activity.[48,68,69] Also, daily consumption of yogurt containing *Lactobacillus* paracasei ssp. *paracase*i, *Bifidobacterium animalis ssp. lactis* and heat-treated *Lactobacillus plantarum* enhances NK cell function. [48,70]

Probiotics and Adaptive Immune System:

1.Probiotics and T Lymphocytes

Probiotics beneficial effect against certain diseases such as allergy or colitis is attributed to their ability to increase the number Treg cells. [48,71] *Bifidobacterium longum* (*B. longum*) improves colorectal colitis in rats by up-regulating the proportion of Treg cells; thus, IL-10, and the ratio of IL-10/IL-12 are increased in the serum while the pro-inflammatory cytokines IL-12, IL-17 and IL-23 are downregulated. [48,72,73] In rats with 2, 4, 6TBS induced colitis, *Bifidobacterium longum* (*B. Longum*) demethylates several CpG sites in Foxp3 providing a stable long-term expression of Treg [48,74]. Similarly, in healthy humans, an upregulation of Foxp3 lymphocytes occurs after consumption of *B. infantis* 35624 which diminishes the proinflammatory cytokines levels in patients with psoriasis or ulcerative colitis. [48,55,75] Probiotics can modulate Tcells response by their metabolites like short-chain fatty acids (SCFAs): involves mainly 17 strains of mixture clostrida within clusters IV, XIVa and XVIII, induce IL-10 and inducible T-cell co-stimulator (ICOS). Acetate, propionate, butyrate and isobutyrate in caecal contents of mice receiving a mixture of SFCA indirectly contribute to immune homeostasis and up-regulation of T reg cells [48,76]. In fact, the short fatty acid butyrate

stimulates extrathymic generation of Treg cells while de novo Treg-cell generation is potentiated by propionate which inhibits histone deacetylase (HDAC). [48,77,78] Butyrate induces Foxp3+ cells even under TH1- and TH17-polarizing circumstances. [48,78] In addition, SCFA activate G-protein-coupled receptor 43 leading to improvements in colitis and allergic diseases; GPR43 on colonic T cells enhances the suppressive function of Foxp3+ Tregs through epigenetic modifications [48,77,78,81,80]. Th17 inflammatory cells can be suppressed by probiotic Th17 as well as (IL)-17A-F, the signature cytokines produced by Th17, are involved in the pathogenesis of different inflammatory conditions like inflammatory bowel diseases. *Bifidobacterium breve*, *B. longum*, *Lactobacillus acidophilus*, *B. longum subsp. infantis*, and *L. gasseri* A5 are known to reduce IL17. Furthermore, *B. breve* and *L. rhamnosus* GG reduce IL-23 which is responsible of the expansion, stabilization and conditioning of Th1741. Several probiotics are known to downregulate the expression and production of TNF-α and INFγ which are downstream Th17 (e.g. *lactobacilli* and *Bifidobacterium* species) [48,82]. *B. longum* JCM 1222T improved IL-27 production which has been stated to suppress the generation of IL-17-producing T cells 42. Probiotics can polarize the immune response towards Th1 instead of Th2.For example, a number of probiotic *Lactobacilli, such as Lactobacillus casei* can promote the production of IL-12, thus polarizing Th1 response and alleviating Th2-related diseases. *Lactobacillus rhamnosus* also suppresses Th2, Th17 and ameliorates clinical signs related to atopic dermatitis (AD), allergic asthma or rhinitis. [48,71,82] In mice the allergic process induced by ovalbumin is modulated by probiotic fermented milk which polarized a Th1 response instead of Th2 profile response and resulted in production of IgG instead of IgE, with increasing levels of IL-10 and IFN-γ responsible immunomodulation. [48,85]

2.Probiotics and B lymphocytes

Probiotics can increase the number of IgA positive cells in Peyer's patches and in the lamina propria leading to an induction of IgA production which plays an essential role in host mucosal protection against mucosal pathogens. IgA prohibits bacterial binding to epithelial cells and counteracts toxins. *Lactobacillus gasseri* SBT2055 (LG2055) activates TLR2 signal which induces IgA production and amplifies the rate of IgA+ cells in Peyer's patch and in the lamina propria of the mouse small intestine. Although B lymphocytes are responsible secreting specific antibodies and are the main player in humoral response, they can negatively regulate immunity by producing IL-10 during autoimmune and infectious diseases [48,83,84] *Clostridium butyricum* in combination with specific immunotherapy can transform antigen specific B-cells to regulatory B-cells in asthmatic patients [48,85]. Probiotics are able to increase the number of IgG + memory B-cells and total IgG + B-cells in response to certain vaccines such as Bifidobacterium longum bv infantis CCUG 52486 and influenza vaccination. [48,85]

CONCLUSION AND FUTURE SCOPE:

Probiotics are generally accepted as being live organisms that, when administered in adequate amounts, imparts a healthy benefit to the host. Information about probiotic effects which are strain-specific and the relationship between the dose, its duration, and effect on a strain-by-strain along with characteristics is the basis which is needed. The probiotics must arrive in therapeutic levels at the site of action to find out the dose-response relationship. Classical microbial species that have traditionally been regarded as safe are used in probiotics. Very few cases of adverse reactions have been reported in humans consuming probiotics on regular basis. Antimicrobial production is an important feature of probiotic organism which are responsible for functioning in the gut. Although production of lactate as well as acetate by Bifidobacteria is important in antimicrobials, bacteriocins can also provide a competitive side for bacteria. The simultaneous administration of probiotics and prebiotics (named symbiotic) may synergistically improve their health-promoting effects in the host. The use of probiotics in medical practice is increasing rapidly. They have been used in the prevention and treatment of various diseases, but are not limited to intestinal infectious diseases. Various mechanisms, such as enhanced immune response, reduction of mutagenic compounds, reduction of intestinal inflammation, have varying levels of supporting evidence. The *in vitro* testing and animal and human studies used for efficacy and toxicity of probiotics sometimes contain limitations that must be considered in the assessment.

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